CLINAM

European Foundation for Clinical Nanomedicine

9/2016

Basel, June 26–29

EUROPEAN & GLOBAL SUMMIT FOR CLINICAL NANOMEDICINE AND TARGETED MEDICINE

Enabling Technologies for Personalized Medicine

CONFERENCE PROCEEDINGS

SUPPORTED BY THE SWISS CONFEDERATION, SWISS DEPARTMENT OF ECONOMIC AFFAIRS, EDUCATION AND RESEARCH

In Collaboration with:

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Swiss Confederation

Federal Department of Economic Affairs, Education and Research EAER
State Secretariat for Education, Research and Innovation SERI

Non Biological Complex Drugs working group

EPMA

EFGCP

E-MRS

NCNST

eppm European Platform for Photodynamic Medicine

FDA

European Commission

FAG Freiwillige Akademische Gesellschaft Basel

APV

University of Basel

nanomedicine European Technology Platform

European Medicines Agency Science Medicines Health

Helmholtz Institute for Pharmaceutical Research Saarland
Helmholtz Zentrum für Infektionsforschung
Universität des Saarlandes

EURO nanotox LETTERS

DIA Develop Innovate Advance

imi innovative medicines initiative

Basel

Basel Landschaft

inet

EMPA Materials Science & Technology

CIMT Cancer Immunotherapy

Swiss Biotech Association

BIO NANONET

NIHS

LUNDBECKFONDEN Center of Excellence NanoCAN

NCL Nanotechnology Characterization Lab. NCI Alliance for Nanotechnology in Cancer

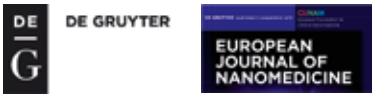
Nanomedicines ALLIANCE

ESNAM European Society for Nanomedicine

Nanosafety Lab. CAS

www.clinam.org

EXIBITORS 2016:



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CLINAM 9/2016

EUROPEAN & GLOBAL SUMMIT FOR CUTTING EDGE MEDICINE: CLINICAL NANOMEDICINE AND TARGETED MEDICINE

The Founders in 2007

The CLINAM-Foundation and 31 collaborators of the Summit have the pleasure to announce CLINAM 9/2016 held in Basel from June 26–29, 2016. This worldwide interdisciplinary Nanomedicine Platform, organized by the CLINAM-Foundation as non-for-profit organization, is the place, where members of the nanomedical community meet to debate in small and large groups the development of Nanomedicine and Targeted Medicine, the building blocks on the road to personalized medicine, with 190 short interventions – and ample time for debates.

The CLINAM Summit is the valuable interaction place to launch collaborations, develop new ideas and learn about novel methodologies and technologies as well as about novel projects, including numerous EU-wide efforts, in Nanomedicine and Targeted Medicine. The field represents the most exciting and promising arena for novel technologies, assisting to combat devastating diseases in developing and industrialized countries and to generate novel concepts for addressing the challenges associated with demographic changes in the European society and worldwide.

Nanomedicine and Targeted Medicine are the catalyst disciplines for developing diagnostics and treatments accounting for the nanoscale, molecular and cellular origin of disease and one of the enabling disciplines for the Knowledge-Based and Personalized Medicine.

The CLINAM-Foundation's primary goal is to support the development and application of Nanomedicine and Targeted Medicine and related fields from the stage of basic research all the way to the clinics for the benefit of the patient and humankind.

This Summit brings together all stakeholders in the field of Nanomedicine and Targeted Medicine, including regulatory authorities from all continents, clinicians, researchers and industrial innovators. This year the Nobel Laureate Prof. Stefan Hell will open the Summit with the topic of the stunning development of the STED-Microscope. As special event, participants can bring their samples to test the Stimulated Emission Depletion (STED) Microscopy within the exhibition.

The conference schedule is divided into four tracks and satellite meetings running in parallel. All contributions to the CLINAM Summits are put online six months after the conference, except for those, whose authors asked, not to make it accessible in the Internet. The Foyer of the Summit is the market place where exhibitors make contacts with decision-makers from institutes, industry, university, hospitals and other research centers.

We look forward towards a fruitful Summit and are glad to welcome you in Basel.

Dr. med. h.c. Beat Löffler, MA
CEO of the CLINAM-Foundation

Prof. Dr. med. Patrick Hunziker
CSO of the CLINAM-Foundation

INTRODUCTION
ON BEHALF OF SWITZERLAND

Over the last nine years, the CLINAM Summit has become the platform to discuss unmet needs in medicine and to explore novel solutions through targeted applications like nanomedicine and related technologies. Thanks to the support and the collaboration of no less than 31 supporting organizations, participating scientists and regulatory authorities from all over the world are convening in Switzerland.

For Switzerland as a country with a very high innovation performance, combined with a historical strength in pharmaceutics, medical technologies and microtechnology projects to promote the development of medicine are of strategic importance. A special emphasis of this Summit is on the potential of nanomedicine and targeted medicine for Personalized Medicine. The development of modular and highly specific therapies promises to bring extraordinary benefits to patients who cannot get help from current therapies, both in industrialized as well as in developing countries. Nanomedicine and targeted medicine involve major enabling technologies for this medical revolution.The State Secretariat for Education, Research and Innovation supports this Summit. We do this because we deeply believe that Switzerland has the privilege and political responsibility, in particular in the European context, to actively promote science and innovation in important fields such as nanomedicine and targeted medicine.

Developments in nanomedicine will contribute significantly to the creation of technology platforms required for personalized diagnosis and treatment. Over the last ten years, Switzerland has already been increasing its investments in these areas. Research and development is intensive at the ETH Zürich and EPF Lausanne, at EMPA as well as at the various Universities and their respective hospitals and at the Universities of Applied Science.

The global players in the pharmaceutical industry and the many start-ups compete for rapid translation to marketable products of the ideas emerging from this field. With all these very different but highly focused institutions and players, nanomedicine is becoming a field of growing importance in our country.

CLINAM started as a small but ambitious Swiss Initiative to create a private non-for-profit foundation to promote nanomedicine and build an international cooperation network. The organizers and founders of the European Foundation for Clinical Nanomedicine some 10 years ago, Dr. Löffler and Prof. Hunziker, have established this meeting as an important yearly summit for the research community. Their vision of a neutral non-for-profit conference has been very successful: excellent short scientific interventions and lively discussions with all stakeholders. CLINAM has become a hub for the translational process from scientific discovery to the development of drugs and devices for diagnosis and therapy. It is the Summit for a “Global Nanomedicine and Related Fields Network” and welcomes all the stakeholders in this highly interdisciplinary field. As a network of trust and cooperation, it has become an ideal stage to present novel and late breaking clinical trials in nanomedicine and targeted medicine.

The CLINAM Platform is already a considerable success at the international level. According to the experts, it has generated more than 25 new cooperation efforts at the European level involving novel technological developments, nanomedicine centers and several new start-up-companies. Four projects supported by the Framework Programme of the European Commission were drawn up at CLINAM Summits. Typically, participants, who met for the first time at the conference, decided to work together and initiated 15 cooperation projects between members of CLINAM.

The mix of clinicians, biochemists, chemists, physicists, pharmacologists, engineers, investors, representatives of industry and regulatory authorities from all continents generates an atmosphere of friendship and mutual respect, which is conducive to accelerating developments for the benefit of patients and society.

I am confident that this Summit will result in new collaborative projects, including more EU-wide efforts, and that it will generate many new ideas for the development of novel methods and technologies involved in nanomedicine.

Dr. Gregor Haefliger, Vice Director, Head of National Research and Innovation Division,
State Secretariat for Education Research and Innovation SERI

CLINAM

European Foundation for Clinical Nanomedicine

9/2016

Basel, June 26–29

CURRICULA VITAE SPEAKERS

Ueli Aebi

Ueli Aebi (born 31 May 1946) holds master degrees in physics and molecular biology. He earned his Ph.D. in biophysics in 1977 from the University of Basel, Switzerland. In 1977/78, he worked as a senior research associate in protein crystallography at the University of California in Los Angeles. In 1979 he joined the faculty at the Johns Hopkins University School of Medicine in Baltimore, holding appointments in the Departments of Cell Biology and Anatomy, and in Dermatology. In 1986 he moved to the Biozentrum, University of Basel where he became part of a world-class structural biology program that integrated X-ray crystallography, NMR spectroscopy, and light, electron and scanning probe microscopies. Until the end of 2011 he was Professor and Director of the M.E. Müller Institute for Structural Biology. He also was a member of the Swiss Nanoscience Institute (SNI) and the National Center of Competence in Research (NCCR) "Nanoscale Science", where he co-directed the program module "Nanobiology and Nanomedicine" (2001-2009).

UA's lab has had a long-standing interest in biomolecular machines by an integrative methods approach that includes light, electron and scanning probe microscopies, X-ray crystallography, molecular cell biology and protein design. His research has focused on (1) the cytoskeleton; (2) nucleocytoplasmic transport; and (3) protein fibrillation. In addition, his group has been working on novel optical and mechanical nano-sensors and -actuators for diagnostics, prevention and therapy by minimally invasive local interventions. According to the Web of Science, as of 31 March 2015 UA's h-index has been 86 with over 500 publications that have been cited almost 22'000 times.

Among the numerous honors and awards, UA is an elected member of the European Molecular Biology Organization (EMBO; 1993) and of the Academia Europaea (AE; 1999). In 2002 he was awarded the G.J. Mendel Medal by the Czech Academy of Sciences; in 2007 he was given a Dr. honoris causae by the 1st Medical Faculty of Charles University in Prague; in 2008 he received the Arne Engström Award by the International Union of Pure and Applied Biophysics (IUPAB); and in 2011 he obtained the Carl Zeiss Lecture Award by the German Society for Cell Biology and was the recipient of the Distinguished Scientist Award by the Microscopy Society of America (MSA). From 2004 to 2008 he was the President of the European Microscopy Society (EMS); and from 2011 to 2014 he served as President of the Swiss Society for Biochemistry.

In addition, UA has over 30 years of business experience. In 1981 he co-founded Protek, Inc. to develop, manufacture, and sell hip and knee prostheses in the USA. From 1986 to 1991 he served on the Technical Board of Protek AG in Switzerland. Since 1996 he has been chairing the Board of Directors of Gehring Cut that develops and manufactures surgical instruments and other precision mechanical components. In 2003 he co-founded Therapeomic, Inc. that focuses on novel protein drug formulations and growth factor enhanced tissue repair. In 2005 he joined the Board of Directors of Alpha-O Peptides, a biotech start-up company that develops novel repetitive antigen display, diagnostic imaging and drug targeting/delivery platforms on the basis of a de novo design of polyhedral peptide nanoparticles. From 2004 to 2011 he served as President of the Basel Tumor Bank Foundation which administers and annotates one of the largest breast tumor registries, and conducts work on biomolecular expression profiling of breast tumors aimed at individualized diagnostics, risk assessment, therapy and follow-up. By the end of 2011 UA has retired from his research and teaching activities at the Biozentrum.

Dong June Ahn

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http://ahngroup.korea.ac.kr

Dong June Ahn received his B.S. and M.S. degrees in Chemical Engineering from Seoul National University, respectively, in 1986 and 1988, and his Ph. D. degree in the field of Interfacial Engineering in Chemical Engineering major from Purdue University in 1993. He worked as a postdoctoral fellow at Purdue University during 1993–1994, and a research scientist of the Center for Advanced Materials at Lawrence Berkeley National Laboratory during 1994–1995. In 1995, he joined the faculty of the Department of Chemical and Biological Engineering at Korea University, where he is a professor and the Director of Institute for Chemical Engineering Convergence. He was a visiting professor at the Bioorganic Chemistry Group of Chiron Research Center during 2001–2002, at the Department of Applied Chemistry and the Nanotechnology Research Center of Waseda University during 2009–2010, and at the Nanyang Institute of Technology in Health & Medicine, Nanyang University in 2013. He serves as the Vice Dean of the KU-KIST Graduate School and as the President of the Korean Society for Nanomedicine.

His research interests include nano-to-macro scale molecular and supramolecular assemblies, surface engineering, and nanobiotechnology. Toward fundamental knowledge, he investigates molecular-level interaction of chemical and biological materials. In applied regime, he develops rapid on-site and small detection devices for chemicals of environmental and IoT interests, and also invents ultra-sensitive labelfree diagnostic sensor chips for DNAs, proteins, and cells. His major scientific contributions have been published in high-profile journals including Science, JACS, Adv. Mater., Acc. Chem. Res., and others.

Zahraa S. Al-Ahmady

I obtained my BSc Degree in Pharmacy with a distinction from the College of Pharmacy, University of Baghdad in 2004. After training as a clinical pharmacist, I was awarded prestigious scholarship to study the Masters in Drug Delivery at the UCL School of Pharmacy, where I won the AstraZeneca Prize for the best overall performance. I completed my PhD studies with the Nanomedicine Lab at the UCL School of Pharmacy on the design, characterization and biological performance of temperature-sensitive vesicles for cancer therapy in 2012. I then joined the NANOSOLUTIONS (FP7-NMP) European project as a postdoctoral research associate at the University of Manchester. My work was mainly focused on the structure – biological function relationship that determines the safety of engineered nanomaterials. I am currently Research Fellow with the North West Centre of Advanced Drug Delivery (NoWCADD) working on the development of innovative therapeutic and *in vivo* imaging approaches against cancer and brain ischemia (stroke).

SELECTED AWARDS AND PRIZES

- Junior Presenter's Award, £200 travel expenses, The Faculty Research Series, 2016.
- Best Poster Presentation Prize at the UKICRS Symposium, Nottingham, 2015.

SELECTED PUBLICATIONS

- Zahraa S. Al-Ahmady, Kostas Kostarelos, Chemical Components for the Design of Temperature-Responsive Vesicles as Cancer Therapeutics. Chemical Reviews (2016).
- Marilena Hadjidemetriou, Zahraa S. Al-Ahmady, Kostas Kostarelos. Time-evolution of *in vivo* protein corona onto blood-circulating PEGylated liposomal doxorubicin (DOXIL) nanoparticles. Nanoscale (2016).
- Marilena Hadjidemetriou, Zahraa S. Al-Ahmady, Mariarosa Mazza, Kostas Kostarelos. Comparison of *in vitro* and *in vivo* formed Protein Coronas: Implication for Targeting and Cellular Internalization of Liposomes, 12 citations. ACSNano (2015).
- Zahraa S. Al-Ahmady, Cheryl L Scudamore, Kostas Kostarelos, Triggered Doxorubicin Release in Solid Tumours from Thermosensitive Liposome-Peptide Hybrids: Critical Parameters and Therapeutic Outcomes, 3 citations. International Journal of Cancer (2015).

SELECTED CONFERENCE PROCEEDINGS

- Zahraa S. Al-Ahmady & Kostas Kostarelos. Targeted Temperature-Sensitive Liposomes: Enhanced *in vivo* Tumor Accumulation by Heat Application. European Workshop on Particulate Systems in Nanomedicine, Utrecht, 2014. Oral presentation.
- Zahraa S. Al-Ahmady, Neus Lozano, Kostas Kostarelos. Smart Tumour-Specific Theranostics for Improved Cancer Therapeutics. The Academy of Pharmaceutical Science (APS), UK, 2014. Oral presentation.

SELECTED OUTREACH

- I have been selected to participate in SoapboxScience that will be taking place in July, Manchester, UK 2016. Below is the link to the event: http://soapboxscience.org/?page_id=2637
- My Research presented recently at NCRI meeting, UK 2015, gained wide national publicity. Below are some of the links to the press release: http://www.bbc.co.uk/news/health-34667804 http://www.dailymail.co.uk/health/article-3297582/The-cancer-grenade-tiny-bubbles-carrying-drugs-blood-blast-tumours.html
- I have contributed to the European Researcher Night at Manchester Museum, UK 2015. Visitors enjoyed hands-on activities using thermo-responsive liposomes.

Khuloud T Al-Jamal

Dr. Khuloud T. Al-Jamal, BSc (Honour), PhD, MRPharmS joined KCL as a lecturer in January 2011. She is currently a Reader in Nanomedicine. She has completed her pre-registration pharmacy training at The University College London Hospital and was awarded the Overseas Research Award Scheme (ORSA) Scholarship from The University of London (2000-2004) to complete her PhD in Drug Delivery from The School of Pharmacy, University of London (currently known as UCL-School of Pharmacy).

She was awarded the prestigious CW Maplethorpe Research and Teaching Postdoctoral Fellowship from The University of London (2005-2007) to explore the use of cationic dendrimers as anti-angiogenic agents for growth inhibition of solid and metastatic tumours.

She has developed an extensive experience in designing and developing novel nanoscale delivery systems including dendrimers, liposomes, quantum Dots (QDs), viral vectors and chemically functionalised carbon nanotubes, for passive, active and magnetic targeting approaches. Her current work involves pre-clinical translation of novel nanomaterials designed specifically for drug, siRNA, plasmid and radionuclide delivery for therapeutic or diagnostic applications. She believes that investigation of structure-activity relationships is a key for successful exploitation of nanomaterials in medicine.

She reported for the first time the intrinsic anti-angiogenic activity of cationic poly-L-lysine dendrimers, and pioneered surface engineering of carbon nanotube-based vectors to deliver siRNA materials to the central nervous system (CNS) and solid tumours *in vivo*.

She was awarded and is managing a number of research projects funded by The Royal Society, Worldwide Cancer Research, EPSRC, BBSRC, Wellcome Trust, FP6, FP7 and ITN Marie Curie research programmes. In February 2012, she was awarded the BBSRC New Investigator award exploring the use of chemically functionalised carbon nano-needles as vectors for delivering therapeutics across the BBB. In 2012, she was awarded the prestigious Royal Pharmaceutical Society Science Award in recognition for her outstanding scientific achievements in the field of Nanomedicine. She was the winner of the Wellcome Trust Science Image Award for three years in row (2014, 2015 and 2016), reflecting her commitment to public engagement in science.

Christoph Alexiou

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www.hno-klinik.uk-erlangen.de/seon-nanomedizin/

Prof. Dr. Christoph Alexiou, received his Ph.D. in 1995 from the Technical University of Munich, Medical school. After finishing his internship in the Gastroenterology Department at the University-hospital of the Technical University he started as a physician and researcher at the Department of oto-rhino-laryngology, head and neck surgery and founded a research group working on the field of local chemotherapy with magnetic nanoparticles (Magnetic Drug Targeting). In the year 2000 he received his degree as an ENT-Physician and 2002 he changed to the ENT-Department in Erlangen, Germany, where he performed his postdoctoral lecture qualification (Habilitation). He is working there as an assistant medical director in the clinic and leads the Section for Experimental Oncology and Nanomedicine (SEON). Since 2009 he owns the Else Kröner-Fresenius-Foundation-Professorship for Nanomedicine at the Universityhospital Erlangen. He receives grants from the European Union, German Research Community (DFG), Ministry of Education and Science (BMBF) and Bavarian State Ministry of the Enviroment and Consumer Protection and is a member of the Executive Board of the European Technology Platform for Nanomedicine (ETPN). His research is addressing the emerging fields of Diagnosis, Treatment and Regenerative Medicine using magnetic nanoparticles and the translation from basic research into clinical trials. He received for his research several national and international renowned awards.

Elke Anklam

E-mail: elke.anklam@ec.europa.eu

Elke Anklam is a chemist by education with specialisation in food, organic and radiation chemistry. After having obtained her PhD from the University Hamburg, Germany, she worked in various European Research Institutions and was a teaching Professor at the Applied University of Fulda, Germany.

Since 1991 she has been working in the European Commission's Joint Research Centre (JRC-EC) From 2006–2012 as Director of the JRC-Institute for Health and Consumer Protection (JRC-IHCP) in Ispra, Italy and since January 2013 as Director of the JRC-Institute for Reference Materials and Measurements (JRC-IRMM) in Geel, Belgium.

María Antonieta Annunziato

Internist-Specialist on Infectious diseases
Founding President of LATNAMCLI
Founding President of Asovenac:
http://www.asovenac.org
Chief Physician/Internist-Infectious disease
BANDESIR: http://www.bandesir.org/
Clinical research Biocontrolled:
http://www.biocontrolled.com

BACKGROUND EDUCATION

- Surgeon, Medical Doctor. University of Carabobo. Valencia/Carabobo Venezuela. "Dr. Enrique Tejera". 1992.
- Specialist in Internal Medicine University of Carabobo. Hospital Universitario "Dr. Angel Larralde". Valencia-Venezuela, 1994-1997.
- Infectious Disease Specialist (Infectious diseases). Central University of Venezuela. University Hospital of Caracas, 1998-2000.

AWARDS AND PRIZES

- Scientist "Dr. Miguel Perez Carreno" Award from

Award from Clinical Study entitled "Asymptomatic bacteriury in institutionalized elderly patients"

LECTURES

- Brainstorming on Infectious Diseases. What is better? staphylococcal infections: beta-lactams versus other drugs. IX Venezuelan Congress of Infectious Diseases "Dr. Pedro Navarro ", Caracas, October 12-15, 2010.
- Nanomedicine: Medical and Clinical Applications. Co participant in the panel of experts at the seminar: New medical technologies and advances in medicine. XXXV Scientific Conference of the National Institute of Hygiene "Rafael Rangel", Caracas October 21-25 2013.
- Ebola. Audirorium Red Cross of Venezuela. Caracas. 04/03/2015
- Nanomedicine: History, medical focus and clinical applications. 1st Symposium of Clinical Nanomedicine managed/coordinated by the Venezuelan and Latin American Associations of Clinical Nanomedicine. Fundación Emanuel. November 30th 2015. Caracas-Venezuela.
- Theranostics: General Issues. 1st Symposium of Clinical Nanomedicine managed/coordinated by the Latin American and Venezuelan Associations of Clinical Nanomedicine.Fundación Emanuel. November 30th 2015. Caracas-Venezuela.

PUBLICATIONS

- Nanotechnology applied to medicine. Science and health. Volume 1 .Nº September 2, 2010.
- Herbal Medicine: empiric use of ancestral development and clinical research. Herbal Medicine magazine. Volume 1. No. 1. Page 9-11. Year 2012
- What is the Human Papilloma Virus (HPV) ?. Infecto News magazine. 2011. Link: http://www.svinfectologia.com/inmunizaciones/preguntasexperto.htm.
- Vitamin D: overall evaluation and possible clinical use in different types of cancer. In publication.
- Evaluation of compared bio-availability of a Test product containing Calcium Dobesilate in tablets 1000 mg, Extended Release. Produced by Laboratorios Leti S.A.V. one dose; versus a reference product, Doxium®, produced by Laboratorios Leti S.A.V., containing 500 mg in capsules of Calcium Dobesilate of Immediate Release, taken 2 times per day, for a total of same dose of 1000 mg, in healthy volunteers. Received by authorities: 20/10/2014. Approved: 21/11/2014. Venezuelan Archives of Pharmacology and Therapeutics.

Marianne Ashford

Upon completion of a PhD into Oral Drug Delivery Systems to the Colon, in the Department of Pharmacy and Pharmaceutical Science, at the University of Manchester, Marianne joined ICI Pharmaceuticals later to become Zeneca/AstraZeneca in Cheshire, UK. Marianne worked in a Pharmaceutical Research group initially looking at formulation approaches for poorly soluble compounds and building up the biopharmaceutics capability. She became Team Leader and then Associate Director of a Preformulation and Biopharmaceutics Group evaluating the product design characteristics of candidate drugs in the Oncology, Inflammation and Cardiovascular therapy areas, supplying pre-clinical formulations as well as providing solid state science and biopharmaceutics support across the Discovery and Development portfolio. In 2005, Marianne moved to a project management role leading the pharmaceutical development of a number of AstraZeneca's Oncology development drugs at all stages of clinical development (e.g Lynparza™, Faslodex®, Arimidex®). In 2011, Marianne returned to a scientific role focused on exploiting drug delivery approaches to improve the therapeutic index of medicines and in particular, has worked closely with the Oncology teams to initiate a number of joint projects and collaborations in the Nanomedicine area to enable new medicines. More recently, Marianne has been responsible for overseeing the evaluation of delivery systems for delivery of nucleic acids into cells both via in house work and external partnerships & supporting our mRNA, ASO & mirRNA portfolios. She is a member of the Oncology iScience Leadership Team. Marianne has authored a number of book chapters, research papers and patents in the pharmaceutical science arena and more recently has given several invited talks and plenaries in the Nanomedicine/Advanced Drug Delivery field. Marianne is keen to use her scientific knowledge and experience to improve therapies for patients and apply drug delivery technologies to enable new and better medicines.

Anthony Amaechi Attama

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Anthony Amaechi Attamais a professor of Pharmaceutics (Drug Delivery and Nanomedicines) at the University of Nigeria. He studied pharmacy at the University of Nigeria, Nsukka where he obtained Bachelor of Pharmacy with distinction in 1994 and Doctor of Philosophy in 2002. He thereafter, proceeded to Institut für Pharmazeutische Technologie, Technische Universität Carolo-Wilhelmina zu Braunschweig, Germany for his postdoctoral research in pharmaceutical nanotechnology. His research interests include among others, development and formulation of novel delivery systems (e.g. micro/nano systems) of bioactive agents for the control of tropical diseases using natural, semi-synthetic and synthetic biomaterials. He supervises postgraduate students in pharmaceutical sciences and has many research articles published in peer-reviewed high impact journals. In a bid to translate research results to products, he has fostered the establishment of some pharmaceutical industries in Nigeria and also serves as a consultant to many pharmaceutical companies in Nigeria.

Simon Baconnier

Simon Baconnier completed his scholar cursus at Université Joseph Fourier Grenoble 1, were he graduated in 2003 with a PhD in Biophysics. Then, he chose to shift his carrier towards international project management and partnership building. After a first mission in the Biotech Start-up environment in Genopole (Evry, France) were he managed the optimisation of biotech integration in European projects, Simon Baconnier integrated Canceropole Lyon Auvergne Rhone-Alpes (CLARA, Lyon, France) for which he was first hired to develop the Europe Funding strategy to support the regional and local oncology stakeholders participation in EU funding programs. CLARA then asked him to set-up and managed a Nanotechnology for Cancer network in the Rhône-Alpes region supported by regional and international experts from both fields, and allows a strong development of his network in both fields. Since 2006, Simon Baconnier is also coordinator assistant in European projects supporting the development of clinical and translational research in the field of soft tissue tumours : "Conticanet" (2005-2011) and "EuroSarc" (2012-2016), Currently, Simon Baconnier is coordinating an international KOL network in a rare cancer area since 2010 (World Sarcoma Network). Simon Baconnier, also has a good knowledge of the field of industry. In 2010, he took the lead of the French affiliate of a Japanese Biotech (OncoTherapy Science Inc.), as Chief Scientific Officer, to manage the early clinical development of an innovative treatment in a rare cancer. This mission reinforced his skills in management and optimization of international partnership and allowed him to acquire a huge experience in early clinical development and investigational drug development in the pharma industry environment.

Mohammed Bakheit

I was born in 1970 in Malliet, grown up in Elfashir, Sudan. I graduated from the Faculty of Veterinary Medicine, University of Khartoum, Sudan, where I obtained my Bachelor degree in the year 1993. Shortly after my graduation, I was appointed a Teaching Assistant at the Department of Parasitology, University of Khartoum, Sudan. During that period, I was involved in the teaching of Parasitology practical classes to the undergraduate students and in the on-going research programs at the Department.

I finished the course of my PhD thesis at the Free University of Berlin, Germany. Thereafter, I carried post-doctoral research at the National Research Center for Protozoan Diseases (NRCPD), Obihiro University, Japan, where I obtained experience in the emerging Loop-Mediated Isothermal Amplification (LAMP) - based diagnostic methods. Then, I joined the scientific staff of Research Center Borstel, Leibniz Center for Medicine and Biosciences in Borstel, Germany, where I was additionally involved in research projects sponsored by the German Research Foundation and the EU. My research interests lie in the fields of serological and molecular diagnosis of parasitic diseases as well as in the biology of parasites of veterinary and human importance. In total, I published over 40 peer-reviewed articles and two book chapters. Other scientific activities include the participation in several international conferences, meetings and symposia, and the organization of several professional training courses.

Currently, within the product development team at Mast Diagnostica GmbH, Germany, I am working towards advancing the LAMP-based diagnostic technologies. Herewith, my team is also involved in several innovative research and development projects such as the EU-sponsored "Disc-shaped Point-of-Care platform for infectious disease diagnosis". Our mission is to revolutionize the point-of-care diagnosis by the development of suited platforms and the implementation of LAMP technology.

Lajos (Lou) P. Balogh

Dr. Lajos (Lou) P. Balogh, Ph.D., is the owner and Chief Scientific Advisor of AA Nanomedicine & Nanotechnology (AANMNT), a consulting firm registered in Essex County, Massachusetts, USA. AANMNT promotes and assists nanomedicine and nanotechnology research, R&D projects, and technology developments by providing concept evaluation and scientific feasibility assessments for businesses and investors, and by offering expert consultation, project evaluation, and technology due diligence for individuals, institutions, private companies, and government agencies in nanomedicine, nanobiotechnology, and nanotechnology. Prof. Balogh also serves as the Executive Editor of Manuscript Clinic, (www.manuscriptclinic.us) which supports scientists and students to successfully publish their research results in the field of Nanomedicine and Nanotechnology by offering pre- and post-submission services. Past and current clients of AANMNT (baloghl@aananomedicine.com) include (NIH, USNSF, USEPA, DoD, DTRA, CAS, SNUH, etc.).

Dr. Balogh received his Ph.D. from the Kossuth L. University in Hungary and held faculty positions at Kossuth University Debrecen, H, the University of Massachusetts Lowell, MA, the Michigan Molecular Institute, Midland, MI, the University of Michigan, Ann Arbor, MI, the Roswell Park Cancer Institute (RPCI), Buffalo, NY, and at Northeastern University, Boston, MA. Between 2008-2015 Lou as Editor-in-Chief made it successful the journal Nanomedicine: Nanotechnology, Biology and Medicine (Elsevier). During this time he has critically evaluated several thousand manuscripts and scientific documents in basic, translational, and clinical nanomedicine.

Dr. Balogh has published 219 scientific papers, gave >220 invited lectures, and is awarded 12 patents in chemistry, drug discovery, materials science, nanomedicine and nanotechnology. Lou has 14 papers with more than 100 citations, and 6 with more than 200 citations (a total of > 5900 today).

Lou is one of the five Founders of the American Society for Nanomedicine, board member of the International Society for Nanomedicine, member of the Nanobusiness Alliance USA, the British Society for Nanomedicine, the Steering Committee of the American National Standard Institute Nanotechnology Panel, the US Technical Advisory Committee to the International Standard Organization on Nanotechnology (TC-229), in addition to numerous USA, European, and International expert committees. He is Recipient of the KOFST Brain Pool Program Award for Renowned Foreign Scientists, Visiting Professorship for Senior International Scientists of the Chinese Academy of Sciences, member of the External Body of the Hungarian Academy of Sciences, etc.

LinkedIn: http://www.linkedin.com/in/lajosbalogh

Google Scholar: https://scholar.google.com/citations?user=jbDlqSwAAAAJ&hl=en

Ian Banks

Retired GP and accident and emergency doctor. For 25 years he represented all doctors for the British Medical Association and founder board member of the Self Care Forum (UK)

Official spokesman on men's health issues for the BMA, president of the European Mens Health Forum, wrote or edited over 150 health books and manuals with a distribution of over 2m. Author of the NHS Direct Healthcare Guide and Web site.

Visiting professor to the Universities of Ulster and Leeds Medical School. Honorary senior lecturer Queens Medical School. Chairs the European Cancer Organisation (ECCO) patient advisory committee. and the European Forum Against Blindness. Trustee pf the European Pharmaceutical Students Association.

Yechezkel Barenholz

Professor Emeritus Yechezkel (Chezy) Barenholz is head of the Liposome and Membrane Research Lab and is also the Daniel G. Miller Professor in Cancer Research at Hebrew University of Jerusalem. He has been on the faculty at Hebrew University since 1968 and has been a Professor there since 1981. He was a Visiting Professor at the University of Virginia School of Medicine, Charlottesville, VA (1973-2005) and the F.C. Donders Chair Professor at the Faculty of Pharmacy, University of Utrecht, The Netherlands (1992). He was also a Visiting Professor at Kyoto University, Japan (1998); La Sapeinza University, Rome, Italy (2006); Jiaotong University, Shanghai, China (2006); King's College, London, UK, (2006); and the Technical University of Denmark, Copenhagen, Denmark (2010). His current research focuses on the development of drugs and nano-drugs based on drug delivery systems (DDS) best exemplified by the anticancer Doxil®, the first liposomal drug as well as the first nano-drug approved by the FDA (1995) and used world-wide. Professor Barenholz is an author of 399 scientific publications totalling more than 23,000 citations. He is a co-inventor in more than 45 approved patent families. He was an executive editor of Progress in Lipid Research, an editor of four volumes of None Medical Applications of Liposomes and four Special Issues, and is on the editorial board of five scientific journals.

Professor Barenholz is a founder of Moebius Medical LTD, PolyPid Ltd, LipoCure LTD, and Ayana LTD. All are in an advanced stage of the development of liposomal drugs based on Professor Barenholz's inventions and expertise. He has been awarded the F.C. Donders Chair at the University of Utrecht, the Kaye Award (1995 & 1997) from Hebrew University, the Alec D. Bangham Award (1998), Teva Founders Prize (2001), an Honorary Doctorate degree from the Technical University of Denmark (2012), the International Controlled Release Society's CRS Founders Award (2012), and the Israeli chapter of the International Controlled Release Society's Award (2014). In 2003, Professor Barenholz founded the Barenholz Prizes from Doxil® royalties to encourage excellence and innovation in the applied sciences of Israeli PhD students.

Professor Barenholz is married to Dr Hanna Barenholz together they have 4 daughters and 12 grand- children.

Jack Barokas

Over 20 years' experience of
maintenance of audio visual equipment
as an owner of
a private business (1975–2002).
Computer and network maintenance
services providing at TAU.
Head of the Educational Digital Media
Applications team at TAU.

BA degree in Learning Technologies and Instructional Design, HIT 2010 Israel.

Building fully online courses to replace Face2Face learning at School of Medicine. Administration of webcast and lecture/course recording services for European projects such as: Nano2Life, NanoEl, NanoSkills, QNano.

Higher Education Reform Expert (HERE) in the framework of Israel National Tempus Office (NTO) (2001-2013)

Currently Local coordinator of EduNano TEMUS project

RECENT PUBLICATIONS

- Danilo Demarchi, Gianluca Piccinini, Mariagrazia Graziano, Jack Barokas, Silvia Schintke, Philippe Morey-Chaisemartin, and Slavka Tzanova, HANDS-ON LABORATORIES IN THE NANOEL PROJECT, 9th European Workshop on Microelectronics Education EWME'12, Grenoble, France, May 11, 2012.
- Philippe Morey Chaisemartin, Slavka Tzanova, Silvia Schintke, Danilo Demarchi, Jack Barokas, Fabian Wleklinski, Jean-Marc Melique, and Eric Beisser, INDUSTRY NEEDS ANALYSIS FOR DEVELOPING NEW SKILLS IN NANO-ELECTRONICS, 9th European Workshop on Microelectronics Education EWME'12, Grenoble, France, May 10, 2012.
- Barokas, J., Ketterl, M. & Brooks, C. (2010). Lecture Capture: Student Perceptions, Expectations, and Behaviors. In J. Sanchez & K. Zhang (Eds.), Proceedings of World Conference on E-Learning in Corporate, Government, Healthcare, and Higher Education 2010 (pp. 424-431). Chesapeake, VA: AACE.

Michal Bassani-Sternberg

Michal Bassani-Sternberg is the head of the Immunopeptidomics unit that is integrated within the Center of Experimental Therapeutic (CTE) in the department of Oncology at the CHUV, the university hospital of Lausanne, Switzerland. The central goal of the unit is to integrate, for the first time, mass spectrometry based immunopeptidomics into the innovative translational clinical strategy of personalized immunotherapy within the department of oncology. Identification by mass spectrometry of the naturally presented private HLA epitopes, including both shared and neo-epitopes, on an individual patient basis, will guide the development of personalized immunotherapy and immune-monitoring for cancer patients, as well as their potential development into biomarkers.

In the last ten years, Dr Bassani-Sternberg has focused her research mainly on the development of methodologies for highly accurate and in-depth immunopeptidomics. She has completed her PhD at the Faculty of Biology at the Technion - Israel Institute of Technology. She was the first to capitalize and show that the repertoire of peptides bound to the circulating soluble HLA molecules in the plasma of cancer patients is a potential new source for biomarkers. She then moved to perform her post-doctoral research at the Proteomics and Signal Transduction Department, headed by Prof. Matthias Mann at the Max Planck Institute of Biochemistry. She was awarded the Minerva and the Alexander von Humboldt fellowship awards. She further developed a high-throughput MS-based workflow that allows stringent and accurate in-depth identification of HLA-p, and has established a very comprehensive database of immunopeptidomes isolated from different cancer types including melanoma, hepatocellular carcinoma and leukemia that uncovered hundreds of new cancer specific epitopes. In combination with exome sequencing data, she further extended the analysis to include the identification of patient's specific neo-epitopes that contain non-synonymous somatic mutations. Currently, she is implementing this platform in a clinical environment that is supported financially jointly by the CHUV and by the Ludwig Institute for Cancer Research. In addition to clinical applications, this platform will spark new fundamental research seeking to understand how tumor cells process antigens, what are the biochemical characteristics of immunogenic antigens, what are the molecular bases of tumor immunogenicity and how can we increase tumor immunogenicity using drugs.

Matthias Baumann

Dr. Baumann, is a R & D professional with more than 25 years of experience in the healthcare industry. He has a strong background in medical sciences and has worked on a broad range of indications and development approaches for chemical and biotechnological compounds.

Before joining NOXXON Pharma AG, Berlin, Germany, as Chief Medical Officer in 2011, he worked in various management positions with increasing responsibility in the international pharmaceutical and CRO industries.

From 2002 to 2010 he served as Chief Scientific Officer and Managing Director of FOCUS Clinical Drug Development, Neuss/Düsseldorf, Germany, a CRO specialized in early clinical studies and exploratory development. In this role he was responsible for the design and execution of integrated programs progressing development compounds from the preclinical candidate stage to clinical proof of concept.

Before joining FOCUS, Dr. Baumann was with Hoffmann-La Roche, Basel, Switzerland, from 1998 to 2002. As the medical officer of the Integrated Healthcare Solutions group, he was instrumental in the planning and conduct of clinical studies for the qualification of biomarkers and companion diagnostics in various therapeutic areas, e.g. cardiovascular, metabolism and oncology.

Dr. Baumann started his career in the pharmaceutical industry in 1990 at Boehringer Mannheim, Mannheim, Germany. Initially he served as a preclinical project manager and pharmacologist for development programs in the field of hematopoietic growth factors and cytokines. From 1993 to 1998 he worked as program manager and department head of the clinical R & D group of Boehringer, dealing with NCEs in osteoporosis and cardiovascular indications and with recombinant growth factors, monoclonal antibodies, gene therapy approaches and medical devices in oncology, infectious diseases and cystic fibrosis.

After receiving his MD degree from the University of Erlangen, Germany in 1984 and before joining the pharmaceutical industry, Dr. Baumann pursued 5 years of postgraduate training in biochemistry and molecular biology at the University of Erlangen, Germany and at the Research Institute of Scripps Clinic, La Jolla, USA. He authored numerous publications in well-recognized, peer-reviewed international journals on topics in oncological and immunological research.

Dr. Baumann joined NOXXON in February 2011 as Chief Medical Officer and Member of the Executive Board.

Shmuel (Muli) Ben-Sasson

Prof. Shmuel (Muli) Ben-Sasson, PhD, Dept. Developmental Biology & Cancer Research, The Hebrew University-Hadassah Medical School, Jerusalem, Israel, is actively conducting translational research in the field of Cancer during the last twenty years. He is Co-founder and Chief Scientific Officer of Tiltan Pharma, a clinical-stage company that developed an oral, synergic drug combination with anti-cancer and anti angiogenic properties. This therapy takes advantage of the multifaceted nature of the angiogenic process and attack it simultaneously from several independent angles. A Phase II study of this therapy against metastatic pancreatic cancer was just concluded, successfuly. Another aspect of translational medicine advanced by Prof. Ben-Sasson and his team is based on the realization of the pivotal role played by an epigenetic chaos in the evolvement and progression of broad spectrum of tumors. They are developing a novel strategy resulting in the development of new chemical entity that target the unique epigenetic makeup of cancer cells. He also served as Visiting Professor at the Harvard Medical School for 10 years, where he worked in close collaboration with key opinion leaders in the field of anti-angiogenesis, including Prof. Judah Folkman, Dept. Surgical Research, Children's Hospital (Boston). Among his significant scientific contributions in the invention and development of the TUNEL assay, now serving as the universal gold-standard for the identification of apoptosis. Muli is the author of numerous scholarly articles and the inventor of 20 granted patents. In addition to Tiltan Pharma, he is the scientific founder of several other biotech companies, including Keryx, Chiasma and Raziel Therapeutics.

Nadia Benkirane-Jessel

Dr. Nadia Benkirane is Research director and head of the " Osteoarticular and Dental regenerative Nanomedicine" laboratory, at INSERM (French National Institute for Health and Medical Research), UMR 1109, Strasbourg, France. She was leader of "Active Biomaterials and Tissue Engineering" team INSERM 977. She received her Ph.D. from University Louis Pasteur, ULP, Strasbourg, France for the work on Development of pseudopeptides as synthetic vaccines. Dr. Jessel (Benkirane) then held a postdoctoral position in collaboration with the Institut Pasteur, Paris, France, working on Immunotherapy HIV, and another postdoctoral position on the application of modified peptides as vaccines against FMDV (Plum Island Animal Disease Center, ARS, USDA, Greenport, NY 11944-0848, USA). She joined the INSERM U595 in 2002 as a post-doc, and received the diploma to direct the research (HDR) in 2004. Dr. Jessel got the permanent position (CR1) in the INSERM 595 laboratory in 2004 and Research Director (DR2) position in the INSERM 977 and head of "active Biomaterials and Tissue Engineering team from 2009 until 2012). Currently Research Director (DR1) in the INSERM UMR 1109 (Osteoarticular and Dental Regenerative Nanomedicine" and heads the team. Dr. Jessel possesses expertise in diverse fields of molecular and cellular biology, immunochemistry, tissue engineering and biomedical engineering. In the last 10 years, she focused her research on the bio-functionalization of multilayered polyelectrolyte architectures with emphasis on the use of these architectures to induce specific cellular responses and gain control over cell proliferation and differentiation. Dr. Benkirane-Jessel have 138 publications (h index: 36) with peer-reviewed publications in high impact factor journals (Proc. Nat. Acad. Sci. USA; Adv. Mater.; Adv. Funct. Mater.; Small; Nanoletters, Biomaterials, ACS Nano), 5 chapters reviews and 5 international patents, she is a regular referee for a number of scientific journals (Nature nanotechnology, Nature Materials, ACS nano, Biomaterials, Nanoletters…). She is under the contract (Interface INSERM/Clinic 2008-2013) and she got also "Prime d'Excellence Scientifique" from the INSERM, 2010-2014 and the PEDR from the INSERM on 2016 for 4 years.

SCIENTIFIC TOPICS:

- Material Science
- Nanomedicine
- Regenerative Medicine
- Tissue Engineering

MOST RELEVANT PUBLICATIONS

- Benkirane-Jessel N, et al., Bioactive coatings based on a polyelectrolyte multilayer architecture functionalized by embedded proteins. Adv. Mater. 2004, 15:692-695 (IF-15.409).
- Benkirane-Jessel N et al., Control of monocyte morphology on and response to model surfaces for implants equipped with anti-inflammatory agents. Adv. Materials, 2004, 16, 1507-1511 (IF-15.409).
- Benkirane-Jessel N et al., Pyridylamino-beta-cyclodextrin as a molecular chaperone for lipopolysaccharide embedded in a multilayered polyelectrolyte architecture. Adv. Materials, 2004, 14, 963-969 (IF-15.409).

- Benkirane-Jessel N et al., Build-up of polypeptide multilayer coatings with anti-inflammatory properties based on the embedding of piroxicam-cyclodextrin complexes. Adv. Funct. Mater. 2004, 14:174-182 (IF-10.439).
- Benkirane-Jessel N et al., Short-time tuning of the biological activity of functionalized polyelectrolyte multilayers. Adv. Funct. Mater. 2005, 15:648-654 (IF-10.439).

François Berger

CLINATEC director, Professor of cell Biology and oncology in Grenoble medical university; Director of the Brain nanomedicine Group, INSERM U1205 CEA-Leti- MINATEC Campus, 17, rue des Martyrs, 38054 Grenoble Cedex 9, France;
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François Berger, MD,PhD had a dual scientific and clinical education in the field of neurology, oncology and molecular and cell biology. He continues to have a dual clinical and research activity has professor of cell biology and neuro-oncology. He develops a translational research activity, trying to validate innovative technologies at the preclinical/clinical level in close collaboration with micro-nanotechnology groups. Exploring the best modalities to accelerate translation of technology innovation at the bedside is the main focus of his research. As Clinatec director he explored the feasibility of clinical research delocalization inside Minatec technology campus. Difficulties to export an academic research mode, cost to manage safely patients outside the hospital, the progressive migration to a techno-centric position and at the end the ethical questioning of this position were the main bottlenecks. The development of an innovative translational strategy to catch disruptive innovation outside the health sector was the main success in the context of CEA excellence in the field of technology. After two years, he came back inside the hospital as director of of a new research unit INSERM U1205 associating INSERM-Grenoble University and Grenoble university hospital. The objectives of this group are to develop innovative technologies for a better understanding and therapy of Brain diseases and cancer. It is also to accelerate the transfer of technology innovation at the bedside implementing innovating translational methodologies from cellular, preclinical to human proof of concept trials. **Research area:** neuro-oncology, neurosciences, biomarkers, nanomedicine

EDUCATION

1990: Poitiers Medical school; MD
1994: Angers Medical school; neurologist Residency
1995–1997: San Diego Salk Institute; Postdoctoral fellowship in the F Gage laboratory
1995: Grenoble University; PhD
1998: Grenoble University; Master of Science

PROFESSIONAL CAREER

2016: Director of the new INSERM research unit U1205 in Grenoble hospital.
2011–2014: Director of Clinatec Lab INSERM UA 01 and CLINATEC INSTITUTE, CEA, Grenoble (Grenoble (Innovative Applications of Micro-Nano-Technologies to Medicine).
Since 2009: scientific advisor of the French technology Institute associating all the public research agencies in France (CEA, INSERM, CNRS, INRIA)
Since 2004: Head of the INSERM research laboratory “Brain nanomedicine group”
Since 2000: Professor of Cell Biology and Oncology- clinical activity in the field of neuro-oncology
1999–2004: Head of the neuro-oncology group in the INSERM research laboratory of AL Benabid.
1994–1995: Assistant in cell biology and neuro-oncology

RECENT PUBLICATIONS

- Accessing to the minor proteome of red blood cells through the influence of the nanoparticle surface properties on the corona compositionZaccaria A, Roux-Dalvai F, Bouamrani A, Mombrun A, Mossuz P, Monsarrat B, Berger F. International Journal of Nanomedicine 2015, 10:1869-1883.
- Appaix F, Nissou MF, van der Sanden B, Dreyfus M, Berger F, Issartel JP, Wion D. Brain mesenchymal stem cells: The other stem cells of the brain? World J Stem Cells. 2014 Apr 26;6(2):134-43.
- Sarraf M, Perles-Barbacaru AT, Nissou MF, van der Sanden B, Berger F, Lahrech H. Rapid-Steady-State-T1 signal modeling during contrast agent extravasation: Toward tumor blood volume quantification without requiring the arterial inputfunction. Magn Reson Med. 2015 Mar;73(3):1005-14. 2014 Apr 14.
- Vilgrain I, Sidibé A, Polena H, Cand F, Mannic T, Arboleas M, Boccard S, Baudet A, Gulino-Debrac D, Bouillet L, Quesada JL, Mendoza C, Lebas JF, Pelletier L, Berger F. Evidence for post-translational processing of vascular endothelial (VE)-cadherin in brain tumors: towards a candidate biomarker. PLoS One. 2013 Dec 16;8(12):e80056.
- Selek L, Seigneuret E, Nugue G, Wion D, Nissou MF, Salon C, Seurin MJ, Carozzo C, Ponce F, Roger T, Berger F. Imaging and histological characterization of a human brain xenograft in pig: the first induced glioma model in a large animal. J Neurosci Methods. 2014 Jan 15;221:159-65.

Erem Bilensoy

Dr. Erem Bilensoy is a full professor of pharmaceutical technology. She graduated from Hacettepe University Faculty of Pharmacy in 1992. She obtained her double Ph.D. degree with a co-tutelle thesis between Université Paris-Sud, France and Hacettepe University in 2002 under the supervisions of Dominique Duchene and Atilla Hıncal on the evaluation of amphiphilic ß-cyclodextrins modified on the primary face as novel excipients in the preparation of nanospheres and nanocapsules. She has been appointed as associate professor at the Department of Pharmaceutical Technology, Hacettepe University in 2007 and received full professor position in 2013.

She is the author of more than 55 scientific articles, 11 international book chapters. She has given several invited lectures, oral and poster presentations receiving more than 800 citations with a current H-index of 21. Erem Bilensoy also wprked as Editor of the book wntitled “Cyclodextrins in the Field of Pharmaceutics, Cosmetics and Biomedicine: Current and Future Industrial Applications” published by John Wiley&Sons in 2012.

Dr. Bilensoy has served as Bioavailability/Bioequivalence Evaluation Commission member within Turkish Medicines and Medical Devices Agancy between 2007-2012 and from December 2015 onwards. She was appointed as Vice Dean of Faculty of Pharmacy between 2010-2013. She is founder member and scientific secretary for EUFEPS Network on Nanomedicine since 2010 and Executive Board Member for European Cyclodextrin Society since 2009. She is Editorial Board Member of the journal Recent Patents in Drug Delivery and Formulation.

Erem Bilensoy joined the European Federation for Pharmaceutical Sciences EUFEPS Executive Committee in 2012 and was recently elected as President of EUFEPS starting from June 2015. She is also on the Consultancy Board for Projects on nanobiotechnology at TUBİTAK Turkish Scientific and Technological Research Council. Erem Bilensoy was elected as Vice Chairman for Hacettepe Technology Transfer Canter Executive Committee on January 2016.

Her current research interests include targeted nanoparticles in cancer therapy, cationic nanoparticles, cyclodextrin-based drug delivery, inkjet and 3D printed drug delivery systems, biomedical applications of nanoparticles and regulatory approaches on bioavailability/bioequivalence. Erem Bilensoy is married and has a daughter ,Deniz aged 12.

Gerd Binnig

Definiens AG, CTO and Founder
Born in Frankfurt, Germany, Dr. Binnig studied at the J.W. Goethe University in Frankfurt, where he received his doctorate degree in 1978. He then immediately joined IBM's Zurich Research Laboratory and stayed with IBM till 2002. During this time Dr. Binnig invented and developed the Scanning Tunneling Microscope, STM, together with his colleague Dr. Heinrich Rohrer. He went on to invent the Atomic Force Microscope, AFM, which he developed together with Calvin Quate and Christoph Gerber during a sabbatical at IBM Almaden Research Center (1985/86) and a guest professorship at Stanford University (1985-88). Additionally, he opened and headed a small IBM research group from 1987 to 1995 within the University of Munich, from which he received an honorary professorship.
Through both techniques, STM and AFM, atoms on the surface of matter are imaged and manipulated so that features of single atoms, such as electronic states (STM) and interaction forces (AFM), can be measured. The potential of investigating and manipulating matter on the atomic scale started the new discipline of nanotechnology. In addition to receiving numerous awards and honors, Dr. Binnig was awarded the Nobel Prize in Physics together with his colleague Dr. Heinrich Rohrer for the invention of the STM.
In 1995, Dr. Binnig together with the journalist Dieter Herold founded a small research group, which was the precursor of Definiens. In 2000 he founded the company Definiens by bringing in investors. With his team at Definiens he developed the Cognition Network Technology, CNT, to automatically understand complex data. This technique was initially applied to image analysis which uniquely enabled Definiens software to analyze large numbers of images automatically, just like the human eye and brain are capable of doing. Later, CNT was extended to the automated analysis of data tables derived from the analysis and rich quantification of tissue images, enabling the novel field of Tissue Phenomics.

Patrick Boisseau

Patrick Boisseau is graduated in biological engineering from the French Elite Schools Institut National Agronomique (1983) and Ecole Nationale du Génie Rural, des Eaux et des Forêts (1985). His career is fully dedicated to academic research, research and development and research management. He entered in 1987 at CEA, a French public research organisation where he occupied several functions in biological research and later in medical technologies.
Patrick Boisseau's current position is Nanomedicine Programme Manager at CEA-Leti, a public nonprofit Research & Technology Organisation, based in Grenoble, France. He has acquired a large expertise of coordination of EU projects like the Network of Excellence Nano2Life (2004-2008), EuroNanoBio, BIBA, TARGET-PDT, and recently the EU-NCL infrastructure on nanocharacterisation. His field of technical expertise is drug delivery, medical imaging and innovative medical technologies.
His experience in strategy and planning made him responsible for the Strategic Planning in Life Sciences and Healthcare Technologies, at CEATech since 2013.
Patrick Boisseau is solicited for the numerous expertise for the European Commission and other national funding agencies.
He has been reelected Chairman of the Board of the European Technology Platform on Nanomedicine in October 2015.

Bastian Bonhoeffer

Doctoral Candidate
Novartis Pharma AG
CH-4002 Basel
SWITZERLAND
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Bastian Bonhoeffer grew up and finished school in the vicinity of Bonn, Germany. He then moved to Mannheim, Germany to study Process Engineering (B.Sc.) and Chemical Engineering (M.Sc.) at Mannheim University of Applied Sciences. During his studies he received a scholarship from the Academic Foundation of the German People. After finishing his studies with distinction he started a position as Doctoral Candidate at Novartis Pharma AG in Basel, Switzerland in 2014. There he works on the processing of drug-nanosuspensions into solid pharmaceutical products. The doctorate is carried out in cooperation with the Institute of Particle Technology at TU Braunschweig, Germany.

Gerrit Borchard

PharmD, Ph.D.
Gerrit Borchard is a licensed pharmacist and obtained his Ph.D. in pharmaceutical technology from the University of Frankfurt (Germany) for his thesis on the interaction of colloidal drug carrier systems with the immune system. After holding several academic posts, including a lecturer position at Saarland University (Germany) and Assistant and Associate Professorships at Leiden University (The Netherlands), he joined Enzon Pharmaceuticals, Inc. (USA) as Vice President Research. In 2005, he was appointed Full Professor of Biopharmaceutics at the University of Geneva (Switzerland), and Scientific Director of the Centre Pharmapeptides in Archamps (France), an international center for biopharmaceutical research and training. Prof. Borchard is (co-)author of over 130 scientific publications and book chapters, co-editor of one book and named as inventor on 7 patents.
From 2008 to 2013, he served as Vice President of the School of Pharmaceutical Sciences Geneva-Lausanne (EPGL) and from 2013 to 2014 as acting president. In 2012 Prof. Borchard joined the Non Biological Complex Drugs (NBCD) working group hosted at Lygature (former Top Institute Pharma (TIP, Leiden, The Netherlands), and became member of the steering committee in 2015. He was nominated Chair of the NBC working party at the European Directorate for the Quality of Medicines & Health Care (EDQM) by Swissmedic in 2013.
Prof. Borchard was nominated Fellow of the Swiss Society of Pharmaceutical Sciences (SSPhS) in 2010, and has been President of the Swiss Academy of Pharmaceutical Sciences since 2014. He also served as Vice President of the European Federation of Pharmaceutical Sciences (EUFEPS) from 2013 to 2015.
Due to his working in both academia and industry, and living in four countries, Prof. Borchard has acquired extensive experience in diverse working and cultural environments, and speaks Dutch, English, French and German fluently. Time allowing, he enjoys roaming the trails and by-roads of the Jura mountains on foot and bike.

Sven Even Borgos

Sven Even Borgos (born 1976) earned both his undergraduate and PhD degrees at the Norwegian University of Science and Technology in Trondheim, which is the main technical university of Norway. His undergraduate was from the Faculty of Physics and Mathematics, specialization in Biophysics and Medical Technology. His PhD, however, was in molecular biology. More specifically, it was concerned with genetic engineering of the antibiotic-producing soil bacterium Streptomyces noursei in order to develop mutants producing derivatives of the clinically important antifungal antibiotic nystatin and related compounds, with improved pharmacological properties. His post doc was earned in systems biology, developing and validating a genome-scale metabolic model of the alginate-producing bacterium Pseudomonas fluorescens. Since 2006, he has been working in SINTEF (Norway), which is the largest independent research institute in Northern Europe. Here, he has been working with advanced analytical chemistry, mainly based on mass spectrometry coupled to chromatography in the Research Group Mass Spectrometry. The last years, he has been specializing in physico-chemical characterisation of nanomaterials, with an emphasis on nanomedicines, using various modalities of mass spectrometry and novel separation methods such as field flow fractionation (FFF). He is working in the European Nanomedicine Characterisation Laboratory H2020 project within the chemical part of the characterisation cascade, as well as leading the work that identifies novel nanomedicines characterisation technologies. He also has a keen interest in the novel field of mass spectrometry imaging (MSI), and is leading this activity within SINTEF. The use of MSI for label-free, spatially resolved analysis of drug biodistribution in tissues is particularly fascinating, e.g. in terms of targeted drug delivery by nanomedicines.

Whenever he is not obsessing with nanomedicine, he is being kept busy with his two kids and enjoys outdoors activities in the Norwegian mountains

Susanne Bremer-Hoffmann

Dr. rer nat Susanne Bremer-Hoffmann, obtained her PhD from the Charite University Hospital Berlin in Germany for her work on T-cell reactions against autologous leukemia cells. After post-doctoral research at the Federal Institute for Risk Assessment in Germany, S. Bremer-Hoffmann joint the Centre for the Validation of Alternative Methods (EURL-ECVAM) hosted by Institute for Health & Consumer Protection (IHCP) at the European Commission's Joint Research Centre JRC in 1995. She coordinated EUR-ECVAM's activities in the area of reproductive toxicity involving the validation on several *in vitro* tests for assessing endocrine disrupter activity as well as the development of *in vitro* toxicity tests based on murine/human embryonic and induced stem cells in the context of the FP6 and FP 7 projects ReProTect, ESNATS and the SEURAT-1 cluster. She joint the Nanobiosciences Unit of the same Institute where she is responsible for the group working on the interaction of nanomaterial with biological systems. S.Bremer-Hoffmann is a member of the EU-NCL project.

Donald Bruce

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Dr Donald Bruce holds doctorates in chemistry and in theology. He is managing director of the independent consultancy Edinethics Ltd., working on the ethics and public engagement of emerging technologies. After working 15 years as a chemist in nuclear energy research, risk regulation, and energy policy, he became Director of the Church of Scotland's Society, Religion and Technology Project (SRT) from 1992-2007. In this role he did pioneering ethical assessment of many emerging technologies including GM crops and animals, cloning and stem cells. He has worked extensively on nanomedicine and related technologies from 2003 to the present, in a series of EC projects Nano2Life, NanoBio-Raise, NanoMedRound, Ethentech (on human enhancement). He is a partner in the NanoAthero EC FP7 project on nanodevices to detect and treat atherosclerosis. An integral part of this work has been in developing and writing public engagement tools with Perry Walker formerly of the New Economics Foundation. He helped develop the Democs/Decide card games and Open-up argument map concepts, on such issues as GM crops, synthetic biology, human enhancement, and stem cells for therapy and for toxicity testing. He will demonstrate a Democs game on nanomedicine written for the NanoAthero project at Clinam 2016. He has also worked on the implications of distributed healthcare for patients, carers, medical staff and the healthcare system, using the ethical matrix method.

He was a former member of the Scottish Science Advisory Committee, the Societal Issues Panel of Engineering and Physical Sciences Research Council, the Public Affairs advisory group of Biotechnology Research Council, the Nanotechnology Engagement Group, and the bioethics working group of the Conference of European Churches. He has been a member of the Advisory Board of the Institute of Nanotechnology and the Edinburgh University Research Ethics Committee.

Reto Brun

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Reto Brun is a well-known parasitologist who mainly worked on malaria, African sleeping sickness and other protozoan diseases. He studied biology and chemistry at the University of Basel and received his Ph.D. in 1973. Thereafter, he worked as a post-doctoral fellow in the laboratory of Prof. Stuart Krassner at the University of California, Irvine, where he studied differentiation processes of the protozoan parasite Leishmania. In 1976 he joined the Swiss Tropical Institute and initiated work on African trypanosomes including collaborations with African partners in East Africa. He is also a co-founder of the Eastern Africa Network for Trypanosomiasis which is doing research and control of sleeping sickness.

During the last 25 years his main interest was in drug discovery and development for diseases caused by protozoan parasites. At the Swiss Tropical and Public Health Institute he established a Drug Screening Centre which was involved in the discovery of most of the clinical candidates for malaria and sleeping sickness which are in clinical development today. From 2003 to 2011 he was a member of the Board of Directors of the Drugs for Neglected Diseases initia-

tive. As a professor at the University of Basel he supervised over 60 M.Sc. and Ph.D. students and as an author he published over 550 research articles, reviews and book chapters. He is the president of the Basel Society of Natural Sciences.

David Bunka

CTO, APTAMER Group, Heslington, York (UK)

David holds a Ph.D. in Molecular Biology from the University of Leeds and is a Molecular Biologist by training. He spent 12 years at the University of Leeds developing and running a high throughput automated aptamer selection system, which enabled him to isolate ~200 aptamers against a wide variety of targets including: small molecule antibiotics, food contaminants, disease associated proteins, several cancer associated cell-lines, viruses and patient tissue samples. He has built up a solid international reputation in the field, jointly authoring a number of papers describing aptamers against a range of different targets and applications. Key publications include invited review articles on the use of aptamers and a book chapter in 2012 for the Royal Society of Chemistry entitled "therapeutic uses of nucleic acid aptamer conjugates". Since co-founding and officially joining Aptamer Group in 2012 David had developed over 50 aptamers to various targets and has development several patentable process variations. David has also given several lectures at a variety of events including the "Nucleosides & Nucleotides" event organised by the Royal Society of Chemistry, the "Oligonucleotide Therapeutics – Bubbling Under Technologies" conference hosted by GSK, the "RNAi Therapeutics" conference in London and the "Annual Gene and Cell Therapy Conference" in New Orleans.

Michel Calame

After graduating in physics at the University of Neuchâtel (Switzerland) in 1993, Michel Calame worked on a PhD in condensed matter physics investigating the electrical transport properties of superconducting thin films in the group of Prof. P. Martinoli. In 1998, he spent six months at the Swiss Federal Office of Metrology METAS (Bern, Switzerland) as a scientific collaborator in the Electricity, Acoustics and Time section headed by Dr. B Jeckelmann working on low-temperature electronics for single electron transistors. After being awarded a Swiss National Science Foundation grant for young researchers, he moved in 1999 to the Center for Studies in Physics and Biology at the Rockefeller University (NY, USA) to join the group of Prof. A. Libchaber for a postdoctoral stay in molecular biophysics. In 2001, he joined the nanoelectronics group of Prof. C. Schönenberger at the University of Basel to work on nanoscale and single molecule devices electronics.
Michel Calame served as a board member of the Mathematics, Astronomy and Physics Platform at the Swiss Academy of Sciences from 2007 to 2012. He received the venia docendi in physics from the University of Basel in 2011 and is a staff member of the Swiss Nanoscience Institute (SNI) since 2013 where he coordinates the SNI Doctoral Programme (about 30 PhD Students). Since 2012, he leads his own research group and focuses on the (opto-)electrical transport properties of hybrid (organic-inorganic) nano-scale devices as well as in chemical and biochemical sensing using nanoscale electronic systems. He is co-author of more than seventy peer-reviewed publications, two book chapters and three patents (full list of publications: http://calame.unibas.ch/publications/).

Aoneng Cao

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Prof. Cao is a full professor and the executive director of the Institute of Nanochemistry and Nanobiology, Shanghai University. He received the B. S. degree in Chemistry from Zhejiang University, China, in 1989, the M. S. degree in Material Sciences from Zhejiang University, China, in 1995, and the Ph. D. degree in Chemistry from Peking University, China, in 1998. After two years of postdoc training with Prof. Harold A. Scheraga at Cornell University, USA, he joined the faculty of the College of Chemistry and Molecular Engineering at Peking University, China, in 2000. He was a visiting associate professor at the Institute for Protein Research at Osaka University, Japan, in 2004. He moved to current institute in 2008.
Prof. Cao's major research interests include: 1) experimental and computational approaches for understanding the basic physical chemistry of the interaction between nanoparticles and biomolecules; 2) structure-based rational design of functional nanoparticles for biomedical applications (bio-imaging and drug-delivery) and bio-catalysts. He has published over 60 peer-reviewed papers and hold 8 patents.

Angel M. Carcaboso

Angel M. Carcaboso is BS Pharm (1999) and PhD (2004) at Universidad del Pais Vasco (Spain); and MBA (2003) at UNED (Spain). He received postdoctoral training at UBA (Argentina; 2004-2008) and Saint Jude Children's Research Hospital (USA; 2008-2010).

RESEARCH BACKGROUND.

Angel is the group leader of the Preclinical Therapeutics and Drug Delivery Research Program at Sant Joan de Deu Research Foundation (Barcelona, Spain). He is author of 30 international publications and inventor of several patent applications in pharmaceutical sciences and cancer research. Since joining Hospital Sant Joan de Déu (2011) he has received competitive funding from prestigious European programs including the Marie Curie International Reintegration Grant and Euronanomed II 2015 call (project Cure2DIPG). He coordinates the initiative Cure2DIPG to accelerate research of a pediatric disease, diffuse intrinsic pontine glioma (DIPG), an incurable brain tumor affecting 300 children yearly in Europe and the United States. The main objective of the project is to develop new nanotechnology-based treatments able to cross the blood-brain barrier in DIPG.
Angel's laboratory is focused in improving therapy for children with solid tumors through preclinical studies. The development of preclinical tumor models from patient biopsies is also a priority in his lab. During the last 3 years his laboratory has developed preclinical DIPG models from patient biopsies that reproduce the K27M mutation discovered in the H3 histones in year 2012. His lab has also established several patient-derived xenografts (PDX) from patients with pediatric solid tumors (neuroblastoma, Ewing sarcoma, rhabdomyosarcoma, retinoblastoma). Angel's group has tested the activity of new treatments and drug delivery systems in such models (Monterrubio et al, Pharm Res, 2015; Monterrubio et al, Biomaterials, 2016). He also collaborates with several international research institutions focused on finding cures for children with cancer.

Werner Cautreels

PhD
President and CEO
Selecta Biosciences Inc.

Prior to joining Selecta Biosciences in 2010, Dr. Cautreels was Chief Executive Officer of Solvay Pharmaceuticals, the pharmaceuticals division of the Solvay Group, in Brussels, Belgium, from 2005 until Solvay Pharmaceuticals was acquired by Abbott Laboratories in February 2010. Before becoming the CEO of Solvay Pharmaceuticals, Dr. Cautreels was its Global Head of R&D from 1998.
Prior to joining Solvay, he was employed by Sanofi, Sterling Winthrop from 1979 to 1994, and Nycomed Amersham from 1994 to 1998 in a variety of R&D management positions in Europe and in the United States. Dr. Cautreels was a director of Innogenetics NV in Gent, Belgium and ArQule Inc., in Woburn, Massachusetts from 1999 to 2006. He currently serves as a director of Seres Therapeutics, Inc. and Galapagos NV, in Mechelen, Belgium. He was the President of the Belgian Luxemburg Chamber of Commerce for Russia and Belarus until June 2010. Dr. Cautreels received his Ph.D. in Chemistry, specializing in Mass Spectrometry, from the University of Antwerp (Antwerp, Belgium), and his financial and business training from the Advanced Management Program at Harvard Business School.

Valentin Ceña

Born in Barcelona (Spain) in 1957. He received his MD degree in 1980 and his Ph.D. degree in 1982. He spent two postdoctoral periods at the Department of Pharmacology at Downstate Medical Center; New York University (3 months) and at the National Institutes of Health in Washington D.C. at NICHD and NIADDK (4 years). In 1987, he was appointed Assistant Professor of Pharmacology at the University of Alicante (Spain) and promoted to Professor in 1995. He moved to University Miguel Hernández in 1997 and to University of Castilla-La Mancha in 2000 to set up the Medical School where he is currently working. In addition to his academic duties, he has been appointed for several management positions including Director of the Research Institute C.R.I.B. of the University of Castilla-La Mancha and Deputy Director for Network and Cooperative Research of the Instituto de Salud Carlos III (Ministery of Health; Spain).
His research interests have been aimed to 3 main areas: a) to study the molecular mechanisms of neurosecretion and the role that ionic channels, mainly voltage-dependent calcium channels subtypes play in such process, b) the molecular mechanisms involved in neuronal death occurring during neurodegenerative diseases such as Parkinson and Alzheimer disease with a special focus on the mitochondrial mechanisms involved in different neuronal death paradigms like excitotoxicity or autophagy-induced death and c) the use of siRNA introduced in the cells by different nanoparticles, mainly dendrimers, as a new therapeutic approach to different diseases, mainly neurodegenerative diseases and cancer with a special focus on glioblastoma.
He has published almost 100 papers and has an h-index of 32.

Mark Chiu

Dr. Mark Chiu is an Associate Director in the Department of Structural Biology of Janssen BioTherapeutics which is part of Johnson and Johnson at Spring House, Pennsylvania. Mark received a B.A. Biophysics from University of California, Berkeley and a Ph.D. Biochemistry from the University of Illinois Urbana-Champaign. He was a post-doctoral fellow at the ETH-Zurich and at the Biocenter at the University of Basel. Mark has worked as a synthetic organic chemist in Microgenics, Assistant Professor of Chemistry at Seton Hall University, and a Research Investigator in the Department of Structural Biology at Abbott Laboratories. Currently, Mark is head of the Biologics, Engineering, and Function group that develops fit for purpose drug candidates based on protein engineering. Outside of work, Mark explores the realms that involve the symbiosis of invertebrates and native flora.

Insung S. Choi

Insung S. Choi is Professor of Chemistry and of Bio and Brain Engineering at KAIST, Korea, and the Director of the Center for Cell-Encapsulation Research (Creative Research Initiative; 2012-). He obtained his BS and MS degrees in Chemistry at Seoul National University in 1991 and 1993, and did his PhD degree in Chemistry at Harvard University in 2000 under the supervision of George M. Whitesides. After postdoctoral work with Robert Langer at the Department of Chemical Engineering of MIT, he joined the faculty at KAIST in 2002. He was awarded KCS-Wily Young Chemist Award (2003), Thieme Journal Award (2003), Presidential Young Scientist Award (2004; KAST), and JANG SEHEE Research Achievement Award (2013; KCS). His research interests include biomimetic chemistry, cell-material interfaces, and biosurface organic chemistry. He has published over 200 peer-reviewed papers. He is the editorial board member of Chemistry-An Asian Journal (Wiley-VCH), ChemNanoMat (Wiley-VCH), and Scientific Reports (NPG).

Gerhard Christofori

Gerhard Christofori (1957) studied Biology at the University of Heidelberg and obtained his Diploma in 1985 and his PhD in 1988 in the laboratory of Prof. Walter Keller at the German Cancer Research Center in Heidelberg and at the Biocenter of the University Basel on the biochemistry of 3' processing and polyadenylation of eukaryotic messenger RNA. He did his postdoctoral training with Prof. Douglas Hanahan at the University of California San Francisco, USA, where he began to study the molecular mechanisms underlying multistage tumor development in transgenic mouse models. In 1994 he became a group leader at the Institute of Molecular Pathology (IMP) in Vienna, Austria. Since 2001 he is Professor and Chair of Biochemistry within the Department of Biomedicine at the University of Basel.

His laboratory uses cultured cancer cell lines *in vitro* and refined mouse models of carcinogenesis *in vivo* to unravel the molecular mechanisms underlying malignant tumor progression and metastasis formation. One major focus in the laboratory is the transcriptional and epigenetic control of epithelial-mesenchymal transition (EMT) during tumor progression and the development of resistance to targeted therapy. Another focus is based on the observation that tumor malignancy is not only induced by changes within the tumor cells themselves but also by the tumor microenvironment.

Daan J.A. Crommelin

PhD

Prof. Daan Crommelin is professor emeritus at the Department of Pharmaceutics at Utrecht University. Until December 2011 he was scientific director of the Dutch Top Institute Pharma in Leiden. He is adjunct professor at the Department of Pharmaceutics and Pharmaceutical Chemistry at the University of Utah. Crommelin is co-founder of OctoPlus, a Leiden based company specialized in the development of pharmaceutical (mainly protein based) product formulations and advanced drug delivery systems. He published extensively and is on the editorial board of 10 peer reviewed journals in the pharmaceutical sciences. He is Editor-in-Chief of the AAPS book series 'Advances in the Pharmaceutical Sciences'. He advises venture capital groups and acts as consultant. He chairs the UCAB Foundation: the Utrecht Center of Excellence for Affordable Biotherapeutics, a WHO supported initiative. He chaired the Board of Pharmaceutical Sciences of the International Pharmaceutical Federation (F.I.P.), was chair of the organizing committee of the Pharmaceutical Sciences World Conference 2007 in Amsterdam. He is past president of the European Federation of Pharmaceutical Sciences (EUFEPS) and past vice-chair of the scientific advisory board of the European Innovative Medicines Initiative (IMI).

Bertrand Czarny

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QUALIFICATIONS

2007: Master degree in biochemistry (CNAM-National Conservatory of Arts and Crafts, Paris)
2008: Advanced Master degree in Structural and Functional Engineering of Biomolecules (University of Paris Descartes, Pharmacy Paris V)
2012: Doctorate Ph.D. in Structural Biology (University of Paris Descartes, Pharmacy Paris V)

PROFESSIONAL APPOINTMENTS

2001: CEA Technician, Department of Engineering and Study of Proteins, France
2012: CEA Researcher, Service of Molecular Engineering of Proteins (SIMOPRO), France
2014: Sabbatical, Senior Research Fellow, Department of Pharmaceutics Utrecht University, Netherlands, and based at National University of Singapore, Department of Pharmacy, Singapore

Kenneth A. Dawson

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University College Dublin Belfield, Dublin 4

EDUCATION

BSc. Chemistry, (QUB) **(1980)**; MSc Mathematics (QUB) **(1981)**; DPhil Chemistry (University of Oxford) **(1984)**

EMPLOYMENT POSITION

Assistant Professor of Chemistry, University of California, Berkeley **1989- 1992** Adjunct Professor of Biophysics, University of California, Berkeley, **1989-present** Chair of Physical Chemistry, University College Dublin **1992**, **1992-**present, Director Centre for BioNano Interactions, University College Dublin.

INNOVATION/COMMERCIALISATION ACTIVITY

- **Patent:** A method for the selective concentration of a specific low abundance biomolecules; European & preliminary US filings – Priority Date 27/02/09
- Patent Preparation: Nanoscale Control of Immune systems (2016) Patent Preparation; Other (2016)
- **Industry collaborations:** Intel, Glantreo, Biotrin, Thermofisher, DePuy, Cellix, Life Technologies. Also, L'Oreal, Intel, Medtronic, DSM, Umicore, Avanti Cell Science, Grimm Aerosol Technik, Bayer Technology Services, Becton Dickinson, Nikon Instruments, Progenika Biopharma, INNOVA, Selective Antibodies, NanoSight, Radisens Diagnostics, Advanced Accelerator Applications, Esoate, Sadosa, Optofluidics, Attana AB.

SUMMARY CURRENT POSITION:

- Founder of modern version of the 'protein corona' hypothesis in which the biological identity of engineered nanoparticles (and other surface-induced effects such as fibrillation) is defined by most slowly exchanged surface biomolecules, now generating validation across a variety of arenas (e.g. Nanobiotechnology:nanoparticle

coronas take shape. Nature Nanotechnol. 2011, 6(1), 11-2.) The 2007 papergenerated Cozzarelli prize of US National Academy. Leader of several EU major grant programs in field.

- Principal PI and Co ordinator of European Infrastructure, QNano for Bionanoscience/Nanosafety. Member of European Commission scientific committees including SCENIHR committee on regulations for new risk in EU, and in European Medicines Agency, Board European Science Foundation. Ad hoc advisor to several governments, and EU, and European Parliament. Represents Ireland in OECD and ISO in field. Editor of several journals, experimental, theoretical, interdisciplinary. Recently conceived, funded, and supported development of National BioNano Centre.

PRIZES:

- Richardson Prize, Harrison Prize (RSC),
- IBM (two prizes, for chemistry and for distributed processing),
- Sloan Fellow (U.S.)
- Dreyfus Fellow (U.S.)
- Packard Fellow (International)
- Canon Professor (Japan)
- Cozzarelli Prize National Academy Science United States 2008 (U.S.) for the protein corona.

PRESENT RESEARCH ORIENTED INTERNATIONAL ACTIVITY:

Recognised (2015) as a Foreign Visiting Professor in Brazil

Paolo Decuzzi

Paolo Decuzzi is a senior researcher and director of the Laboratory of Nanotechnology for Precision Medicine (https://www.iit.it/research/lines/nanotechnology-for-precision-medicine) at the Italian Institute of Technology in Genova – Italy. Dr. Decuzzi earned his M.Sc. degree in Mechanical Engineering from the Politecnico di Bari (Italy) in 1997 and his Ph.D. degree in Mechanical Engineering from the University of Naples – Federico II (Italy) in 2000, with a thesis on friction and adhesion at the nanoscale. In 2002, he was nominated Assistant Professor of Machine Design at the Politecnico di Bari and, in 2005, he became Associate Professor in the School of Medicine of the University 'Magna Graecia'. There, he co-founded BioNEM - the laboratory of BioNanotechnology and Engineering for Medicine - one of the first nano-engineering laboratories built in a School of Medicine. In October 2007, he joined The University of Texas Health Science Center in Houston as an Associate Professor of Biomedical Engineering. In October 2010, he moved to the Houston Methodist Hospital where he served as a Professor of Biomedical Engineering till July 2015. There, he founded the Center for the Rational Design of Multifunctional Nanoconstructs, with the financial support of the Cancer Prevention and Research Center of Texas and the US National Cancer Institute; and served first as the co-chair of the Nanomedicine Department and then as the interim chair of the Translational Imaging Department. In July 2014, Dr. Decuzzi was awarded a 5-year European Research Council "Consolidator Grant" to design, synthesize and develop nanoconstructs for imaging and therapy in brain cancer.

Dr. Decuzzi has been a visiting scientist at the Department of Theoretical and Applied Mechanics at the University of Michigan - Ann Arbor (1998, 1999 and 2001); and a visiting professor at the Princeton Material Institute – Princeton (2003); the Heart and Lung Institute at the Ohio State University (2003 and 2004); the University of Texas Health Science Center (2006). Dr. Decuzzi has published over 150 papers in international peer-reviewed journals, international conferences and book chapters. He holds over 5 patents in the field of Nanomedicine. He co-founded NEMB – NanoEngineering for Medicine and Biology – committee of the American Society for Mechanical Engineers and is involved in multiple dissemination activities to foster the collaboration between biomedical scientists and engineers. He serves on multiple NIH, NSF, ESF, and Italian Government study sections and his research activity is primarily supported by NIH, DOD, CPRIT in USA; ESF and ERC in EU.

Decuzzi's lab mission is to i. rationally design polymeric nanoconstructs for multi-modal imaging and combination therapy in cancer, cardiovascular and neurological diseases; ii. fabricate microfluidic chips for the rapid screening of novel molecular and nano-based therapeutic agents; iii. develop multi-scale, hierarchical computational models for predicting the transport and therapeutic efficacy of nanoconstructs; iv. organize dissemination activities at the interface between engineering and biomedical sciences; and v. promote the professional development of lab members in a highly multi-disciplinary environment.

Neil P. Desai

PhD

Neil Desai is Founder/CEO of AADi, LLC, a clinical stage start-up developing targeted mTOR therapeutics for oncology/cardiovascular applications, Founder/CEO of Aadigen, LLC, a company focused on delivery of nucleotide therapeutics, and VP of Strategic Platforms at Celgene Corp. He was formerly SVP of Global R&D at Abraxis Bioscience (Los Angeles, California, USA, acquired by Celgene in 2010 for approximately $3B) where he led the development of Abraxane®, the company's flagship nanotechnology product. Dr. Desai is an inventor of the nanoparticle-albumin bound (nab®) drug-delivery platform and Abraxane, which is approved for metastatic breast cancer, non-small cell lung cancer and pancreatic cancer with sales of $967M in 2015. Prior to Abraxis, Dr. Desai held positons of increasing seniority at American Bioscience, Inc., VivoRx, Inc. and VivoRx Pharmaceuticals, Inc. (predecessor companies of Abraxis), where he worked on the early discovery and development of Abraxane, developed novel encapsulation systems for living cells and was part of the team that performed the world's first successful encapsulated islet cell transplant in a diabetic patient. Dr. Desai has over 25 years of experience in novel therapeutic delivery systems with over 100 issued patents, over 40 peer-reviewed publications and book chapters, and over 200 presentations at scientific meetings. He is reviewer for several scientific journals, an active participant in FDA and EU Nanotechnology initiatives and a member of the Steering Committee for the National Cancer Institute (NCI) Alliance for Nanotechnology in Cancer. He holds board and advisory positions in various start-ups. Dr. Desai recieved a M.S and Ph.D. in Chemical Engineering from the University of Texas at Austin, USA, and a B.S. in Chemical Engineering from the University Institute of Chemical Technology in Mumbai, India.

László Dézsi

Ph.D., Dr. habil.
Senior Research Associate, leading scientist (*in vivo* small animal lab.)
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LPhD, DrHabil., Senior Research Associate, leading scientist of the *in vivo* small animal laboratory, Nanomedicine Research and Education Center, Institute of Pathophysiology at Semmelweis University since 2012 in Budapest, Hungary.

He obtained his MSc degree in biology at Eötvös Loránd Univer-

sity and his PhD in physiology at Semmelweis University Medical School, Budapest, Hungary. He conducted teaching and research activities at Semmelweis University (1981-1999), and meanwhile he received fellowships at Albert Ludwigs Universität, Freiburg, Germany (Prof. E. Bassenge) working in the field of local regulation of blood flow in skeletal and cardiac muscle; as well as at the University of Pennsylvania, Philadelphia, USA, Cerebrovascular Research Center (Prof. M. Reivich, Dr. J. H. Greenberg) working in the field of cerebral blood flow/metabolism and cerebral ischemia/reperfusion in stroke models in animals. He had been head of laboratory, CRO monitor and research project manager in vascular and safety pharmacology at Gedeon Richter Pharmaceutical Plc. (1999-2012). He was manager of Analgesic Research Laboratory (2006-2012), a joint venture of Gedeon Richter and University of Pécs, Department of Pharmacology (Prof. J. Szolcsányi). He was involved in curriculum development and had been Secretary of Biomedical Engineering (BE) Course Committee (1994-2000), now member of the MSc BE Committee at Technical University, Budapest. He made his habilitation at Semmelweis University in 2005 and became Adjunct Professor of physiology in 2006. He established his own teaching course in 2008 entitled "Cardiorespiratoric and neurophysiological measuring techniques" at the Department of Human Physiology and Clinical Experimental Research (Prof. Z. Benyó). He participates in Postgradual Education in Nanomedicine (Prof. L. Rosivall).

Currently he is working in the field of nanomedicine investigating cardiopulmonary and immunological effects of nanoparticles in various *in vivo* models of complement activation related pseudoallergy (CARPA) and participates in the development of new models under the supervision of Prof. J. Szebeni. He works as a member of the EU FP7 "NanoAthero" Consortium (head: Didier Letourneur).

Gilles Divita

Dr. Gilles DIVITA obtained a doctoral degree in Biochemistry from Claude Bernard University in Lyon France in 1992. He worked, then as an Associate Scientist at Protein Structure Department of the Max Planck Institute for Medical Research in Heidelberg, Germany. In 1996, Dr. DIVITA joined, as Principal Investigator, the Centre de Recherche en Biochimie Macromoléculaire at the French National Center for Scientific Research (CNRS) in Montpellier France. From 1999 to 2001, he worked as Associate Professor at the Molecular Biology Department of the Scripps Research Institute in La Jolla, CA-USA. Since 2002, Dr. DIVITA is CNRS Research Director, head of Chemical Biology and Nanotechnology for Therapeutics Team at the CNRS, Montpellier-France. Since 2014, He is consultant at Aadigen LLC, California (USA).

Dr Divita's work focuses on strategies to probe and perturb the behaviour of biomolecules in physiological and pathological settings. Dr. DIVITA is the pioneer of the "non covalent cell penetrating peptide-based strategy" for therapeutic delivery and has a strong expertise in drug delivery systems, peptide-drugs and fluorescence-biosensors. His team developed multifunctional nanoparticles for efficient targeted delivery of candidate drugs, adjuvants and bioprobes, that can be applied to therapeutic strategies in various diseases, diagnostics, as well as for cosmetic applications. . Dr. DIVITA is author of over 180 articles in peer reviewed scientific journals and of 12 patents. He is member of the Editorial Board of Nucleic Acid Research, Open Access Nanomedicine and of BMC Biophysics.

Marina Dobrovolskaia

At the Nanotechnology Characterization Laboratory (NCL) operated by Leidos Biomedical Research Inc. for the US National Cancer Institute, Dr. Dobrovolskaia directs characterization related to nanomaterials' interactions with components of the immune system. She leads a team of scientists and technicians to develop, validate and qualify performance of *in vitro* and ex vivo assays to support preclinical immuotoxicity characterization of nanoparticles, to monitor nanoparticle purity from biological contaminants such as bacteria, yeast, mold and endotoxin, to conduct structure activity relationship and mechanistic studies explaining nanoparticle immunotoxicity. Dr. Dobrovolskaia is a member of several working groups on Nanomedicine, Oligonucleotide Safety and Endotoxin Detection. She has published more than 40 peer-reviewed papers regarding nanomaterial interactions with the immune system, prepared and edited two editions of a Handbook of Immunological Properties of Engineered Nanomaterials. Dr. Dobrovolskaia is an invited speaker to numerous national and international nanotechnology-related conferences. She has served as a Special Associate Editor in Immunology for the "Nanomedicine: Nanotechnology, Biology and Medicine" Journal published by Elsevier and is currently an editorial board member for the Journal of Nanotoxicology and Nanomedicine by IGI Global. Prior to joining the NCL, Dr. Dobrovolskaia worked as a Research Scientist in a GLP laboratory at PPD Development, Inc. in Richmond, VA, where she was responsible for the design, development and validation of bioanalytical ligand-binding assays to support pharmacokinetic and toxicity studies in a variety of drug development projects. She received her M.S. degree from the Kazan State University in Russia, her Ph.D. from the N.N. Blokhin Cancer Research Center of the Russian Academy of Medical Sciences in Moscow, Russia, MBA degree from Hood College in Frederick MD, and completed two postdoctoral trainings in immunology at the National Cancer Institute in Frederick, MD and the University of Maryland in Baltimore, MD. Her areas of expertise include cell signaling, innate immunity, immunogenicity of drug products, analytical methodology, and endotoxin detection and quantification.

Christopher Drewell

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13355 Berlin
christopher.drewell@campus.tu-berlin.de

My name is Christopher Drewell, I was born on March 6th 1985 in Berlin, Germany. I studied Biotechnology at the Technical University Berlin and finished this study in 2012 with the degree of Diplom-Ingenieur (Dipl.-Ing.). My diploma thesis was performed at the Max-Planck-Institute for Infection Biology, Berlin, Germany in the group of Fritz Melchers and is entitled „The influence of hPax5 expression levels on lymphoid vs. myeloid development of pro/preB cells".

Already in 2011 I started working in the Department of Medical Biotechnology at the Technical University Berlin, first as a student assistant, then as a PhD student. For my PhD project I had the opportunity to follow my deep interests in tissue engineering and became responsible for the development of the kidney equivalent in the context of the Human-on-a-Chip project of the TissUse company.

For the funding of my PhD project I was awarded a scholarship by the Berlin-Brandenburg School of Regenerative Therapies (BSRT).

PUBLICATIONS

• Simmons S, Knoll M, Drewell C, Wolf I, Mollenkopf H-J, Bouquet C, Melchers F: Bi-phenotypic B-lymphoid/myeloid cells expressing

low levels of Pax5: potential targets of BAL development. Blood
- Maschmeyer I, Lorenz AK, Schimek K, Hasenberg T, Ramme AP, Hübner J, Lindner M, Drewell C, Bauer S, Thomas A, Sambo NS, Sonntag F, Lauster R, Marx U: A four-organ-chip for interconnected long-term co-culture of human intestine, liver, skin and kidney equivalents. Lab Chip 15: 2688–2699, 2015

Lukas Engelberger

Since 2014, Dr. iur., LLM, Lukas Engelberger (CVP) is a member of the Government of the Canton Basel-Stadt and Head of the Public Health Department. After his studies and doctorate in law at the universities of Basel, Fribourg and Bern, he was practising as an atorney at Bär & Karrer in Zürich (2003–2005) and as a legal counsel at Hoffmann-La Roche Ltd. in Basel (2005–2014). He is member of the executive committee of the Swiss Conference of the State Ministers of Public Health (GDK). He is married and father of three children.

Bastiaan Evers

Bastiaan Evers was born on the 3rd of January 1977 in Groningen, the Netherlands. After high school, he studied biotechnology and process engineering at Wageningen university where he graduated with honors in 2002 with specializations in molecular/cellular biotechnology and business administration. In 2003 he started his Ph.D under supervision of Prof. Jos Jonkers at the Netherlands Cancer institute on novel therapeutic strategies for hereditary breast cancer. On a Dutch Cancer Foundation research grant, he then performed postdoctoral research in the lab of Prof. Thomas Helleday at the university of Oxford and later ran a small research team at the Karolinska Institute in Stockholm, Sweden. Since 2013, he's back in Amsterdam, working in the lab of René Bernards on identifying synthetic lethal interactions for therapeutic exploitation in bladder cancer.

Bengt Fadeel

Bengt Fadeel is a Professor of Medical Inflammation Research at the Institute of Environmental Medicine, Karolinska Institutet, Stockholm, and Adjunct Professor of Environmental and Occupational Health, University of Pittsburgh. He is a Fellow of the Academy of Toxicological Sciences (ATS). He received his M.D. in 1997 and Ph.D. in 1999 from Karolinska Institutet. Dr. Fadeel is the past coordinator of FP7-NANOMMUNE, an EU-funded consortium focused on hazardous effects of nanomaterials on the immune system, and currently engaged in several other EU-funded nanosafety projects as well as the Flagship Project GRAPHENE, and a member of the national MISTRA Environmental Nanosafety consortium. Dr. Fadeel chairs the working group on systems biology in the EU nanosafety cluster and he is a member of the WHO-IPCS panel on immunotoxicity testing of engineered nanomaterials.

Omid Farokhzad

Omid Farokhzad is an Associate Professor at Harvard Medical School (HMS) and a physician-scientist in the Department of Anesthesiology at Brigham and Women's Hospital (BWH). Dr. Farokhzad directs the Laboratory of Nanomedicine and Biomaterials at BWH. He is a faculty member of the Brigham Research Institute Cancer Research Center. He is additionally a member of the Dana Farber/Harvard Cancer Center Programs in Prostate Cancer and Cancer Cell Biology. Dr. Farokhzad's research is focused on the development of therapeutic nanoparticle technologies; most notably, he pioneered the high throughput combinatorial development and screening of multifunctional nanoparticles for medical applications. Dr. Farokhzad has authored approximately 130 papers (~24,000 citations; H-Index 62) and holds more than 145 issued/pending US and International patents. The technologies that Dr. Farokhzad has developed with collaborators at HMS and MIT formed the basis for the launch of four biotechnology companies: BIND Therapeutics (NASDAQ: BIND), Selecta Biosciences, Tarveda Therapeutics (formerly Blend Therapeutics), and Koan Biotherapeutics, which are translating the aforementioned academic innovations toward commercialization and societal impact. Dr. Farokhzad has served in various capacities on the Board of Directors and the Scientific Advisory Board of these companies. He was a recipient of the 2013 RUSNANOPRIZE, one of the largest international nanotechnology prizes, for the development and industrialization of nanoparticle technologies for medical applications. In 2014, he received the Golden Door Award from the International Institute of New England for his societal and economic impact as a naturalized USA citizen. In 2015, he was named as one of The Worldview 100 by Scientific American, which recognized visionaries who shape biotechnology around the world. In 2016, he was among the recipients of the Ellis Island Medal of Honor for his scientific, societal and economic contributions to America as an immigrant. Dr. Farokhzad was elected to the College of the Fellows of the American Institute of Medical and biological Engineering. He was selected by Thomson Reuters among the Highly Cited Researchers in 2014 and 2015. The Boston Globe selected him among the top innovators in Massachusetts and the Boston Business Journal selected him among the Health Care Champions for his innovations. In 2012, he was among the regional Ernst & Young Entrepreneur of the Year awardees. Dr. Farokhzad completed his post-graduate clinical and post-doctoral research trainings, respectively, at the BWH/HMS and MIT in the laboratory of Institute Professor Robert Langer. He received his M.D. and M.A. from Boston University School of Medicine and his M.B.A. from the MIT Sloan School of Management.

Delphine Felder-Flesch

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Delphine Felder-Flesch obtained her PhD in supramolecular organic chemistry from the University of Strasbourg, France in 2001. After an ERASMUS training period at the University of York, England, she started post-doctoral research at the ETH-Zürich, Switzerland, in the group of Prof. Dr. Francois Diederich. Since October 2003 she is a CNRS research scientist at the Institute of Physics and Chemistry of Materials, Strasbourg. Her main research interests include (ME)MRI or nuclear medicine dendritic nanoprobes for efficient *in vivo* tumor targeting and cancer early diagnosis imaging. She also

develops, in collaboration with solid state chemists, dendronized nanoparticles (metal oxides) as MRI nanoprobes and for magnetic hyperthermia treatment of cancers.

FIELD OF EXPERTISE:

Organic chemistry_Functional materials _Dendrimers_Nanoprobes for biomedical imaging

Xavier Fernàndez-Busquets

Xavier Fernàndez-Busquets started his career as a trainee student at the CIBA-GEIGY Zentrale Forschungslaboratorien in Basel. He graduated in Biochemistry at the Universitat Autònoma de Barcelona, where he obtained his PhD in Molecular Biology. Between 1992 and 2001 he held several postdoctoral positions, among which those at the Friedrich Miescher Institut (Novartis AG, Basel) and at the Woods Hole Marine Biological Laboratory. In 2001 he obtained a 5-year tenure track Ramón y Cajal position at the Universitat de Barcelona. In 2006 he became Senior Researcher at the IBEC and since 2010 he is Head of the Nanomalaria Joint Unit (IBEC/ISGlobal).

PRESENT POSITIONS AND AFFILIATIONS:

- Associate Researcher, Head of Nanomalaria Joint Unit, Institute for Bioengineering of Catalonia (IBEC), Barcelona Science Park, Baldiri Reixac 10-12, ES-08028 Barcelona, Spain. www.ibecbarcelona.eu.
- Assistant Research Professor, Head of Nanomalaria Joint Unit, Barcelona Institute for Global Health (ISGlobal, Hospital Clínic-Universitat de Barcelona), Rosselló 132, ES-08036 Barcelona, Spain. www.cresib.cat.
- Coordinator, Nanomalaria Group, Nanoscience and Nanotechnology Institute (IN2UB), University of Barcelona, Martí i Franquès 1, E-08028 Barcelona, Spain. Tel: +34 93 227 5400 (ext. 4581), E-mail: xfernandez_busquets@ub.edu

CURRENT RESEARCH: NANOBIOMEDICINE

- Single-molecule studies of proteoglycan and glycosaminoglycan interactions.
- Application of nanotechnology to the study of functional amyloids.
- Development of nanovectors for the targeted delivery of antimalarial drugs.

ACADEMIC BACKGROUND

1986: Graduate in Biological Sciences, area of Biochemistry. Universitat Autònoma de Barcelona, Spain.
1988: Dissertation for University degree, area of Enzymology/Organic Chemistry. CIBA-GEIGY AG, Basel, Switzerland/Universitat Autònoma de Barcelona.
1988: Master in Biochemistry and Molecular Biology. Universitat Autònoma de Barcelona.
1992: PhD Thesis in Biological Sciences. Universitat Autònoma de Barcelona.
2004: Diploma in University Teaching, Institut de Ciències de l'Educació, Universitat de Barcelona.

POSITIONS HELD

November 2001–November 2006: 5-year tenure track Ramón y Cajal Position. Research Center for Bioelectronics and Nanobioscience. Barcelona Science Park, Universitat de Barcelona, Spain.
May 1999–November 2001: Postdoctoral position. Plant Biotechnology Group. Department of Biochemistry and Molecular Biology, School of Pharmacy, Universitat de Barcelona, Spain.
April 1993–April 1999: Postdoctoral position. Novartis AG-Friedrich Miescher Institut, Basel, Switzerland, and Marine Biological Laboratory, Woods Hole, USA.
October 1992–March 1993: Postdoctoral position. Institute of Agroalimentary Research and Technology (IRTA), Cabrils, Spain.
February 1987–September 1992: PhD Thesis. Department of Biochemistry and Molecular Biology, Universitat Autònoma de Barcelona, Spain.
July–October 1985 and July–December 1986: Trainee student. Zentrale Forschungslaboratorien, CIBA-GEIGY AG, Basel, Switzerland.
PEER-REVIEWED PUBLICATIONS: 81; **CONFERENCE CONTRIBUTIONS**: 133

Lino Ferreira

EDUCATION/TRAINING

1989-1994: BSc, University of Coimbra, Coimbra, Portugal
1999-2003: PhD, University of Coimbra, Coimbra, Portugal
2004-2007: Postdoc, MIT (USA)

POSITIONS AND EMPLOYMENT

Lino Ferreira is Coordinator Investigator (eq. Full Professor) at Center of Neurosciences and Cell Biology, and the head of the Biomaterials and Stem Cell-Based Therapies research group. He holds a Ph.D. in Biotechnology (2003) from the University of Coimbra (Portugal). He did postdoctoral work at MIT (USA) in the laboratory of Professor Robert Langer in the areas of human embryonic stem cells, micro- and nanotechnologies. He joined the Center of Neurosciences and Cell Biology (CNC, University of Coimbra) and Biocant (Innovation Center, Portugal) in October 2007. He has published more than 80 peer reviewed papers and has 18 issued or pending patents– 7 of which have been licensed to companies in the biomedical industry. He has supervised more than 6 PhD thesis and he is a founder of 2 spin offs (Matera, HumanPred). He is the CNC coordinator of the MIT-Portugal Program. In 2012 he was awarded with a prestigious European Research Council starting grant. His research group has two main avenues of research: (i) development of bioengineering platforms to modulate the differentiation and maturation of stem cells and (ii) development of nanomedicine platforms to modulate the activity of stem cells and their progenies.

AWARDS AND PRIZES

2015: FCT Investigator award.
2013: Member of the Jury of Pulido Valente Ciencia 2012 prize.
2013: FCT Investigator award.
2012: European Research Council- Starting Independent Researcher Grant (StG, Grant nº 307384)
2008: Crioestaminal Prize, in recognition of the best biomedical basic research project carried out in Portugal in 2008.

CONTRIBUTION TO SCIENCE

The development of a wide spectrum of nanotechnologies (referred as Nanomedicine by National Institutes of Health for applications in the biomedical area) during the last years are very promising for the study of stem cell biology and to control exogenous and endogenous stem cells for regenerative medicine. My group is particularly interested to use these tools to induce *in vivo* stem cell differentiation and to mobilize stem cells from their niches to treat diseases. For this purpose, we are developing nanomaterials that release efficiently small molecules, proteins or non-coding RNA (miRNAs) to manipulate stem cells or their progenies.

1. Gomes, RSM, Neves, R, Cochlin, L, Lima, AF, Carvalho, R, Korpisalo, P, Dragneva, G, Turunen, M, Liimatainen, T, Clarke, K, Yla-Herttuala, S, Carr, CA, Ferreira, L. "Nanoparticles for simultaneous cell tracking and pro-survival/angiogenic miRNA delivery in an ischemic animal model". ACS Nano 2013, 7(4), 3362-3372.
2. Santos, T, Ferreira, R, Maia, J, Agasse, F, Xapelli, S, Cortes, L, Bragança, J, Malva, JO, Ferreira, L*, Bernardino, L*. "Polymeric nanoparticles to control the differentiation of neural stem cells in the subventricular zone of the brain". ACS Nano 2012, 6(12), 10463-10474. *Authors contributed equally.

3. Maia, J, Santos, T, Aday, S, Agasse, F, Cortes, L, Malva, JO, Bernardino, L*, Ferreira, L*. “Controlling the neuronal differentiation of stem cells by the intracellular delivery of retinoic acid-loaded nanoparticles”. ACS Nano 2011, 5(1), 97-106. *Authors contributed equally.
4. Ferreira L*, Karp JM, Nobre L, Langer R*. “New opportunities: The use of nanotechnologies to manipulate and track stem cells”. Cell Stem Cell 2008, 3, 136-146. *Corresponding authors.

Another area of interest in my group is related to the development of biomaterials that have biomedical application. We have done significant contributions for in the development of biomaterials with adhesive properties and elastomers.

1. N. Lang*, M.J. Pereira*, I. Friehs, N. Vasilyev, E. N. Feins, K. Ablasser, E. O’Cearbhaill, C. Xu, A. Fabozzo, Y. Lee, R. Padera, S. Wasserman, F. Freudenthal, L.S. Ferreira, R. Langer, J.M. Karp, P. J. del Nido. “A biocompatible light-activated adhesive for minimally invasive repair of cardiovascular defects”. Science Translational Medicine 2014, 6(218), 218ra6.
2. Pereira MJ, Ouyang B, Sundback CA , Lang N, Friehs I, Mureli S, Pomerantseva I, McFadden J, Mochel MC, Mwizerwa O, del Nido P, Sarkar D, Masiakos PT, Langer R, Ferreira LS*, and Karp JM*. A highly tunable biocompatible and multifunctional biodegradable elastomer. Advanced Materials 2012, Feb 25;25(8):1209-15.
3. Mahdavi A*, Ferreira L*, Sundback C, Nichol JW, Chan EP, Carter JD, Bettinger C, Patanavanich S, Chignozha L, Ben-Joseph E, Galakatos A, Pryor H, Pomerantseva I, Masiakos P, Faquin W, Zumbuehl A, Hong S, Borenstein J, Vacanti J, Langer R, Karp JM. “A biodegradable and biocompatible Gecko-inspired tissue adhesive”. Proceedings of the National Academy of Sciences (COVER FEATURE), 2008 Feb 19;105(7):2307-12.*Authors contributed equally.

Joachim Fischer

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Dr. Joachim Fischer received his PhD on superresolution lithograDr. rer. nat. Joachim Fischer (Dipl. Phys.)
In 2003, Joachim began studying physics at the Karlsruhe Institute of Technology (KIT) in Karlsruhe, Germany and graduated with honors in 2008. He did his diploma thesis entitled “Fabrication and characterization of elastic three-dimensional micro structures for biological cell-experiments” in Prof. Martin Wegener’s group at the Institute for Applied Physics (APH) in collaboration with Prof. Martin Bastmeyer’s at the Institute I. In this interdisciplinary work, tailored micro-/nanostructres were fabricated using direct laser writing (DLW). Isolated primary chicken cardiomyocytes were cultivated inside these structures and, after some days, started to periodically deform these structures significantly. Corresponding cell forces were deduced by determining the structure stiffness with atomic-force-microscope measurements, yielding values consistent with literature reports of single cardiomyocyte forces.
In 2008, he started working on his PhD thesis entitled “Three-dimensional optical lithography beyond the diffraction limit” and graduated with honors in 2012. Here, two-photon DLW – a lithography approach allowing to create almost arbitrary three-dimensional structures out of polymers – was combined with the concept of stimulated-emission-depletion (STED) microscopy in order to increase its resolution beyond the limitations impinged by the wave-nature of light. This new super-resolution lithography approach enabled the fabrication of structures with resolutions exceeding the diffraction limit – both, in lateral xy-direction or in axial z-direction. Using STED-DLW, formerly impossible applications in nano-photonics research such as three-dimensional cloaking at visible frequencies or complete photonic band gaps at visible frequencies have been realized. As a result, his thesis was awarded “Best dissertation in 2012” by the German Society for Applied Optics.
Between 2012 and 2014, Joachim worked as a postdoc at the Institute of Nanotechnology (INT) at the KIT. Topics included in-situ temperature measurements using upconverting nanoparticles, monitoring polymerization-kinetics on the micro-nano-scale, placing and integrating single-photon emitters in DLW-fabricated microstructures, and developing novel photoresist systems allowing for nano-lithography below the diffraction limit.
Thereafter, Joachim joined the group of Prof. Stefan W. Hell (meanwhile Nobel laureate) in Heidelberg as a postdoc. He worked on super-resolution imaging and the development of novel microscopy methods, including methods based on 4Pi microscopy and adaptive optics.
In 2015, he joined Abberior Instruments GmbH to continue on his work on super-resolution microscopy in a commercial context.
So far, Joachim has authored 20+ publications in peer-reviewed scientific journals and filed 5 patent applications.

Sara Fortuna

Sara Fortuna is the coordinator of the Theory section of the Molecular NAnotechnology for LIfe Science Applications (MONALISA) group at the Department of Biological and Medical Sciences, University of Udine (UniUD), and contract professor of Practical Informatics at the Department of Chemical and Pharmaceutical Sciences, University of Trieste. Sara Fortuna has been awarded her PhD in Chemistry from Warwick University (Coventry, UK) in September 2010 with a thesis on “Modelling Tecniques for the study of molecular self-organisation” (Supervisor: Prof.Troisi). Following this she took up a postdoctoral appointment in the group of Dr. Fabris (SISSA, Trieste) using density functional to study metal-supported organometallic nanostructures. In October 2012 she started her present appointment at the University of Udine where, under the mentorship of Prof.G.Scoles (Donner professor of Science, Emeritus at Princeton), she is in charge of coordinating a multidisciplinary collaboration aiming to provide novel theoretical solutions to problem of medical and biological interest, with particular interest on the development of new peptide-based nanodevices for protein recognition.
Her area of expertise is in the multiscale modelling of non-covalently bound molecular systems. She looks at surface adsorbed molecular layers, peptides and minibody design for protein recognition, including new molecular architectures for biosensing. In her career to date she has gained experience in applying both classical molecular simulation (lattice and off-lattice models, Monte Carlo and molecular dynamics simulations) and quantum chemical (DFT) calculations to these systems. This combination of techniques gives her the ability to meet the needs of her collaborators both by suggesting new (macro)moleculas designs and by interpreting their results. Her only-theory work focuses on coding coarse-grained and lattice models to unravel the design principles underpinning molecular organisation and recognition.Sara Fortuna is author of 13 papers published in peer reviewed journals (J.Phys.Chem, Langmuir, J.Am.Chem.Soc., PloS ONE, J.Chem.Theory Comput., Sci.Rep.), collecting 140+ citations and an H-index of 8 as indexed by ISI (160+ citation, H-index 9 according to Google Scholar), plus a number of divulgative publications. As a PI she has been awarded a number of prizes, travel grants, and computational grants. She has been invited speaker at 2 workshops, gave 9 seminars, and 7 contributed talks at conferences. She is member of the Institute of Physics (and committee member of IOP Liquids and Complex Fluids Group) and the Royal Society of Chemistry, and has reviewed articles for the American Chemical Society journals (J.Phys.Chem, J.Phys.Chem.Lett., J.Chem. Theory Comput.).For further information visit www.sarafortuna.eu and http://monalisa.uniud.it

Frycek Rudolf

Rudolf Fryček, PhD (M) is the CEO of AMIRES and he has more than 12 years' experience in the European project management and consultancy. He was a consultant to several SMEs in the field of production, innovation and company development, including preparation of project for governmental incentive and for several business oriented bank loans. In 2006 he was nominated as a Seconded National Expert to the European Commission, Directorate General for Industrial Technologies. Beside his technological expertise and daily project officer work (more than 13 projects under his responsibility) he has been active in the policy structuring for exploitation and commercialization of EU framework projects. He helped to analyze the overall nanotechnology unit project portfolio in terms of generated IPR and also co-organized the workshop with European Patent Office and US Patent and Trade Office on IPR in nanotechnology – lessons from experiences worldwide, held in Brussels. He was a Scientific Coordinator of the EuroNanoForum 2009, the bi-annual conference financed by the European Commission. Since 2011 he is an accredited coach of Innovation Platform – PLATINN, which provides hands-on coaching to SMEs. Rudolf is a cooperation coach, which helps companies to increase their innovation capacity. Rudolf is a founder of AMIRES company having 2 main locations, Prague, Czech Republic and Neuchâtel, Switzerland.

Rudolf is active in ETP Nanomedicine (between 2005 - 2006 he was a member of the mirror group) and he regularly visits the general assembly and helps to formulate the strategic research agenda. His activity is particular in the field of regenerative medicine or in-vitro diagnostics, where AMIRES follows several ongoing project (e.g. iONE-FP7, ULTRAPLACAD) and it has a long-term cooperation contract with an SME – CONTIPRO operating in this field since 20 years. CONTIPRO is a medium-size company focused on production of active pharmaceutical ingredients and active substances for cosmetics industry. CONTIPRO launches at least one new final product every year, especially in veterinary medicine and wound healing area. CONTIPRO has an exceptional focus on innovation due to strong basic and applied R&D background and due to established partnerships. CONTIPRO has been recently elected as a co-chairing organization of the ETP Nanomedicine, Working Group Regenerative Medicine.

Alberto A. Gabizon

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Shaare Zedek Medical Center, and
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Alberto Gabizon received his M.D. degree from the School of Medicine, University of Granada, Spain (1974), and his Ph.D. in Cancer Immunology from the Weizmann Institute of Science, Rehovot, Israel (1979). He later completed his training and certification in Radiation and Medical Oncology at Hadassah-Hebrew University Medical Center, Jerusalem, Israel (1985). During his research fellowship at the Cancer Research Institute of UCSF Medical Center, San Francisco, CA (1986-89), he pioneered the development of a new generation of long-circulating liposomes known as Stealth liposomes which have greatly improved stability and selective accumulation in tumors.

Dr. Gabizon's inventorship and research contribution played a key role in the development of DOXIL (pegylated liposomal doxorubicin, also known as Caelyx), a unique anticancer formulation extensively used in the clinic (ovarian cancer, breast cancer, and other cancer types) with important pharmacologic and safety advantages over conventional chemotherapy. Gabizon was one of the first researchers to identify the cardioprotective effect of liposome delivery on doxorubicin-based chemotherapy. His most recent invention currently in clinical studies is PROMITIL (pegylated liposomal mitomycin-C prodrug), a formulation with improved safety over the parent drug mitomycin C, that may be particularly useful in DNA repair-deficient tumors.

In 2011, he founded Lipomedix Pharmaceuticals Inc., a start-up company aimed at developing PROMITIL and other inventions in the field of cancer nanomedicine. Dr. Gabizon has received the university graduation Spain National Prize of Medicine (1975), the Research Career Award of the Israel Cancer Research Fund (1989), the Hebrew University Kaye Innovation Award (1997) for the invention "Liposomal Doxorubicin for Cancer Treatment", the Tel Aviv University Sarnat Lectureship (2000), the Professorship Award of the Israel Cancer Research Fund (2008), and the Alec Bangham Life Time Achievement Award of the International Liposome Research Society (2010).

Dr. Gabizon is active in the medical oncology field in clinical practice and clinical trials, as well as in preclinical pharmacology research with special emphasis on applications of liposomes in drug delivery, targeting of drugs, and experimental cancer therapy. He has published over 150 articles and specialized book chapters, and is an inventor of 10 USPTO-approved patents.

Since 2002, Dr. Gabizon is Director of the Oncology Institute and Laboratory of Experimental Oncology at Shaare Zedek Medical Center, and Professor of Oncology at the Hebrew University-Faculty of Medicine in Jerusalem.

Jérôme Galon

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Dr Jérôme Galon is Research Director first class at INSERM (National Institute of Health and Medical Research) and head of an INSERM laboratory (Integrative Cancer Immunology) at the Cordeliers Research Center in Paris, France. He was trained as an immunologist at the Pasteur Institute and at the Curie Institute (Paris, France). Between 1997 and 2001 he worked at the NIH (National Institute of Health, Bethesda, USA) on functional genomics, bioinformatics and immunology on fundamental and clinical research. In 1999, he received the fellow Award for Research Excellence at NIH (USA).

Since his full-tenured position at INSERM in 2001, Dr Galon directs interdisciplinary research. Works from his laboratory on comprehensive analysis of the tumor-microenvironment and bioinformatics demonstrated that the adaptive immune reaction within the tumor was a better predictor of survival than traditional staging based on cancer's size and spread (N Engl J Med, Science, Science Transl Med, Cancer Res, JCO, Gastroenterology, Immunity, Nat Cancer Rev). He defined the concept of cancer immune-contexture, defined the Immunoscore and is PI of the Immunoscore worldwide consortium. Dr Galon was awarded for his work on cancer research, by the French foundation (Schaeverbeke Award 2008), by the Medical Research Foundation (Rose Lamarca Award 2008). He received the William B. Coley Award for Distinguished Research in Basic and Tumor Immunology (Cancer Research Institute, New York, USA 2010), and Award from the National Academy of Science (Simone et Cino del Duca Cancer Research Award, 2011), and Award from the National Academy of Medicine (Gallet et Breton Award, 2011), Award from the French Society of Immunology (Jacques Oudin Award, 2014). He gave the Annual B. Benacerraf Lecture in Immunology (Harvard, USA, 2014). Jérôme Galon is the co-founder of the company, HalioDx, and is the Chairman of its scientific council.

Anne Gauthier

Anne Gauthier received her MSc in Chemical Engineering and Biotechnology from the Swiss Federal Institute of Technology in Lausanne (EPFL) in 2013. She did her Master's Thesis at Imperial College London on the effects of temperature on antibody production, cell cycle and apoptosis on the GS-NS0 cell line under the supervision of Prof. A Mantalaris. She is currently doing her PhD at Novartis Pharma in the Parenteral and Topical Formulation and Process Development department where she is developing liposomal formulations for the delivery of polarizing compounds to macrophages. Her academic supervisors are Profs. Twan Lammers from RWTH Aachen University and Gert Storm for the University of Utrecht.

Christoph Gerber

www.nccr-nano.org/nccr/contact/
en.wikipedia.org/wiki/Christoph_Gerber

Christoph Gerber is a titular professor at the Department of Physics, University of Basel, Switzerland. He was a founding member and Director for Scientific Communication of the NCCR (National Center of Competence in Research Nanoscale Science). He was formerly a Research Staff Member in Nanoscale Science at the IBM Research Laboratory in Rueschlikon, Switzerland, and has served as a project leader in various programs of the Swiss National Science Foundation and in the European Framework 6. For the past 35 years, his research has been focused on Nanoscale Science. He is a pioneer in Scanning Probe Microscopy, and he made major contributions to the invention of the Scanning Tunneling Microscope and the Atomic Force Microscope (AFM), he is also a co-inventor of Biochemical sensors based on AFM Technology. He is the author and co-author of more than 165 scientific papers that have appeared in peer-reviewed journals and has been cited more than 28'000 times in cross-disciplinary fields. He belongs to the one hundred worldwide most cited researchers in Physical Sciences. He has given numerous plenary and invited talks at international conferences. His work has been recognized with multiple honorary degrees and various awards and appeared in numerous articles in daily press and TV coverage. He is a Fellow of the American Physical Society, a Fellow of the World Technology Network and a Fellow of the IOP Institute of physics UK. He serves in the advisory board of several nano institutes and has chaired and co-chaired various international conferences. His IP portfolio contains 37 patents and patent publications. His private interest range from literature (scientific and a good novel) to art and sports (he is a passionate skier and plays an acceptable round of golf).

Nicolas Gouze

Nicolas Gouze has an engineer's degree in optronics from the University Paris XI and studied Innovation Management at the University of Valenciennes (France).
Nicolas possess more than 10 years of professional experience as a senior consultant in the Department for Future Technologies and Europe at VDI/VDE-IT. He has a strong track record in the management of projects on the European level and in depth knowledge in the field of innovation management, technology transfer and exploitation of research results. He has involved in the management of the ETP Nanomedicine since 2010 and Head of its Secretariat between 2012 and 2015. In that role Nicolas has been supporting the platform's and the international Executive Board as well as formulating research and policy recommendations for the European Commission.Nicolas has also proven project management skills as coordinator of the ENATRANS project, a CSA to provide the nanomedicine community with a series of instruments and services to overcome the hurdles encountered during the translation process of innovative medical technologies from the lab to the bedside. He furthermore coordinated the European project NANOMED2020. Under his leadership concrete recommendations to push forward the field of nanomedicine under HORIZON 2020 were delivered to the European Commission. In addition to his management skills, as innovation officer Nicolas has proven his ability to function as a facilitator for technology transfer, EU funding and innovation issues within the Innovation Relay Centre (IRC) and Enterprise Europe Network (EEN). He supported transnational Technology Transfer Agreements and shown his ability to translate strategic thinking into operational action plans.

Jennifer Grossman

Ph.D.
www.linkedin.com/in/grossmanjennifer/

Dr. Jennifer Grossman is a Senior Scientist at the National Cancer Institute (NCI)'s Nanotechnology Characterization Laboratory (NCL), a collaboration among NCI, the National Institute of Science and Technology (NIST), and the Food and Drug Administration (FDA). The NCL is an interdisciplinary team of scientists with expertise in complex drug and dosage form R&D. NCL formulates and tests nanotech drugs and diagnostics in collaboration with academia, industry, and government. Dr. Grossman leads NCL's alliance, project, and data management. She has established and managed productive collaborations within NCI, FDA, NIST and a network of over 100 drug development labs in industry and academia. She analyzes preclinical data on nanomaterial cancer therapeutics and has contributed to development of analytical/bioanalytical and physicochemical characterization methods linked to *in vivo* drug performance. Dr. Grossman areas of expertise include nuclear magnetic resonance (NMR) of proteins and nanoparticles, biophysical modeling of nanoparticle structures and interactions, and regulatory approaches to non-biological complex drugs. Dr. Grossman has experience in a variety of issues related to drug discovery, development and regulation and is a member of several working groups related to nanobioinformatics, nanomedicine, and other nanotechnology issues.

Olivier T. Guenat

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Olivier T. Guenat is the Head of the Organs-on-Chip Technologies Group at the ARTORG Center at the University of Bern in Switzerland. He is associated with the Pulmonary Medicine and the Thoracic Surgery Divisions of the University Hospital of Bern. His research focuses on the development of organs-on-chip, in particular lung-on-chips that mimic the healthy and diseased in-vivo cellular microenvironments of the lung. Prior to his position at the University of Bern, he held a position at the Swiss Center for Electronics and Microelectronics (CSEM), at the Ecole Polytechnique de Montréal (QC, Canada), before which he performed a post-doc at Harvard Medical School in Boston and at the University of Neuchâtel in Switzerland. He is the founder of AlveoliX, a biotech start-up that aims at bringing organs-on-chip on the market, for which he recently received the Ypsomed and the Venturekick Awards.

Heinrich Haas

Vice President RNA Formulation & Drug Delivery, BioNTech RNA Pharmaceuticals GmbH, An der Goldgrube 12, 55131 Mainz, Germany

Heinrich Haas has more than 20 years of experience in academic research and industrial pharmaceutical development. After he received his Ph.D. in physical chemistry, Dr. Haas researched lipid membranes and organized biomolecular systems. His professional focus is on colloidal/nanoparticulate formulations for targeted drug delivery with therapeutic and diagnostic applications. After joining BioNTech RNA Pharmaceuticals GmbH in 2010, he helped build the formulation development and analytics unit, which develops formulations for delivery of RNA and small molecules. Dr. Haas is also the Deputy Head of IMP manufacturing at BioNTech RNA Pharmaceuticals GmbH.

Gregor Haefliger

Deputy-Director at the State Secretariat of Education, Research and Innovation SEFRI (Federal Government). Background: Studies in Philosophy and Mathematics (Master [1985]; PhD [1989]). Until 1993 teacher/lecturer at the University of Fribourg. From 1992 to 1998 his research-focus was in Science Studies (applied research, bibliometric analysis, evaluation). Since 1999 he is working at the Ministery as scientific counsellor and in different positions in the R&D-Management. At the moment he is Head of the division National Research and Innovation at the SEFRI. In this position his main responsibilities include science policy and governance of Swiss funding agencies (Swiss National Science Foundation; Funding-Commission of Technology and Innovation), Federal research institutions/national research infrastructures as well as special federal research programs and initiatives.

Stefan Halbherr

Ph.D.
Manager Research and Development
InnoMedica
Studied Biochemistry at the University of Bern/Switzerland. At the University Institute for Immunology in the Insel hospital in Bern, he investigated disease-specific antibody signatures in Hemophilia patients using Designed Ankyrin Repeat Protein (DARPin) technology. During his PhD, he developed genetically engineered RNA vectors for vaccination of poultry against avian influenza A (e.g. H5N1). During his doctoral studies already, he joined in 2013 the biomedical research team of InnoMedica and contributed to the initiation of the lead project "Talidox", a novel glycan-targeted liposomal formulation of doxorubicin. In his role as Manager Research and Development he brought the research concepts of the acquired Yamazaki DDS, Ltd. to a marketable product, introducing many innovations in the processes of industry-scale liposome assembly, drug loading, and especially addition of linkers and surface-glycan ligands. At the same time, he was involved in the creation of the SwissMedic approved liposome manufacturing facility of InnoMedica in Marly/Switzerland. With his research and development team, Stefan Halbherr is leading InnoMedica to create and clinically translate a new type of glycan-targeted drug delivery platform suited for a number of key medical applications like chemotherapeutic treatment of cancer, management of bacterial infections, and control of autoinflammation.

Jens Hasskarl

Senior Global Clinical Leader, Cell & Gene Therapies Unit, Novartis Pharma AG, Switzerland
Jens Hasskarl, MD, obtained his Doctorate in Medicine at the German Cancer Research Center and the University of Heidelberg, Germany. He completed his training at the University Hospital Freiburg as board certified hematologist and oncologist and subsequently became faculty. From 2000 until 2003 he accomplished a post-doctoral fellowship in the field of molecular carcinogenesis at Harvard Medical School, Boston, USA. He is continuing his work on genetic instability. Dr. Hasskarl subsequently shifted focus to clinical research and fined Novartis Oncology in 2009 in clinical development. He joined the CTL019 team in 2014 to develop CD19-directed CAR therapies in lymphomas.

Stefan W. Hell

Stefan W. Hell is a director at the Max Planck Institute for Biophysical Chemistry in Göttingen, where he leads the Department of NanoBiophotonics. He is an honorary professor of experimental physics at the University of Göttingen and adjunct professor of physics at the University of Heidelberg. Since 2003 he also led the Optical Nanoscopy division at the German Cancer Research Center (DKFZ) in Heidelberg.
Stefan W. Hell received his diploma (1987) and doctorate (1990) in physics from the University of Heidelberg. From 1991 to 1993 he worked at the European Molecular Biology Laboratory, also in Heidelberg, and followed with stays as a senior researcher at the University of Turku, Finland, between 1993 and 1996, and as a visiting scientist at the University of Oxford, England, in 1994. In 1997 he was appointed to the MPI for Biophysical Chemistry in Göttingen as a group leader and was promoted in 2002 to director.
Stefan W. Hell is credited with having conceived, validated and applied the first viable concept for overcoming Abbe's diffraction-limited resolution barrier in a light-focusing fluorescence microscope. For this accomplishment he has received several awards: most recently he shared the 2014 Kavli Prize in Nanoscience and the Nobel Prize in Chemistry.

Clemens Helmbrecht

Head of Research and Development
Particle Metrix GmbH, Diessen
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Neudiessener Str. 6, 86911 Diessen
Fax: +49 (0) 8807 94355
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2013–:

- Head of "Research and Development" at Particle Metrix GmbH Nanoparticle Tracking Analysis (NTA) for measurement of particle size, zeta potential, concentration and fluorescence combined with multivariate statistics

2009–2012:

- Groupleader of "Laserbased Separation Techniques" at Technische Universität München
- Development of techniques for separation and characterization of nanoparticle suspensions
- Teaching activity: Analytical Chemistry, Analytical Chemistry Lab, Advanced Statistical Methods for Analytical Chemistry

2009:

- PhD in Chemistry (Technische Universität München, Institute for Hydrochemistry),
- Research on photophoresis (migration induced by the forces of light),
- Photophoretic velocimetry for nanoparticle characterization, development of a photophoretic separator for nanoparticle suspensions

Paul Herrling

Chairman of the Board of the Novartis Institute for Tropical Diseases.

Paul Herrling is Chairman of the Board of the Novartis Institute for Tropical Diseases, a long-term endeavour to advance medical research in tropical infectious diseases, which historically have received little drug-research funding. He is also a consultant to Novartis Pharma AG since January 2012 after his of ficial retirement.

Prior to his current position, he was Head of Novartis Institutes for Developing World Medical Research (NIDWMR) in Novartis Institutes for Biomedical Research (NIBR), a position he assumed in February 2010.

Before that Paul Herrling was Head of Corporate Research in Novartis supervising 4 institutes, Head of Global Research of Novartis Pharma and a member of the Pharma Executive Committee (PEC). In this capacity, he directed the integration of the research organizations of Sandoz and Ciba-Geigy following their merger in 1996 to form Novartis.

Paul Herrling joined Sandoz Pharma in 1975 and held various positions in research at both Sandoz in Basel, Switzerland and Wander in Bern, Switzerland. In 1985, he became Head of the Sandoz Research Institute in Bern and Head of the Preclinical CNS Research Department at Sandoz Pharma in Basel. In 1992, he was made Head of Preclinical Research Basel for Sandoz Pharma and, in 1994, Head of Pharma Corporate Research.

He is also a Professor of Drug Discovery Science at the University of Basel, Switzerland. In addition to scientific editing activities, he serves on several boards, most notably, University Council of the University of Basel , Board of Trustees of the Foundation for NIH and the Scientific Advisory Committee of the Drugs for Neglected Diseases Initiative (DNDi). Since January 2008 he is also the Vice-President of the ETH Board (Swiss Federal Institutes of Technology).

Paul Herrling obtained his Ph.D. in 1975 from the University of Zurich, Switzerland and was a post-doctorate fellow at the Neuropsychiatric Institute at the University of California, Los Angeles (UCLA) in the USA.

Inge Herrmann

Inge Herrmann (1985) studied Chemical and Bioengineering at the ETH Zurich with a stay at the TU Delft in 2007. During her PhD studies in the Stark lab at the ETH Zurich, she pioneered a nanomagnet-based blood purification technology in collaboration with the University Hospital Zurich. After her PhD in biomedical engineering (2010) and postgraduate studies in clinical trials management, she held various positions at the Centre of Clinical Research at the University Hospital in Zurich, the University of Illinois and in the Stevens lab at the Imperial College London. Since 2015, she heads the Particles 3D group at the Swiss Federal Laboratories for Materials Science and Technology (Empa). Her research interests include the design of particle-based approaches for diagnostics and therapy, the development of point-of-care devices and translational nanomedicine.

www.empa.ch/web/s403/particles-3d

Gesine Heuck

Gesine Heuck, (Ph.D. Pharm.D. eq) is a field application scientist at Precision NanoSystems Inc with 10 years of experience in the development and translation of nanomedicines. Gesine completed her Pharmacy degree at the Universities of Kiel and Regensburg, Germany, and the University of Michigan, Ann Arbor, USA. During her Ph.D. at the University of Geneva, Switzerland, she worked on the nanoencapsulation of the commercial drug Hexvix® for photodynamic therapy and fluorescence photodetection, and investigated the heme pathway in gram-negative bacteria in a joint project with the Institut Pasteur, Paris.

Simone E. Hieber

Dr. Simone E. Hieber is a research group leader at the Biomaterials Science Center, University of Basel. She was educated in various disciplines in three countries. She gained a diploma in engineering cybernetics from the University of Stuttgart, Stuttgart, Germany, a master's degree in mathematics from the Michigan Technological University, Houghton, MI, USA, and a PhD in computer science from ETH Zurich, Zurich, Switzerland. During her education she focused on projects in computational modeling and simulations of various systems, ranging from nonlinear turbo charger over metabolic networks and stochastic sprays to large-scale simulations of a swimming fish. After graduation she coordinated the Swiss-wide PhD training network of the Swiss Institute of Bioinformatics. Moreover, she has been supervising the PhD studies in biomedical engineering at the University of Basel.

Since 2012 she has been leading the research group Computational Analysis of Tissues in Health and Disease at the Biomaterial Science Center. The main focus of research includes investigating characteristic parameters of soft and hard tissues based on high-resolution X-ray images. Disease currently considered are arthrosclerosis, epilepsy, stroke and cancer.

She has published more than 30 peer reviewed scientific articles as well as several reviews and book contributions. She received several academic awards including one for the best oral presentation at Bernd Spiessl Symposium in Basel, Switzerland. Dr. Simone E. Hieber is married and has one daughter.

Hans Hellmuth Hirsch

MD, MSc, Professor, FMH Infectious Diseases, FMH Internal Medicine, FAMH Medical Microbiology, Department Biomedicine, University of Basel, and Infectious Diseases & Hospital Epidemiology, University Hospital Basel, Switzerland.

Hans H. Hirsch studied medicine in Freiburg (Germany) and biochemistry at Oregon State University (OR, USA). He then specialized in internal medicine, infectious diseases and medical microbiology at the University of Basel and the University Hospital Basel. In 2004, the Medical Faculty of the University of Basel appointed him Professor. Currently, Professor Hans H. Hirsch is director of the Division of Infection Diagnostics and leads the research group Transplantation & Clinical Virology. He holds a senior clinical appointment as consultant in infectious diseases at the University Hospital Basel. He has a special focus on HIV-infected patients and

patients with other viral diseases, including after transplantation. HH Hirsch has published more than 200 peer-reviewed papers and has been invited to relevant international conferences in virology and infectious diseases, and contributed to international guidelines in transplantation and infectious diseases such as ASTIDCOP, CIBMT, ECIL-4, ECIL-6, ESGICH, and KDIGO.

AWARDS AND HONOURS:

1981: Exchange Fellowship State Baden-Wurttemberg, Germany - Oregon, USA

Heinrich Hofmann

Professor for Powder Technology, Ecole Polytechnique Fédérale Lausanne, Institute of Material Science

Hofmann Heinrich, Prof. Dr.-Ing. Studied first foundry engineering at the Applied University of Duisburg followed by a study of Material Science and Engineering at the Technical University of Berlin. 1983 he got his PhD in Material Science with a thesis prepared at the Powder Metallurgy Laboratory at the Max Planck Institute in Stuttgart. Between 1983 and 1985, he was senior scientist at the same Max Planck Institute working on novel hard metals and composites. In 1985 he joined the R&D center of Alusuisse-Lonza Services AG, at Neuhausen-am-Rheinfall. He was first involved in the development of new alumina powders for ceramic application. In a second part, he developed a new titania stabilized zirconia powder as well as a pilot plant for a first fabrication of such powders in industrial quantities. In parallel, he also developed carbothermic processes for the fabrication of silicon nitride powders. In 1993 he joined the Swiss Federal Institute of Technology as Professor and Director of the Powder Technology Laboratory at the Department of Materials Science and Engineering. His research area includes the synthesis of nanostructured materials based on nanoparticles and the modification of surfaces with nanoparticles using colloidal methods. The applications of such materials are in the medical and biological field (drug delivery, hyperthermia, cell separation, biosensors), electronics and sensors as well as coating of medical devices, turbine blades and paper. He is member of several professional organizations as well as of the "Europäische Akademie für Technikfolgen Abschätzung" (technology assessment) and Member of the Swiss Federal working group "Nanoregulation". From 2006 to 2011 he was director of the research unit "Surface, Coating and Particle Engineering" SPERU of the Competence Centre of Material Science. Since 2010 he is member of the Steering commmitte of the National Science Program 64 "Opportunities and risks of nanotechnology". He is member of various scientific advisory boards in Japan, China and Thailand, all related to nanomaterials and nanotechnology.. Since 2008 he is a cofounder of a company developing nanocomposites for cancer treatments (ANTIA Therapeutics). His publication list comprises over 90 Publications in reviewed journals, 33 publications in proceedings, co-author of 4 books and co-editor of 2 MRS proceedings and he is co-inventor of 15 patents or patent applications. Additionally he has given more than 20 invited and key note lectures on particles synthesis, modification and nanoparticles in biomedical applications in EU, US Australia and Asia. He supervised 25 PhD students in the period 1994 – 2011.

Main topics of his research today are synthesis and functionalisation of superparamagnetic iron oxide nanoparticles for application like molecular imaging, stem cell tracking, protein separation and hyperthermia. Additionally he is working in the field of colloidal behaviour of nanoparticles in complex environment including transport phenomena. Development of nanocomposites, mostly based on polymers is an other topic where he is using the core competencies of his lab: colloidal chemistry.

Patrick Hunziker

Patrick Hunziker has studied Medicine the University of Zurich, Switzerland. He received a doctoral decree based on thesis work in experimental immunology from the University of Zurich and did further research in experimental haematology at University Hospital in Zurich, Switzerland. He earned specialist degrees in Internal Medicine, Cardiology and Intensive Care Medicine. As a fellow the Massachusetts General Hospital, Harvard Medical School, worked on cardiac imaging in a joint project with the Massachusetts Institute of Technology, Cambridge.

His professional activities in Europe, the U.S., Africa and China gave him a broad insight into the needs for the medicine of the future in a variety of settings. Hunziker became involved in medical applications of Nanoscience in the late nineties and has been the pioneer physician in Nanomedicine in Switzerland since then. With improved prevention, diagnosis and cure of cardiovascular disease as his main research topic, he worked in the nanoscience fields of atomic force microscopy, nanoptics, micro/nanofluidics, nanomechanical sensors and polymer nanocarriers for targeting.

He is the co-founder and president of the European Society of Nanomedicine, co-founder of the European Foundation for Clinical Nanomedicine and co-initiator of the European Conference for Clinical Nanomedicine and is clinically active as deputy head of the Clinic for Intensive Care Medicine at the University Hospital Basel, Switzerland. In November 2008 Patrick Hunziker became professor for Cardiology and Intensive Care Medicine at the University of Basel.

Jörg Huwyler

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Full professor and head of the division Pharmaceutical Technology, Department of Pharmaceutical Sciences, University Basel

PROFESSIONAL TRAINING AND EXPERIENCE

09/2010: Appointment as full professor of Pharmaceutical Technology, University of Basel, Switzerland, Pharmaceutical Sciences, http://pharma.unibas.ch
Head of the Division of Pharmaceutical Technology: Drug Targeting, Drug Delivery

2006 - 2010: Professorship at the Institute of Pharmaceutical Technology, University of Applied Sciences NorthWestern Switzerland ('Fachhochschule Nordwestschweiz, Hochschule für Life Sciences') Drug Targeting, Drug Delivery

1999 – 2006: Permanent position Pharmaceuticals: F.Hoffmann-LaRoche Ltd., Pharmaceuticals Division, Basel, Switzerland.
Scientific specialist for pharmacokinetics (preclinical research)

1998 – 1998: Project leader and deputy group leader, University Hospital Basel, in the research group of Prof. Dr. Alex Eberle
Research interest: Drug targeting

1997 – 1998: Team leader: Department of Research, University Hospital, Basel
Research interest: Drug transport across the blood-brain barrier

1996 – 1997: Post-graduate researcher: UCLA School of Medicine, Los Angeles, in the research group of Prof. Dr. William Pardridge
Research interest: Vector-mediated delivery of drugs to the brain

1993 – 1995: Project leader: Department of Research, University Hospital, Basel, in the research group of Prof. Dr. Jürgen Drewe
Research interest: *In vitro* models of the blood-brain barrier

ACADEMIC AND PROFESSIONAL MILESTONES

2010: Appointment as full professor of Pharmaceutical Technology
2006: Professorship at the University of Applied Sciences (FHNW), Switzerland
2003: Habilitation at the University of Basel, Faculty of Pharmacy.
1992: Ph.D. Thesis: Biocenter of the University of Basel, under the guidance of Prof. Dr. Joseph Gut Research topic: Interactions between halothane-metabolism and the homeostasis of leukotrienes in the liver
1988: Diploma in Biochemistry, Biocenter of the University of Basel

AWARDS

2015: Nomination**:** Fellow of the Swiss Academy of Pharmaceutical Sciences.
2007: Pfizer Nephrology Research Award for the publication: Tuffin et al., 2005, J. Am. Soc. Nephrol. 16, 3295-3305.
2004: Amedis Award for the publication: Schnyder et al., 2004, Biochem. J. 377, 61-67.

PATENTS AND PUBLICATIONS

- 117 peer reviewed research articles between 1990 and 2016
- 12 reviews, reports and book articles
- 11 patents

Simon Ittig

Biozentrum, University of Basel
Klingelbergstr. 50-70
CH-4056 Basel
Phone: +41 61 267 2200
E-mail: simon.ittig@unibas.ch

After his bachelor studies in Biochemistry at the University of Bern, Switzerland, Simon completed his master studies in Biotechnolgy at the tri-national ESBS in Strasbourg, France. Simon then joined the group of Prof. Guy Cornelis at the Biozentrum in Basel for a PhD on the surface structures of Capnocytophaga canimorsus and their importance in pathogenesis. For his PhD, Simon was elected as fellow of the Werner Siemens Foundation Excellence PhD program at the Biozentrum of the University of Basel. In this work and further collaborations he focused on the structure – activity relation of lipopolysaccharide, one of the most pro-inflammatory bacterial compounds. In his following time as a PostDoc in the group of Prof. Arrieumerlou at the Biozentrum, he studied the interplay of bacterial effectors and the host defense. To this end, his task was to develop a tool allowing a proteome wide analysis of the impact of single bacterial effector proteins on host cells. To reach a synchronized and very short termed onset of the effects, he engineered diverse bacterial and even eukaryotic proteins in a way to be secreted by a Yersinia strain via its type 3 secretion system. Data generated during this time allowed him to team up with Profs Erich Nigg (Biozentrum, Uni Basel) and Gerhard Christofori (DBM, Basel) to exploit the potential of this technology for treatment of solid tumors. For his work, Simon has received the Novartis University of Basel Excellence Scholarship in 2014 and a research award by the Dr. Arnold U. und Susanne Huggenberger-Bischoff foundation. Simon is co-founder and CEO of a spin-off company, T3 Pharmaceuticals, which aims at developing this microbial therapy into the clinics.

Kewal Jain

Professor K. K. Jain is a neurologist/neurosurgeon by training. He received graduate training in both Switzerland as well as North America and has held academic positions in several other countries. He passed the specialist examinations in neurosurgery in USA, Canada and Australia, attaining Fellowships of the Royal College of Surgeons of Canada and the Royal Australasian College of Surgeons. After his retirement from neurosurgery, he started a second career in the biotechnology/biopharmaceuticals industry as a consultant and founded Jain PharmaBiotech. He became a Fellow of the Faculty of Pharmaceutical Medicine of the Royal Colleges of Physicians of UK. Although his main interests are research, writing and teaching in biotechnology, he is also a consultant in neurology. He is Associate Editor of MedLink Neurology (San Diego, California), an accredited continuing education program in neurology, which contains over 1100 regularly updated articles by 450 authors. He is responsible for writing and yearly updating of 144 articles that include neuropharmacology and application of biotechnology in neurology.
Prof. Jain's 447 publications include 26 books (21 as author + 5 as editor) and 50 special reports, which have covered important areas in neurosciences, biotechnology, cell/gene therapy and biopharmaceuticals. In 1970s, he invented a technique for sutureless microvascular anastomosis using lasers described in his "Handbook of Laser Neurosurgery" published by Charles C Thomas in 1984. His "Textbook of Gene Therapy" was translated into Chinese language in 2000. The "Textbook of Hyperbaric Medicine" (6th ed 2016), which contains several chapters on neurological disorders, has been a standard reference on the subject for over 25 years. Prof. Jain has edited "Drug Delivery to the Central Nervous System" (Springer 2010) and "Drug Delivery Systems" (Springer), the 2nd ed was released in 2014. His recent books include "Textbook of Personalized Medicine" 2nd ed, (Springer 2015), "Handbook of Biomarkers" (Springer 2010), Handbook of Neuroprotection (Springer 2011), Drug-induced Neurological Disorders, 3rd ed (Hogrefe 2011), "Handbook of Nanomedicine", 2nd ed (Springer 2012), "Application of Biotechnology in Neurology" (Springer 2013), and "Application of Biotechnology in Oncology" (Springer 2014). Chinese editions were also published of the Handbook of Nanomedicine and Handbook of Biomarkers. Textbook of Personalized Medicine has been translated into Japanese.
The main interest of Prof. Jain is development of personalized medicine and he wrote the first monograph on this topic in 1998, which has evolved into a textbook. He believes that the concept of personalized medicine is the best driver of translation of new technologies and integration into clinical medicine. Of all the new biotechnologies besides Omics, he considers nanobiotechnology to have the most potential for facilitating personalized medicine. He published the first paper about role of nanobiotechnology in personalized medicine more than a decade ago and has numerous publications and books on this topic since then.

Su Chul Jang

Su Chul Jang received his Ph.D. degree at Pohang University of Science and Technology, South Korea at 2014. His major is Life Science, especially extracellular vesicle's biology. He has been studied about extracellular vesicles, exosomes and exosome-mimetic nanovesicles for 10 years including his Ph.D degree. He has been first invented and described about exosome-mimetic nanovesicles as use of drug delivery vehicles. Since 2015, he has joined the Jan Lotvall's group in University of Gothenburg, Sweden and is working about extracellular vesicle's biology. During his research career, he has been published 17 peer-reviewed papers and 13 granted patents worldwide.

PUBLICATIONS

1. Noninvasive imaging of radiolabeled exosome-mimetic nanovesicle using 99mTc-HMPAO. Scientific Reports. 5:15636. 2015.
2. Gut microbe-derived extracellular vesicles induce insulin resistance and thereby impair glucose metabolism in skeletal muscles. Scientific Reports. 5:15878. 2015.
3. Small RNA deep sequencing discriminates subsets of extracellular vesicles released by melanoma cells - evidence of unique microRNA cargos. RNA Biology. 12(8): 810-823, 2015.
4. Large oncosomes contain distinct protein cargo and represent a separate functional class of tumor-derived extracellular vesicles. Oncotarget. 6(13): 11327-11341, 2015.
5. Bacterial Protoplast-Derived Nanovesicles as Vaccine Delivery System against Bacterial Infection. Nano Letters. 15(1): 266-274, 2015.

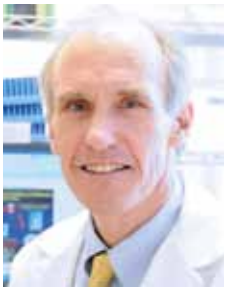
PATENTS

1. Microvesicles derived from cell protoplast, and use thereof. Japan. Patent number: 5814363. 2015
2. Microvesicles derived from cell protoplast, and use thereof. US. Patent number: 09149542. 2015
3. Method for treating and diagnosing cancer by using cell-derived microvesicles. China. Patent number: 103079592. 2015
4. Microvesicles derived from cell protoplast, and use thereof. US. Patent number: 9084830. 2015
5. Method for treating and diagnosing cancer by using cell-derived microvesicles. US. Patent number: 9066971. 2015

Olivier Jordan

Olivier Jordan is Senior Lecturer at the School of Pharmacy, University of Geneva. He graduated in engineering and received his PhD from EPFL working on the micro-encapsulation of insulin-secreting cells for the treatment of diabetes. In the team of Prof Aebischer at the Lausanne University Hospital, he focused on biomaterials engineering for cartilage and nerve prosthesis.

Moving to the School of Pharmacy at Geneva University, he developed projects in the field of novel delivery carriers for drugs, protein and therapeutic heat, based on in situ forming implants, nano- or microparticles. He has strong interests in the field of interventional oncology, e.g. using drug-eluting embolization materials to treat liver cancer through anti-angiogenic strategies, or delivering superparamagnetic iron oxide nanoparticles formulations for tumor thermotherapy. He is the author of 55 peer-reviewed publications and holder of 8 patents.



Carl H. June

Perelman School of Medicine at the University of Pennsylvania

Carl June is the Richard W. Vague Professor in Immunotherapy in the Department of Pathology and Laboratory Medicine. He is currently Director of the Center for Cellular Immunotherapies at the University of Pennsylvania, and is an Investigator of the Abramson Family Cancer Research Institute. He is a graduate of the Naval Academy in Annapolis, and Baylor College of Medicine in Houston, 1979. He had graduate training in Immunology and malaria with Dr. Paul-Henri Lambert at the World Health Organization, Geneva, Switzerland from 1978-79, and post-doctoral training in transplantation biology with E. Donnell Thomas and John Hansen at the Fred Hutchinson Cancer Research Center in Seattle from 1983 - 1986. He is board certified in Internal Medicine and Medical Oncology. He founded the Immune Cell Biology Program and was head of the Department of Immunology at the Naval Medical Research Institute from 1990 to 1995 before joining the faculty of the Perelman School of Medicine in 1999. He maintains a research laboratory that studies various mechanisms of lymphocyte activation that relate to immune tolerance and adoptive immunotherapy for cancer and chronic infection. He has published more than 350 manuscripts and is the recipient of numerous prizes and honors, including election to the Institute of Medicine in 2012, the William B Coley award, the Richard V Smalley Memorial Award from the Society for Immunotherapy of Cancer, the AACR-CRI Lloyd J. Old Award in Cancer Immunology, the Hamdan Award for Medical Research Excellence, the Karl Landsteiner Memorial Award from the AABB, and the Paul Ehrlich and Ludwig Darmstaedter Prize (shared with J. Allison). In 2014 he was elected to the American Academy of Arts and Sciences.

Nidhi Jyotsana

PhD
Department of Hematology, Hemostasis, Oncology and Stem cell transplantation
Hannover Medical School
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30625 Hannover
Germany

2010–2015: PhD Molecular Medicine, Hannover Biomedical Research School, Department of Hematology and Oncology, Hannover, Germany
2007–2009: Masters of Engineering Biotechnology, BITS-Pilani Rajasthan, India., CGPA: 9.45 (Out of 10)
2003–2007: Bachelor of Technology Biotechnology, College of Engineering Technology, Moradabad, India, Aggregate Marks: 83%
2002: Higher Secondary Certificate Examination Subjects: Physics, Chemistry, Biology, Mathematics, English, Board: Central Board of Secondary Education, Aggregate Marks: 67%

RESEARCH EXPERIENCE

October 2010–June 2015: **Doctoral Research** Department of Hematology, Hemostasis, Oncology and Stem cell transplantation Hannover Medical School, Germany
November 2009–August 2010: Project fellow (Junior) Indian Institute of Science, TIFR, Bangalore, India **Title:** The significance of the interaction of DNAJB6 with TSC1 and TSC2.
January 2009–October 2009: Masters Dissertation National Centre for Biological Sciences, TIFR, Bangalore, India **Title:** Monitoring of CREB mediated transcriptional activation downstream of 5HT2A receptor and differential expression of 5HT2A receptor in transgenic mice.

June 2008–July 2008: Summer Research fellowship program All India Institute of Medical Science, New Delhi, India **Title:** Molecular diagnosis of neurodegenerative disease and its susceptibility to Diabetic patients.

PUBLICATION LIST

1. Constitutive IRF8 expression inhibits AML by activation of repressed immune response signaling. Sharma A, Yun H, Jyotsana N, Chaturvedi A, Schwarzer A, Yung E, Lai CK, Kuchenbauer F, Argiropoulos B, Görlich K, Ganser A, Humphries RK, Heuser M. Leukemia. 2015 Jan;29(1):157-68. doi: 10.1038/leu.2014.162. Epub 2014 May 20.
2. Abstract: "Efficient siRNA delivery *in vivo* in a humanized mouse model, Keystone conference, Feb 2014. Nidhi Jyotsana, Anuhar Chaturvedi, Colin Walsh, Euan Ramsey, Florian Kuchenbauer, Amit Sharma, Christian Rathert, Robert Lindner, Fatih Noyan, Michaela Scherr, Keith Humphries, Pieter Cullis, Michael Heuser.
3. Impact of MLL5 expression on decitabine efficacy and DNA methylation in acute myeloid leukemia. Yun H, Damm F, Yap D, Schwarzer A, Chaturvedi A, Jyotsana N, Lübbert M, Bullinger L, Döhner K, Geffers R, Aparicio S, Humphries RK, Ganser A, Heuser M. Haematologica. 2014 Sep;99(9):1456-64. doi: 10.3324/haematol.2013.101386. Epub 2014 Jun 3.
4. Mutant IDH1 promotes leukemogenesis *in vivo* and can be specifically targeted in human AML. Chaturvedi A, Araujo Cruz MM, Jyotsana N, Sharma A, Yun H, Görlich K, Wichmann M, Schwarzer A, Preller M, Thol F, Meyer J, Haemmerle R, Struys EA, Jansen EE, Modlich U, Li Z, Sly LM, Geffers R, Lindner R, Manstein DJ, Lehmann U, Krauter J, Ganser A, Heuser M. Blood. 2013 Oct 17;122(16):2877-87. doi: 10.1182/blood-2013-03-491571. Epub 2013 Aug 16.
5. Identification and screening of lactic acid bacteria for the presence of naturally occurring plasmids. Kumar N, Jyotsana N, Paul S, Das A. International Journal of Integrative Biology. 2011 Apr 21; 11(2):85-89.

Keon W. Kang

Seoul National University, Republic of Korea

Dr. Keon W. Kang, a nuclear medicine physician, is Professor, Department of Nuclear Medicine, Seoul National University College of Medicine (2007-). He received M.D. degree from Seoul National University College of Medicine (1991). He was trained as an intern and a resident for Internal Medicine at Seoul National University Hospital (1991-1996). He received Ph.D. in Medical Science at Seoul National University College of Medicine (2001). He has worked as Chief, Department of Nuclear Medicine, National Cancer Center, Korea (2000-2007). He studied molecular imaging and researched "Preclinical efficacy of the c-Met Inhibitor from Pfizer Inc. by small-animal PET" with Pf. Sam Gambhir as a visiting scientist of Molecular Imaging Program at Stanford (2003-2004). His research areas are *in vivo* molecular imaging of cancer using PET & nanoparticles. He is studying (1) clinical trials using angiogenesis PET for cancer, (2) translational research using multifunctional nano-particles for optical/PET/MRI imaging, (3) *in vivo* cell trafficking of stem cells or immune cells using bioluminescence, fluorescence, radio-labeled technology, and (4) test biodistribution, pharmacokinetics, and efficacy of nano drug delivery systems using *in vivo* imaging.2014 he was elected to the American Academy of Arts and Sciences.

POSITIONS AND EMPLOYMENT

2003-2004: Visiting Scientist, Molecular Imaging Program at Stanford University, Stanford, CA
2007-2012: Associate Professor, Department of Nuclear Medicine, Seoul National University College of Medicine, Seoul, Korea
2007-: Executive Vice Director, Cancer Research Institute, Seoul National University, Seoul, Korea
2012-: Professor, Department of Nuclear Medicine, Seoul National University College of Medicine, Seoul, Korea
2014-: Chairman, Department of Nuclear Medicine, Seoul National University Hospital

OTHER EXPERIENCE AND PROFESSIONAL MEMBERSHIPS

2009-: Lifetime Member, the American Society for Nanomedicine
2011-: Editorial Board Member, Nanomedicine (Lond)
2012-2014: Member, Steering Committee & Program Committee, World Molecular Imaging Society
2013-: Member, Committee 3 (Medicine), International Commission on Radiological Protection
2015-: President Elect, the Korean Society for Nanomedicine

HONORS

1995: Distinguished Young Investigator's Award from Asia and Oceania, The Japan Society of Nuclear Medicine, Yokohama, Japan
1999: The Best Poster Award, The Korean Society of Nuclear Medicine, Korea
2000: Travel Award, The Organization for Human Brain Mapping, San Antonio, TX
2001: The Department of Health & Welfare Secretary's Award, Korea
2002: The Korean Federation of Science and Technology Societies Award, Korea

PEER-REVIEWED PUBLICATIONS

(Excerpt from 145 peer-reviewed publications)

- Chung T, Youn H, Yeom CJ, Kang KW, Chung JK. Glycosylation of Sodium/Iodide Symporter (NIS) Regulates Its Membrane Translocation and Radioiodine Uptake. PLoS One. 2015 Nov 23;10(11):e0142984.
- Lee SJ, Seo HJ, Kang KW, Jeong SY, Yi NJ, Lee JM, Chung JK, Edmund Kim E, Paeng JC, Cheon GJ, Lee DS. Clinical Performance of Whole-Body 18F-FDG PET/Dixon-VIBE, T1-Weighted, and T2-Weighted MRI Protocol in Colorectal Cancer. Clin Nucl Med. 2015 Aug;40(8):e392-8.
- Kim YH, Youn H, Na J, Hong KJ, Kang KW, Lee DS, Chung JK. Codon-optimized Human Sodium Iodide Symporter (opt-hNIS) as a Sensitive Reporter and Efficient Therapeutic Gene. Theranostics. 2015 Jan 1;5(1):86-96.
- Kong SH, Noh YW, Suh YS, Park HS, Lee HJ, Kang KW, Kim HC, Lim YT, Yang HK. Evaluation of the novel near-infrared fluorescence tracers pullulan polymer nanogel and indocyanine green/γ-glutamic acid complex for sentinel lymph node navigation surgery in large animal models. Gastric Cancer. 2015 Jan;18(1):55-64.
- Yoon HJ, Kang KW, Chun IK, Cho N, Im SA, Jeong S, Lee S, Jung KC, Lee YS, Jeong JM, Lee DS, Chung JK, Moon WK. Correlation of breast cancer subtypes, based on estrogen receptor, progesterone receptor, and HER2, with functional imaging parameters from 68Ga-RGD PET/CT and 18F-FDG PET/CT. Eur J Nucl Med Mol Imaging. 2014 Aug;41(8):1534-43.

Jens M. Kelm

Dr. Jens M. Kelm, Chief scientific officer and co-founder of InSphero AG, Zurich, Switzerland and co-founder of the Swiss competence center for "Tissue Engineering for Drug Development TEDD": 14 years' experience in 3D cell culture using a wide variety of cells and technologies, previously director at the Center for Applied Biotechnology and Molecular Medicine at the University of Zurich CABMM. Co-founder of the Swiss competence center for "Tissue Engineering for Drug Development TEDD" and steering committee member of the bi-annually 3D cell culture conference organized by the German Biotech association DECHEMA.

Fabian Kiessling

Since 2008 Professor Dr. Fabian Kiessling is leading the Institute of Experimental Molecular Imaging at the Helmholtz Institute for Biomedical Engineering at the RWTH-University in Aachen. Aim of his research is the development of novel diagnostic, theranostic and therapeutic probes as well of advanced imaging technologies and image analysis tools. In this context, the main focus of his research is on the investigation of angiogenesis-related processes including tumor development and spread. Fabian Kiessling studied Medicine and did his thesis at the University in Heidelberg. Until the end of 2002, he worked as resident in the Department of Radiology at the German Cancer Research Center (DKFZ) in Heidelberg. In 2003 he changed to the Department of Medical Physics in Radiology of the DKFZ as leader of the Molecular Imaging group. In parallel he did his clinical training at different Departments of the University of Heidelberg and received the board certification as Radiologist in 2007. Fabian Kiessling did his habilitation in experimental radiology in 2006. In 2008 he founded the invivoContrast GmbH together with Matthias Braeutigam.

Fabian Kiessling is author of more than 240 scientific publications and book chapters, edited two books and received many research awards, among those the „Emil Salzer Price for Cancer Research" and the "Richtzenhain Price".

Professor Kiessling is in the Editorial board of several scientific journals including Radiology, European Radiology, and the American Journal of Nuclear Medicine and Molecular Imaging.

He is founding member of the European Society for Functional and Molecular Imaging in Radiology (ESMOFIR), currently treasurer of the European Society for Molecular Imaging (ESMI), founding member of the ESMI working group "Image Guided Therapy and Drug Delivery (IGTDD)" and he was chairman of the "Molecular Imaging" subcommittee of the European Society for Radiology (ESR). Furthermore, he was program chair of the European Molecular Imaging Meeting (EMIM) in 2014 and he is program chair of the World Molecular Imaging Conference (WMIS) in 2016.

Moritz Kircher

Moritz Kircher studied medicine in Würzburg, Berlin and at Harvard Medical School, and graduated from the Humboldt University in Berlin, Germany (Charité – Universitätsmedizin Berlin) with highest honors. He subsequently moved to the United States as a DFG-funded fellow to pursue postdoctoral training at the Center for Molecular Imaging Research of Harvard Medical School/Massachusetts General Hospital (with Ralph Weissleder). This was followed by an Internship year in Surgery at the Cleveland Clinic and a four-year Residency in Diagnostic Radiology at Harvard Medical School/Beth Israel Deaconess Medical School (where he also served as chief resident). For his clinical sub-specialization, Dr. Kircher completed a fellowship in Magnetic Resonance Imaging at Stanford University. At the same time, he was also a postdoctoral fellow in the Molecular Imaging Program at Stanford (with Sam Gambhir). After completion of his training in 2010, Dr. Kircher joined the faculty of the Department of Radiology of Memorial Sloan Kettering Cancer Center (MSKCC) (chair: Hedvig Hricak) as a physician-scientist. His clinical expertise is in the interpretation of oncologic imaging studies of chest, abdomen and pelvis performed with MRI, CT and ultrasound with a sub-specialization in the imaging of the hepatobiliary system. As the head of the Kircher Lab, he focuses on the emerging imaging methods of "Surface-Enhanced Raman Scattering" (SERS) and Photoacoustic Imaging. The main goal of his NIH-funded lab is to develop new molecular imaging probes and techniques that will allow the *in vivo* detection of the macro- and microscopic extent of cancer with so far unparalleled precision. Such new technologies are needed for both earlier cancer detection and to help achieve more complete and precise tumor resections, especially in challenging tumor sites involving the brain, pancreas, prostate, soft tissues or reticuloendothelial system. Dr. Kircher is an Associate Member and Associate Attending at MSKCC, and a Member of its Center for Molecular Imaging & Nanotechnology (CMINT), Brain Tumor Center, MRI Committee, and Governing Committee of the Center for Integrated Metabolic Imaging (CIMI). He also serves as Vice Chair of the Radiology Research Committee. Dr. Kircher received multiple national and international awards for his research, including the Lawrie B. Morrison Research Award of Harvard Medical School/BIDMC, 2011 Dana Neuroscience Scholar Award, 2012 Young Investigator Award from the World Molecular Imaging Society, 2013 Walter-Friedrich-Award from the German Society of Radiology (DRG), 2014 Damon Runyon-Rachleff Innovation Award, and 2015 Pershing Square Sohn Prize. He is the founding chair of the "Molecular Imaging in Nanotechnology and Theranostics" (MINT) Interest Group of the World Molecular Imaging Society and co-founder of the recently incorporated startup company "RIO Imaging". In 2016, Dr. Kircher was elected into the Young Leaders Club of the International Society for Strategic Studies in Radiology (IS^3R).

Barbara Klajnert-Maculewicz

Barbara Klajnert-Maculewicz has completed her Ph.D in 2002 from the University of Lodz, Poland and postdoctoral studies from the McMaster University, Ontario, Canada. She is a full professor at the University of Lodz, Poland and an external scientific member at Leibniz IPF in Dresden, Germany. She is a co-author of 2 books and 10 chapters in monographs. She has published more than 100 papers in reputed journals (h-index 27). In the years 2009–2012 she was the Management Committee Chair of COST Action TD0802 "Dendrimers in biomedical applications" that gathered 24 countries. She has been awarded L'Oréal-UNESCO Fellowship for Women in Science. Her research interests are focused on the study of biological properties and biomedical applications of dendrimers.

Ingrid Klingmann

MD, PhD, FFPM, FBCPM
European Forum for Good Clinical Practice (EFGCP), PHARMAPLEX bvba

Dr. med. Ingrid Klingmann specialized in General Medicine, Clinical Pharmacology and Pharmaceutical Medicine.

After having joined pharmaceutical industry as medical advisor, she held senior management positions in different international contract research organisations and was responsible for operational, scientific, regulatory and business aspects of international clinical research projects from Phase I to Phase IV.

Since January 2003 she has her own pharmaceutical development and site management support consulting company. For 6 years she was also CEO of two investigative sites in London, UK, performing clinical trials in acute and chronic pain as well as musculo-skeletal diseases.

Dr. Klingmann is Chairman of the Board of the European Forum for Good Clinical Practice (EFGCP). On behalf of EFGCP she was Coordinator of the FP7-funded ICREL Project, Work Package Leader of the FP7-funded PatientPartner Project and at presently in her consultancy of the FP7-funded paediatric "LENA" Project. She is currently

President of PharmaTrain Federation, the successor organization of the IMI Project PharmaTrain where she was Coordinator, and Work Package Leader of the IMI Project EUPATI, responsible for developing the EUPATI Network, the EUPATI National Platforms and the Ethics Panel.
Dr. Klingmann chairs the clinical research module of the post-graduate Master in the Regulatory Affairs course at the University of Bonn, Germany, co-founded and is lecturer in the Diploma Course in Clinical Trial Practices at the University of Basel, Switzerland, and is lecturer in the ECPM course at University of Basel and in the Pharmed course at Université Libre de Bruxelles, Belgium.

Sven Klussmann

Sven Klussmann, Chief Scientific Officer of NOXXON Pharma AG, is an expert in the field of therapeutic oligonu-cleotides and co-founder of NOXXON. Starting at the Free University Berlin, he demonstrated how to identify L aptamers (so-called Spiegelmers) and developed this technology into a robust, therapeutic-lead generating process. From 2006 to 2008, Dr. Klussmann served NOXXON in a two-fold function as Chief Scientific and Chief Executive Officer. In this dual role he initiated NOXXON's transformation from a technology-focused drug dis-covery company into an innovation-driven drug development organization. As a result, three drug candidates have successfully passed Phase I and IIa clinical studies. Since 2008, he has served as Chief Scientific Officer. During the past years he has authored more than 80 scientific articles and over 30 patents and patent applica-tions on oligonucleotides and their uses. Furthermore, Dr. Klussmann was significantly involved in the early stage financing, and in attracting more than €100 million from private investors.

SELECTED PUBLICATIONS:

- Oberthür, D., Achenbach, J., Gabdulkhakov, A., Buchner, K., Maasch, C., Falke, S., Rehders, D., Klussmann, S., & Betzel, C. (2015) Crystal structure of a mirror-image L-RNA aptamer (Spiegelmer) in complex with the natural L-protein target CCL2. Nat. Comm. Vol. 6, 6923.
- Yatime, L., Maasch, C., Hoehlig, K., Klussmann, S., Andersen, G. R. & Vater, A. (2015) Structural basis for the targeting of complement anaphylatoxin C5a using a mixed L–RNA/L-DNA aptamer. Nat. Comm. Vol. 6, 6481.
- Hoehlig, K., Johnson, K. W., Pryazhnikov, E., Maasch, C., Clemens-Smith, A., Purschke, W. G., Vauléon, S., Buchner, K., Jarosch, F., Khiroug, L., Vater, A. & Klussmann, S. (2015) A novel CGRP-neutralizing Spiegelmer attenuates neurogenic plasma protein extravasation. Brit. J. Pharmacol., accepted.
- Hoehlig, K., Bethge, L. & Klussmann, S. (2015) Stereospecificity of Oligonucleotide Interactions Revisited: No Evidence for Heterochiral Hybridization and Ribo-zyme/DNAzyme Activity. PLoS One Vol. 10(2), e0115328.
- Steculorum, S. M., Collden, G., Coupe, B., Croizier, S., Lockie, S., Andrew, Z. B., Jarosch, F., Klussmann, S. & Bouret, S. G. (2015) Neonatal ghrelin programs development of hypothalamic feeding circuits. J. Clin. Invest. Vol. 125(2), 846-858.

Felix Kratz

Felix Kratz, Ph.D., Vice President of Drug Discovery, CytRx Corporation

Dr. Kratz is a medicinal chemist with more than 25 years of pertinent experience in the preclinical development of anticancer drugs, prodrugs and protein conjugation chemistry and profound knowledge of translational research from the laboratory to the clinic. He has successfully transferred aldoxorubicin, CytRx clinical phase 3 lead compound, from bench to bedside that is based on an innovative drug delivery platform exploiting circulating albumin as a tumor-specific drug carrier.
Felix Kratz graduated in Chemistry from the University of Heidelberg. Prior to joining CytRx Corporation he established the Division of Macromolecular Prodrugs at the Tumor Biology Center Freiburg. He serves on the Editorial Board for Bioconjugate Chemistry, Current Medicinal Chemistry, Current Bioactive Compounds, and Pharmacology & Pharmacy and has authored approximately 260 scientific publications, book articles and proceedings and is the inventor of over 20 patents and patent applications. Since 2014 as Vice President he heads the CytRx Drug Discovery Branch located in the Innovation Center Freiburg, Germany.

Silke Krol

Fondazione I.R.C.C.S. Istituto Neurologico Carlo Besta, Via Amadeo 42, 20133 Milan, and Istituto Tumori IRCCS "Giovanni Paolo II", Viale Orazio Flacco, 65,70124 Bari, Italy
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In 2016 Silke Krol started a new laboratory with focus on translation of nanomedicine for oncology into clinical practice. She will develop new detection systems for cancer as well as different types of nanoparticles as delivery system for chemotherapeutic drugs. She is still with the 2009 funded Laboratory of Nanomedicine@Fondazione I.R.C.C.S. Istituto Neurologico "Carlo Besta" in Milan, Italy. There her main research focus is on studying the transport mechanisms for differently functionalized gold nanoparticles across the blood brain barrier and how this is influenced by blood derived proteins. Moreover different novel metallic and non-metallic delivery systems for various other diseases (cardiovascular, prion disease, epilepsy, glioma, lymphomas, viral diseases) were designed for projects funded by Italian and European foundations. Her group develops multifunctional polymer/nanogold based drug or drug delivery systems as well as diagnostic tool for medical applications. Moreover, the multilayer-nanocoating is used for encapsulation and immune protection of living cells like e.g. pancreatic islets. She has several pending patents for possible future drugs for prion disease and cancer treatment, viral diseases, and cancer diagnostics.
She is still infrequently lecturing as contract professor for "Nanomedicine" at the University of Trieste and as guest lecturer for "Nanotoxicology" in Turin. In 2009 she worked as an expert consultant for the United Nations and serves as external expert reviewer for National projects in France, Italy, Georgia and Greece. Recently she was announced as project technical advisor for 3 EU-FP7 projects. She is member of the advisory board of "Euro-Nanotox-Letters" and the international advisory committee of the International scientific spring conference in Islamabad, Pakistan. She is member of the advisory board of the CLINAM-Foundation of the journal "Euro-Nanotox-Letters", associate editor of "Frontiers in Nanobiotechnology" and adjunct faculty member at the Pakistan Institute of engineering and applied science. Recently she became consultant and Member of General Scientific Advisory Board at Midatech Pharma PLC.

Twan Lammers

Twan Lammers, PhD, DSc
Dept. of Nanomedicine and Theranostics
Institute for Experimental Molecular Imaging
RWTH Aachen University Clinic
Pauwelsstrasse 30, 52074 Aachen, Germany
tlammers@ukaachen.de

TTwan Lammers obtained a DSc degree in Radiation Oncology from Heidelberg University in 2008 and a PhD degree in Pharmaceutics from Utrecht University in 2009. In the same year, he started the Nanomedicine and Theranostics group at the Institute for Experimental Molecular Imaging at RWTH Aachen University. In 2014, he was promoted to full professor. He has published over 100 research articles and reviews (>4500 citations; h-index 34), and has received several awards. He is associate editor for Europe for the Journal of Controlled Release, and serves on the editorial board member of several other journals. His primary research interests include drug targeting to tumors, image-guided drug delivery and tumor-targeted combination therapy.

Cornelia Lass-Flörl

Univ. Prof. Dr. med. Cornelia Lass-Flörl, Head Division of Hygiene and Medical Microbiology, Department of Hygiene, Microbiology and Social Medicine, Medical University of Innsbruck, Schöpfstraße 41, 6020 Innsbruck
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2000: Medical specialization for Hygiene and Microbiology
2001: Habilitation for "Hygiene, Microbiology and Preventive Medicine", Habilitation thesis: Epidemiology and prevention of nosocomial aspergillosis
2002: Associate professor at the Medical University Innsbruck, Department of Medical Microbiology and Hygiene

SPECIALIZATION:

Medical Mycology, Hospital Infection Control

SCIENTIFIC FOCUS OF INTEREST:

- Epidemiology, prevention and therapy of fungal infections Diagnostic of fungal infections
- Antifungal drug resistance
- Aspergillus and innate immunity
- Antifungal susceptibility testing
- Epidemiology and prevention of hospital infections

PRICES:

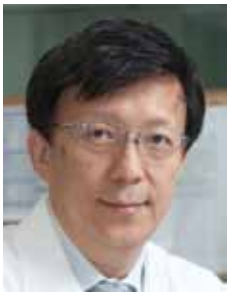
1999 Austrian Microbiology Award
2001 Meteka-Hospital Hygiene Award
2007 Science Award–German Speaking Society of Mycology

INTERNATIONAL CONGRESS ORGANISATION

2013 Executive Committee, European Congress of Clinical Microbiology and Infectious Disease, Berlin, Germany
2014 European Congress of Clinical Microbiology and Infectious Disease, Executive Committee, Barcelona, Spain
2015 International Committee Trends in Medical Mycology, Lissabon, Portugal

PUBLICATIONS (PEER-REVIEWED):

278 Originals, 22 Reviews, 5 Books, 8 Book-Chapters; Total Impact-factor: 1046,87.



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Dong Soo Lee is the Professor in the Department of Nuclear Medicine of Seoul National University (SNU) and SNU Hospital. He is also the Professor and Chairman of the Department of Molecular Medicine and Biopharmaceutical Sciences and is the Director of Bio-MAX/N-Bio of SNU. His major is Nuclear Medicine (Neurology and Cardiology) and Molecular Imaging, Human Brain Mapping and Radionanomedicine. He was the President of the Korean Society of Nuclear Medicine and the President of the Korean Society for Nanomedicine and the President of Korean Society of Human Brain Mapping. He acquired the M.D. from Seoul National University in 1982 and the Ph.D. in 1990. He has been serving in the Editorial Board of Journal of Nuclear Medicine, European Journal of Nuclear Medicine and Molecular Imaging, Journal of Nuclear Cardiology and was Special Associate Editor of Nanomedicine: Nanotechnology, Biology, and Medicine and is Editor-in-Chief of Nuclear Medicine and Molecular Imaging. He is also the Fellow of American College of Cardiology and Member of Korean Academy of Medical Sciences and Member of National Academy of Medicine of Korea.

Claus-Michael Lehr

Prof. Dr.
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Prof. Dr. Claus Michael Lehr is Professor at Saarland University as well as co-founder and head of the department "Drug Delivery" of the Helmholtz Institute for Pharmaceutical Research Saarland (HIPS). Additionally, Prof. Lehr is cofounder of Across Barriers GmbH and PharmBioTec GmbH. He studied pharmacy in Germany, PhD (1991) from Leiden University (The Netherlands), postdoctoral training at USC (Los Angeles, USA, 92), other appointments at Leiden University (93) and Marburg University (Germany, 94). The main focus of research of Prof. Lehr's team over the past 15 years has been on the one hand exploring the biological barriers, in particular the gastro-intestinal tract, the skin and the lungs, and on the other hand developing the appropriate carriers capable of crossing these epithelial barriers and deliver the active molecule to the target.
Prof. Lehr is (co)author of more than 300 papers with >10.000 citations (h-index = 56). He was the recipient of the CRS Young Investigator Award (2001), the APV Research Award 2006 for outstanding achievements in the Pharmaceutical Sciences and the biannual International Price 2008 of the Belgian Society for Pharmaceutical Sciences. In 2011, his team was awarded the German national research award on alternatives to animal testing. Prof. Lehr is Fellow of the American Association of Pharmaceutical Scientists (AAPS, 2010) and corresponding honorary member of the French Academy of Pharmaceutical Sciences (2012). He serves on different national and international scientific and editorial committees and is co-editor of the European Journal of Pharmaceutics and Biopharma-

ceutics. He is regularly involved in the organization of international conferences. In particular, he has been the initiator of an international workshop and conference on "Biological Barriers" at Saarland University, taking place in 2016 for the 11th time with more than 200 participants. In 2015, the British magazine "The Medicine Maker" rated him as one of the top 100 most influencing drug researchers in the world.

Caroline Lemarchand

PhD
Preclinical and Pharmaceutical Development Director, Onxeo, France

Dr Caroline Lemarchand has over 15 years international professional experience in drug product development in both pharmaceutical & biotechnology industry. She demonstrated a strong ability to coordinate drug product development from proof of concept to the registration (FDA/EMA) having actively contributed to the registration of the two first products of the company Onxeo.
She has a broad expertise in pharmaceutical technology from solid drug delivery system to nanoparticles including the industrial development, scaling up, validation and transfer of manufacturing process.
Currently, she leads non-clinical and CMC development activities for oncology products at Onxeo. She previously served as pharmaceutical director, project director and project manager at Onxeo and technical project leader at Novartis. She graduated in Pharmacy and obtained her master degree in collaboration with Sanofi and her PhD in Pharmaceutical Sciences (nanotechnology) at Paris XI University. She is author of international publications and patents.

Didier Letourneur

INSERM U 1148, LVTS – X. Bichat Hospital, Paris, University Paris Diderot and Paris Nord, France
Didier LETOURNEUR, engineer, doctor in chemistry, is Research Director at CNRS. In 2002, he founded a research structure Inserm-University Paris 13, focused on the use of biomedical polymers for 3D structures and contrast agents for vascular imaging. Since 2005, he leads the team of Cardiovascular Bioengineering at Inserm (CHU X Bichat, University Paris Nord and Paris Diderot). He is now the Director of the Laboratory for Vascular Translational Science (LVTS–Inserm U1148 http://www.u1148.fr) with about 160 persons.
D Letourneur is actively involved in several national grants, in Health regional cluster Medicen, and since 2013 as European coordinator of NMP "NanoAthero" large scale project (16 partners, 10 countries - http://www.nanoathero.eu). He was also involved in several FP7 projects (Health 2007-2013 "FAD" Large scale coordinated by its Research Unit, Health 2010-2014 Prestige (WP2 coleader), and NMP 2009-2012 "Nanoantenna").
D Letourneur is the author of 139 international publications (H-index 31), inventor of 16 patents, and won several prizes "Coup d'Elan for Research" Foundation Bettencourt 2001, Diderot Innovation Award 2009 CNRS-University Paris 7, Cardiovascular Innovation Award 2011 from FRM (Medical Research Foundation), and OSEO/BPI emergence 2012 & Creation-Dev 2013 for start-up creation. In 2016, he found the start-up SILTISS for the development of innovative orthopedic implants. In 2016 he obtained the G Winter Award, the highest recognition from the European Society for Biomaterials. He has more than 100 invited lectures and seminars and is the co-organizer of numerous national and international conferences (India, Tunisia, Canada) and two Inserm training workshops for Regenerative Medicine (2009 and 2012). He serves from 2013 at AVIESAN-ITMO for Health technologies in the scientific council. He was vice-chairman for Regenerative Medicine at the European Technology Platform for Nanomedicine and is now General Secretaire. Since 2009, he is President of BIOMAT, French Society for Biomaterials.

Julianna Lisziewicz

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For the past 25 years, I haveDr Lisziewicz led research teams that develop immunotherapy products that enable human immune systems to conquer some of the most deadly diseases, including HIV and cancer. I She started at NCI as the head of the Antiviral Unit in Robert Gallo's laboratory, where she pioneered gene and antisense therapies for HIV/AIDS. In 1995, Dr Lisziewicz co-founded and then directed the Research Institute for Genetic and Human Therapy, Inc. (RIGHT), a non-profit corporation, at which we investigated how the immune system controls HIV and conducted primate and human trials. Her findings regarding immune control of HIV led to our development of DermaVir HIV immunotherapy, a DNA-based synthetic nanomedicine applied topically to target Langerhans cells. Her team showed in a SIV-infected macaque model that the DermaVir immunotherapy could control virus replication. Then, she founded Genetic Immunity Inc., a for profit company, for the clinical development and commercialization of DermaVir. Several clinical trials, one in collaboration with the NIH, demonstrated the safety, immunogenicity and preliminary efficacy of DermaVir in HIV-infected people.
After Genetic Immunity was sold in 2013, Dr Lisziewicz co-founded eMMUNITY, Inc. in order to investigate how HLA genes regulate immune responses. eMMUNITY started by developing the first ever computational immuno-oncology technology to determine subjects' inherent T-cell responses to tumor antigens. Using this computational tool, her team found the correlation between HLA genotype and clinical outcome of immunotherapies. Computational immuno-oncology was developed in its entirety by her team at eMMUNITY. This core proprietary platform technology enables eMMUNITY to develop precision cancer vaccines and *in vitro* medical devices for companion diagnostic and cancer screening. The same computational technology has also supported the non-clinical development of PolyPEPI precision cancer vaccines by providing calculated predictions of the vaccines' safety and clinical response rate as well as providing a means of identifying likely responders to the vaccines. Dr Lisziewicz's aim, through eMMUNITY, to bring to market precision cancer vaccines that can start to make curing cancer a reality.

SELECTED PUBLICATIONS:

- Calarota SA, Weiner DB, Lori F, Lisziewicz J. Induction of HIV-specific memory T-cell responses by topical DermaVir vaccine. Vaccine. 2007; 25(16):3070-4
- Somogyi E, Xu J, Gudics A, Tóth J, Kovács AL, Lori F, Lisziewicz J. A plasmid DNA immunogen expressing fifteen protein antigens and complex virus-like particles (VLP+) mimicking naturally occurring HIV. Vaccine. 201; 29(4):744-53
- Lisziewicz J, Bakare N, Calarota SA, Bánhegyi D, Szlávik J, Ujhelyi E, Tőke ER, Molnár L, Lisziewicz Z, Autran B, Lori F. Single DermaVir immunization: dose-dependent expansion of precursor/memory T cells against all HIV antigens in HIV-1 infected individuals. PLoS One. 2012; 7(5):e35416
- Rodriguez B, Asmuth DM, Matining RM, Spritzler J, Jacobson JM, Mailliard RB, Li XD, Martinez AI, Tenorio AR, Lori F, Lisziewicz J, Yesmin S, Rinaldo CR, Pollard RB. Safety, Tolerability, and Immu-

nogenicity of Repeated Doses of DermaVir, a Candidate Therapeutic HIV Vaccine, in HIV-Infected Patients Receiving Combination Antiretroviral Therapy: Results of the ACTG 5176 Trial. J Acquir Immune Defic Syndr. 2013; 64(4):351-9
- Tőke ER, Lőrincz O, Csiszovszki Zs, Somogyi E, Felföldi G, Molnár L, Szipőcs R, Kolonics A, Malissen B, Lori F, Trocio J, Bakare N, Horkay F, Romani N, Tripp CH, Stoitzner P and Lisziewicz J. Exploitation of Langerhans cells for *in vivo* DNA vaccine delivery into the lymph nodes. Gene Ther. 2014; 21(6):566-74

Beat Löffler

Beat Löffler, MD h.c., MA humanities, was born in Basel. After a study visit in the USA, he studied Philosophy, Communication Sciences and Politics at the University of Basel and the Free University Berlin (FU) graduating with an MA magna cum laude. In 2014, he received an MD h.c. from the University of Basel.–1984 he co-founded an Agency for New Media. From 1988 to 1994, he was Head of the International Hightech Forum Basel organizing congresses on new technologies in mobility, energy, CFD and medical technology. 1994, he founded his own company "Concept Engineering" for translation of science-based visions, giving them a strategy and elaborated mission and implementing them. CE works further in the application and establishment of worldwide networks. He was for 6 years secretary general and coach of the Trinational BioValley Promotion Team, with the mission of establishing the trinational Upper-Rhine Biotechnology network. From 2003 to 2006, he worked for 4 years for NEC Hightech Performance Computing as Lead Consultant Life Sciences Business Development in Biology and Medicine. He co-founded the European Foundation for Clinical Nanomedicine in 2007. The aim of the foundation is the research and development of nanomedicine with regard to its use as an innovative technology, better medical care in the future and the establishing an international network in nanomedicine and related fields. Today is his ninth scientific summit on clinical nanomedicine under the name CLINAM 9/2016 (Clinical Nanomedicine) as a neutral platform CLINAM is the presently worldwide largest network for Clinical Nanomedicine and Targeted Medicine and related fields as meeting point of all stakeholders. He launched the European Journal of Nanomedicine of which he is the Managing Editor. He co-founded the European Society for Nanomedicine and the International Society for Nanomedicine. He is head of dissemination in presently two EU framework programme projects.

Marko Loparic

Dr. med. Marko Loparic, MD-PhD
Chief Medical Director
Nuomedis
Liestal, Switzerland

Marko studied medicine at the Medical Faculty in Zagreb, Croatia. Upon obtaining MD degree in Zagreb in 2005, he pursued MD-PhD studies in the Biozentrum, Basel at the Department of Structural Biology and Biophysics in the group of Prof. Ueli Aebi, where he developed nanomechanical approaches using atomic force microscopy (AFM) to study tissue engineered cartilage. He graduated in 2010. From 2011-2014 Marko was a project manager on two KTI project with the aim to develop automated and easy to use AFM for tissue diagnostics. During these KTI projects, prototype of the *in vitro* diagnostic device that's is based on AFM was designed and prototype was developed. The successes of the two KTI projects has led to founding a University of Basel spin off company called Nuomedis in 2014. Marko is one of the founding members of the company and currently acts as a Chief Medical Director within the Nuomedis.

Volker Mailänder

Univ.-Prof. Dr. med.
Volker Mailänder studied medicine at the University of Ulm supported by a stipend from the Studienstiftung des Deutschen Volkes and was in the graduate program "Molecular Biology". He worked in the Blume/Negrin lab in Stanford, California, on natural killer cells and was involved in patient care in the bone marrow transplantation unit. Afterwards he received training in internal medicine (haematology/oncology) in the Charité hospital in Berlin. After relocating to the Institute for Clinical Transfusion Medicine, University Clinic of Ulm, he worked on stem cell manipulation, the interaction of nanoparticles with cells and especially uptake mechanisms and the intracellular pathway. He was board certified in transfusion medicine. Further work focused on using polymeric nanoparticles for labelling or manipulation of stem cells and other cell types. Since 2008 he is leading a joint research group between the University Medical Clinic, III. Medical (hematology, oncology and pulmonology) and the MPI for Polymer Science in Mainz. He oversees the procedures of manipulating, freezing and storing stem and immune cells for patients care as the head of production and qualified person in the stem cell unit in the III. Medical Clinic. He is active in several cooperative projects (SFB1066 "Nanodimensional polymeric therapeutics for tumor therapy", BMBF projects) and is vice speaker of the center BiomaTiCS (Biomaterials, Tissues and Cells in Science) of the University Medical Center. Since 1.1.2016 he is W2 professor at the University Medicine Mainz and heads the Center for Translational Nanomedicine – CTN.

PUBLICATIONS

- Schöttler S, Becker G, Winzen S, Steinbach T, Mohr K, Landfester K, Mailänder V, Wurm FR.: Protein adsorption is required for stealth effect of poly(ethylene glycol)- and poly(phosphoester)-coated nanocarriers. Nat Nanotechnol. 2016 Apr;11(4):372-7
- Schöttler S, Klein K, Landfester K, Mailänder V.Protein source and choice of anticoagulant decisively affect nanoparticle protein corona and cellular uptake. Nanoscale. 2016, 8: p. 5526-5536
- Hofmann, D., et al., Drug delivery without nanoparticle uptake: delivery by a kiss-and-run mechanism on the cell membrane. Chemical Communications, 2014. 50(11): p. 1369-71.
- Paven, M., et al., Super liquid-repellent gas membranes for carbon dioxide capture and heart-lung machines. Nature Communications, 2013. 4.
- Lerch, S., et al., Polymeric nanoparticles of different sizes overcome the cell membrane barrier. European Journal of Pharmaceutics and Biopharmaceutics, 2013. 84(2): p. 265-274.

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EDUCATION:

1973: Doctorat es sciences Université Paul Sabatier, Toulouse
1973-1974: Post-doc University of East-Anglia, Norwich (UK)

POSITIONS:

- From «Attaché de Recherche» CNRS (1972) to «Directeur de Recherche Classe Exceptionnelle» CNRS (1997) and finally «Directeur de Recherche CNRS Emerite» (2007-)
- Vice-Director «Laboratoire Chimie de Coordination» Toulouse (France) 1998-2002

- Director of a French Polish joint International Laboratory (LEA 1999 – 2007)
- Editor in Chief «New Journal of Chemistry» (2005 - 2009)
- Co-Founder of the company Dendris 2009
- Co-founder of the company Biodendrimer International 2011-
- Director of the Department of " Dendrimers, design and applications", INANOTECH -MASCIR institute Rabat (Morocco) 2010-2012
- Supervisor of 62 PhD

FIELDS OF INTEREST:

Design and properties of dendrimers from biology and medicinal chemistry to material sciences, and catalysis.

AWARDS:

2004: Fellow Royal Society of Chemistry
2005: Member of the Akademie der Wissenschaften,(German Academy of Sciences),
2007: Nanqiang Lecture Award, China
2008: Grand Prix Emile Jungfleisch Académie des Sciences, France
2014: Doctor Honoris Causa, University of Lodz (Poland)

5 RECENT PUBLICATIONS:

- Dendrimer nanodrugs: the scaffold matters ! Caminade A.M., Fruchon,S. Turrin C. O. ,Poupot M.,Ouali,A., Maraval,A, Garzoni,M., Maly,M.,Furer,V., Kovalenko, V., Majoral,J.P., Pavan, G. Poupot R., Nature Commun. 2015, 6, 7722
- Dendrimer Space Exploration: An Assessment of Dendrimers/ Dendritic Scaffolding as Inhibitors of Protein-Protein Interactions, a Potential New Area of Pharmaceutical Development Mignani, S.; El Kazzouli, S.; Bousmina, M. ; Majoral, J.P., Chem. Rev.,2014, 114 (2), 1327-1342
- Dendrimer space concept for innovative nanomedicine: A futuristic vision for medicinal chemistry Mignani S.; El Kazzouli S.; Bousmina, M.; MajoralJ.P., Progress in Polymer Science 2013, 38, 993-1008
- Expand classical drug administration ways by emerging routes using dendrimer drug delivery systems: A concise overview. Mignani, S; El Kazzouli, S; Bousmina, M; Majoral, J.P. Adv. Drug Delivery. Rev, 2013, 64, 1316-1330
- Mannodendrimers prevent acute lung inflammation by inhibiting neutrophil recruitment. Blattes, E.; Vercellone, A.; Eutamene, H.; Turrin, C.O.; Theodorou, V.; Majoral,J.P.; Caminade,A.M.; Prandi, J.; Nigou, J.; Puzo, G. Proceedings of the National Academy of Sciences of the United States of America, Early Edition 2013, (May 13, 2013), 1-6

Frank Malinoski

Dr. Frank Malinoski is a native of the US where he received a BA degree from Colby College (1976) and trained in microbiology (PhD; Rutgers University, 1981) and medicine (MD; Albany Medical College, 1985) serving an internship and then clinical research activities in vaccines with the US Army in Texas and Maryland including the clinical development of vaccines against infectious disease threats of military importance and defense against biological weapons. Frank served as an inspector in both the Soviet Union (1990 &1991) and in Iraq with the United Nations after the first Gulf War in 1991 and retired from reserve military medical service in 2007.

In 1992, he joined Lederle-Praxis Biologicals (Rochester, NY, USA) as Director of Clinical Research conducting prelicensure clinical evaluation of vaccine candidates for bacterial and viral diseases of children, including candidates for RSV, meningococcus and pneumococcus and various combination vaccines. His activities contributed to the licensure of Prevenar (the first vaccine to achieve over $1B in sales in it's first year of launch), Meningitec, and Tetramune vaccines. In 1996 he moved to Nabi Biopharmaceuticals in Rockville, MD, USA as Senior Vice President of Clinical and Medical Affairs where he supervised the conduct of prelicensure and post-licensure activities in support of vaccines and immunotherapy. Research at Nabi included designing and implementing the Phase III program for a staphylococcus vaccine & post-marketing support in the area of transplantation, hematology, and immunology. In 1999 he joined Axis Genetics, directing the clinical and regulatory support for novel human vaccines delivered through plant expression of antigens. In 2000, he joined Wyeth as Vice President of Medical Affairs to direct the Medical Affairs activities for therapy areas in vaccines, transplantation, rheumatology, oncology, and infectious diseases. He then spent 1 year as the head of Business Development for the Wyeth Vaccines division before taking on consulting roles in 2004 to 2005 to include the roles of (1) Chief Medical Officer and Senior Vice President of Development for an immunotherapeutics company, Oxxon Therapeutics, Inc, (2) PATH program director to identify pneumococcal common protein vaccines for the developing world, and (3) senior consultant to Biosyn, a company developing microbicides for the developing world. From December 2005 to March 2009 he has had the positions of Vice President and then Senior Vice President of Medical and Scientific Affairs at MedImmune, LLC with responsibilities for post-approval support to licensed products, including policy activities with payers and government agencies, in support of product sales over $1B annually.

Frank consults with both public and private sector in the clinical and regulatory evaluation of vaccine and medicines of global need. He has directed or consulted on numerous vaccine & infectious disease projects in both developed and emerging market countries, including field vaccine efficacy trials in the US, Europe, the Republic of South Africa and The Gambia. He is a member of two investment consulting groups (Gerson Lehrman Group Leaders program and Tribeca Insights) and has taught courses at the Institute Merieux in Annecy, France Advanced Course in Vaccinology course for 5 years, has directed courses with PERI for industry education, has authored or co-authored multiple scientific and vaccine related publications, and is a member of several editorial boards. He served on the board of LigoCyte Pharmaceuticals, Inc., a biotech vaccine company in Bozeman, MT from March 08 to March 09 and is involved with the North Carolina Biotechnology Center in Wilmington, NC near his home in Leland, NC. Frank has served on an annual medical mission trip to needy parts of Nicaragua with Latin American Ministries and Journey's Crossing Church of Gaithersburg, MD.

Harald Mangge

Harald Mangge is a Medical Doctor and Professor at the Department of Laboratory Medicine of the Medical University of Graz, Austria. His research focuses on cardiovascular and metabolic diseases with emphasis on immune-mediated inflammation. Another focus is Nanomedicine, where an improved diagnosis and treatment of atherosclerotic vascular lesions is investigated (http://www.nanoathero.eu/). In the framework of the STYJOBS/EDECTA cohort project, Harald Mangge conducts a large prospective, observational study to improve the understanding of metabolic and cardiovascular risk in obesity (http://clinicaltrials.gov/ct2/show/NCT00482924). Recently, the activities are extended to oncologic research focusing new metabolic risk profiles of pancreatic ductal carcinomas. Further, Harald Mangge holds since October 1, 2014 the position of an interim Head of the Clinical Institute of Medical and Chemical Laboratory Diagnosis and the function of a Vice speaker of the Cardiovascular Research Field of the Medical University of Graz. He is also consultatnt for the BioTechMed Graz initiative, an interdisciplinary strategic joint project of the three large universities (Technical-, Medical-, and Comprehensive-University) at the location of Graz, Austria.

Mira Marcus-Kalish

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Dr. Mira Marcus-Kalish is currently the Director of International Research Collaborations at Tel Aviv University. Her main areas of interest are mathematical modelling, converging technologies and data mining.
Dr Kalish holds a Ph.D. in Operations Research from the Technion - Israeli Institute of Technology, where she developed one of the first computerized systems for electrocardiogram (ECG) diagnosis. She did her post-doctoral training at the Harvard University, the MBCRR (Molecular Biology Computer Research and Resource) laboratory and at the Dana Farber Cancer Institute. Her B.Sc. is in Statistics and Biology from the Hebrew University of Jerusalem.
Upon her return to Israel, she joined the Tel Aviv University Recanati Business School establishing the Medical Management Program focusing on Medical Informatics. Then joined the Weizmann Institute of Science working with Prof Ephraim Katzir, mainly on protein interactions, specificity and sensitivity. She moved with Prof Katzir back to Tel Aviv University, to the Biotechnology Department taking active part in NBIC, Converging Technologies and contributing to the recent EU-US Wtec-NBIC2 activities.
She was involved at the private business enterprise and served as the scientific advisor and later as the head of the Enterprise Marketing Department of IBM Israel.
Dr. Kalish took an active part in many of the EU framework projects, such as the Nano2Life Network of Excellence, SkinTreat, ReNaChip, EpoCan, NanoAthero and recently the Human Brain Project (HBP) flagship
Leading the Medical Informatics Sub-Project as deputy leader. Other recent EU projects are GLAM, ENATRANS and Brainomics.
Recent projects focused on personalized medicine, skin treatments, rehabilitation of the discrete sensory motor, learning function, cerebellar motor learning, protein- protein interactions, drug toxicity analysis, learning machine systems, data mining and medical informatics.

Marco Marradi

Dr. Marco Marradi got his Degree in Chemistry in 2001 at the University of Florence (Italy) working on the synthesis of nitrones by reaction of carbohydrates with hydroxylamines. After graduation, he was contracted by the pharmaceutical company NicOx S.r.l. to develop nitric oxide-donating drug-candidates.
Then, he started his doctoral studies and got the PhD in Chemistry Sciences in January 2005 at the University of Florence under the supervision of Prof. Goti. The PhD work was a continuation of the MSc thesis and was focussed on the manipulation of new chiral nitrones for stereoselective syntheses of biologically active natural products and their analogues. During his PhD he was teaching assistant in the academic course of Organic Chemistry IV hold by Prof. F. M. Cordero and spent four months at the University of Zaragoza in the group of Prof. P. Merino through a doctoral visiting grant of MIUR. He reinforced his expertise in asymmetric synthesis and organometallic chemistry.
In February 2005, he left to Germany for a post-doctoral position at the Institute of Organic and Biomolecular Chemistry of the Georg-August University (Göttingen) in the group of Prof. A. de Meijere. His research was there focussed on the synthesis of β-lactams and β-amino acids starting from bicyclopropylidene. He was also teaching assistant in the academic course "Reaction Mechanisms in Organic Chemistry" hold by Prof. U. Diederichsen.
Due to the will of enriching his background in organic chemistry with the modern challenges of nanotechnology, he moved to Spain where he joined the group of Prof. S. Penadés as a Marie Curie Fellow in the "GlycoGold" RTN funded by the FP6 during the years 2006 (IIQ-CSIC, Seville) and 2007 (CIC biomaGUNE, San Sebastián). He was trained in the preparation and characterization of water-dispersible and biocompatible gold nanoparticles functionalized with biomolecules as multivalent systems to study biological interactions.
In January 2008, he was contracted by CIBER-BBN as research associate in the Biofunctional Nanomaterials Unit at CIC biomaGUNE (Spain). His main research areas comprised the design and application of metallic nanoparticles (NPs) and the exploration of their potential in biomedical fields, including the development of microbicides/vaccines against HIV and other parasites and the use of Gd-based paramagnetic NPs for molecular imaging of glioma in mice. He got expertise in assembling NPs coated with biomolecules and other compounds (luminescent dyes, magnetic components) to obtain biofunctional inorganic-organic hybrid nanomaterials for nanomedicine and multimodal imaging.
In April 2014, he started to work at the interface of the Biosurfaces (group of Dr. S. Moya) and Molecular Imaging (group of Dr. J. Llop) Units at CIC biomaGUNE as project assistant, taking care of the management of the EU VIROMA project (Layer By Layer technology for the design of colloidal sensors based on virus-modified particles) and developing dual labeled nanoparticles for SPECT discrimination of NPs fate *in vivo*.
Finally, at the end of October 2014 he joined the Biomaterials Unit (group of Dr. I. Loinaz) of IK4-CIDETEC (San Sebastián, Spain) where he currently works as senior researcher in the design, development and application of single chain polymer nanoparticles in nanomedicine. He has directed 3 PhD Thesis and supervised 4 PhD students. During the academic years 2010-2014, he has been teaching in the Master in Nanoscience of the Basque Country University (UPV/EHU). He was the Spanish Delegate in the Management Committee of the European COST Action CM1102 "Multivalent Glycosystems for Nanoscience" (MC Chair: B. Turnbull).
He is author of 43 publications in international refereed journals, being first or second author in >70% of them, and 4 book chapters. His h-index is 19. He has participated in more than 10 competitive projects and he is currently in charge of the EU project PneumoNP and two national non-competitive projects. He has given more than 20 talks, either oral communications to conferences or invited seminars.

Yoko Matsumoto

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B.S.:	Chemistry (Kumamoto University, Japan 1979)
Ph.D.:	Pharmaceutical Sciences (Kyushu University, Japan 1986)
1988-1995:	Assistant Professor Kumamoto Institute of Technology, Japan
1996-2001:	Associate Professor Kumamoto Institute of Technology, Japan
2002-Present:	Professor Sojo University (Former name: Kumamoto Institute of Technology)
2009-Present:	Director Japan Nanomedicine Society
2012-Present:	Councilor Japanese Association for Molecular Target Therapy of Cancer
2013-Present:	Chairman Department of Life Science, Graduate School, Sojo University

- Award for Encouragement of the Society for Synthetic Organic Chemistry, 1994
- Award for Excellent Original Paper, Japanese Society for Pediatric Surgeons, 2005
- Outstanding Female Researcher Award (Society of Chemical Engineering), 2013

Field of Expertise: medicinal chemistry, medical engineering

Scott E. McNeil

Dr. McNeil serves as the Director of the Nanotechnology Characterization Laboratory (NCL) for Leidos Biomedical Research at the Frederick National Laboratory for Cancer Research, where he coordinates preclinical characterization of nanotech cancer therapeutics and diagnostics. At the NCL, Dr. McNeil leads a team of scientists responsible for testing candidate nanotech drugs and diagnostics, evaluating safety and efficacy, and assisting with product development – from discovery-level, through scale-up and into clinical trials. NCL has assisted in characterization and evaluation of over 300 nanotechnology products, several of which are now in human clinical trials.

Dr. McNeil is a member of several working groups on nanomedicine, environmental health and safety, and other nanotechnology issues. He is an invited speaker to numerous nanotechnology-related conferences and has several patents pending related to nanotechnology and biotechnology. He is also a Vice President of Leidos Biomedical Research.

Prior to establishing the NCL, he served as a Senior Scientist in the Nanotech Initiatives Division at SAIC-Frederick where he transitioned basic nanotechnology research to government and commercial markets. He advises industry, State and US Governments on the development of nanotechnology and is a member of several governmental and industrial working groups related to nanotechnology policy, standardization and commercialization. Dr. McNeil's professional career includes tenure as an Army Officer, with tours as Chief of Biochemistry at Tripler Army Medical Center, and as a Combat Arms officer during the Gulf War. He received his bachelor's degree in chemistry from Portland State University and his doctorate in cell biology from Oregon Health Sciences University.

Kuo-Ching Mei

Mei obtained his BSc degree in Pharmacy from Taipei Medical University, Taiwan (2006). He then spent few years working at the Medical and Pharmaceutical Industry Technology and Development Centre (PITDC, Taiwan) and United Biomedical Inc., Asia (UBIA) as a formulator, specialised in the development of controlled-release solid dosage forms and fluid bed coating technology. He obtained his MRes degree (distinction) in nanomedicine and targeted drug delivery from The School of Pharmacy, University of London (now UCL School of Pharmacy) in 2011. During his MRes degree he was involved in a research project focussed on the development of thermosensitive liposome-nanoparticle hybrids. He worked as research assistant at University College London for few months before joining Impax Laboratories Inc., Taiwan in 2012, as an associate formulation scientist. He was awarded the Graduate School International Research Award (GSIR) from King's College London in 2013 to continue his PhD studies at the Institute of Pharmaceutical Science. He is now working on the development of carbon nanostructures-based multimodal delivery systems for brain targeting.

Josbert (Bart) Metselaar

PhD
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Josbert (Bart) Metselaar, Pharmacist and a PhD in Pharmaceutics, Utrecht University started the company Enceladus Pharmaceuticals in 2005. With the help of grants, investments and non-equity funding, he managed to perform a series of preclinical and clinical trials focusing on three different liposomal nanomedicine products. Since 2012, he has worked as a part-time assistant professor at the University of Twente. In 2015, he took up a part-time group leader position at RWTH Aachen, to extend his work on the development of nanomedicines for the treatment of inflammatory disorders and cancer.

Angel Millan

PhD
Tenure Researcher CSIC
Instituto de Ciencia de Materiales de Aragón, C/ Pedro Cerbuna 10, 50009 Zaragoza, Spain
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Angel Millan was graduated in chemistry in the University of Zaragoza in 1982. He got the PhD in chemistry in the University of Balearic Islands in 1990. The subject of his doctoral thesis was the formation of calcium oxalate renal calculi and the main results were the development of a photometric method for the analysis of citrate in urine, a HPLC chromatographic method for the analysis of oxalate in urine, the inhibition of oxalate crystal growth by polycarboxylic acids, and the finding of an outstanding inhibitor that was patented, commercialized and used in therapy. He studied the kinetics of growth of calcium oxalate during a 4 month postdoc stay in the Institute of Inorganic Chemistry of Czechoslovakia, and the morphology of calcium oxalate monohydrate during a 2 years stay in the University of Nijmegen. Then he was contracted for two years by the University of Nijmegen to carry out a project for the firm Agfa-Gevaert on the mechanisms of formation of tabular silver halide crystals that derived in two international patents for the production of tabular photographic emulsions. He stayed, within the human capital and mobility programme of the EC, in the IMP-CNRS of Perpignan working on the development of refrigerators based on ammoniac, and in the Odeillo solar furnace in the simulation of chemical vapour deposition multicrystalline coatings. In 1996 he moved to the Institute of Materials Science of Aragón and stayed there until nowadays. He was working on the synthesis of semimagnetic-semiconductors, and molecular magnetic materials. Then he developed synthesis methods for the production of iron oxide nanoparticles in polymer matrixes, and strategies for the coating, liquid dispersion and multifunctionalization of magnetic nanoparticles. In 2007 he became a permanent member of CSIC in 2007. Recently he developed in cooperation with the University of Aveiro a nanoobject that it is at the same time a nanothermometer and a nanoheater. Actually his main area of interest is the application of this tool in the development of new hyperthermia therapies for cancer.

SCIENTIFIC AND TECHNOLOGICAL INDICATORS

- 8 International and 3 National Research Projects as principal investigator
- 32 National and International Research Projects as co-investigator
- 84 Articles in ISI journals and 6 book chapters. 1360 citations. H index: 18

3 International patents and 4 Spanish patents

- 90 participations in international congresses, chairment in 3 congresses, 15 invited talks, 23 oral presentations.

TEACHING AND SPREADING

- Director of dept of physics and chemistry in several high schools from 1984 to 1990.
- Supervisor of 2 doctoral thesis and 1 master thesis.
- Training 3 postdoctoral researchers, 3 doctoral students and 3 predoctoral students.
- Doctoral courses in nanostructure materials materials for technological applications in 2004-2006, 2007 and 2008; in Nanomedicine 2010, 2011.
- Courses in International Schools of Molecular Nanoscience 2008&2012, Advanced Nanotechnological Materials In Science And Industry 2012, NanoBioCatalysts 2014, and Nanoparticles: from Fundamentals to Applications in Life Sciences 2016.
- Participation in 1 divulgative TV program, 3 science documentaries, 5 radio programs, and several activites for young students.

Marijana Mionic

Scientific collaborator
Centre hospitalier universitaire vaudois (CHUV) and Laboratory of Powder Technology (LTP) at Ecole Polytechnique Federale de Lausanne (EPFL)
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Marijana Mionić Ebersold received B.Sc. in Physics and M.Sc. in Mechanical Engineering from the University of Kragujevac, Serbia, in 2001 and 2006, respectively. In 2011, she obtained Ph.D. degree in Solid state Physics with focus on nanotechnology from the École polytechnique fédérale de Lausanne (EPFL), Switzerland. After Ph.D. she joined Department of Material Science and Engineering at EPFL. Since 2013, she is scientific collaborator at University Hospital of Lausanne (CHUV).

Moien Moghimi

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Prof. Moein Moghimi is at Durham University (UK) where he serves as Professor and Chair in Pharmaceutics at the School of Medicine, Pharmacy and Health. He is also a Full Affiliate Member at the Department of Translational Imaging, Houston Methodist Research Institute, Houston Methodist Hospital Systems (Houston, Texas, USA) and Adjoint Professor at the Department of Pharmaceutical Sciences, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of Colorado-Denver Medical Center (USA). In 2015, Moein was a Visiting Professor at Università Degli Studi Di Padova (Italy). Between 2008 and March 2016, he was based at the University of Copenhagen (Denmark) serving as Professor of Nanomedicine at the Department of Pharmacy, Professor of Pharmaceutical Nanotechology at the NanoScience Centre, and the Founder and Director of the multi-million Dollar Centre for Pharmaceutical Nanotechnology and Nanotoxiocology. His earlier appointments included a Senior Lectureship position in Biopharmacy and Molecular Pharmaceutics at the School of Pharmacy, University of Brighton (UK) and The Nottingham University Research Fellowship in Advanced Drug Delivery Systems at the Department of Pharmaceutical Sciences. Between 2008 and 2010, he further served as the Honorary Professor of Nanomedicine at the Multidisciplinary Research Center, Shantou University (China).

He is also cofounder of S & M Discovery Group (London, UK); a nanomedicine R&D and consulting venture. Moein's research activities are focused on pharmaceutical nanoscience, nanomedicine and nanosafety. More specifically, Moein has made a major contribution to design and surface engineering of nanoparticles and functional nanosystems for parenteral site-specific targeting/drug delivery and imaging modalities (splenotropic entities, lymphotropic agents, 'phagocyte-resistant' nanoparticles, cerebral endothelial cell specific nanoplatforms and anti-cancer nanomedicine) as well as mechanistic understanding of nanomaterial/polymer-mediated infusion reactions and cell death processes. Moein has been the recipient of numerous awards as well as securing over €10 million in competitive research funds as principal investigator. Furthermore, he has partnered large-scale competitive European Commission FP-7 programmes in translational nanomedicine/drug delivery, addressing Alzheimer's disease and atherosclerosis.

Moein has over 200 peer-reviewed publications/patents to his credit. He functions as the Editor-in-Chief of Current Bionanotechnology (Bentham), Associate Editor for Journal of Biomedical Nanotechnology (American Scientific Publishers) as well as Immunology Section Editor for Current Pharmaceutical Biotechnology. Moein also features on the editorial board of several high-impact peer-reviewed scientific international journals to include Advanced Drug Delivery Reviews, Nanomedicine-UK (Future Medicine), Journal of Controlled Release (Elsevier) and Molecular and Cellular Therapies (BioMed Central).

Following completion of secondary education at d'Overbroeck's College Oxford (UK), Moein studied biochemistry at the University of Manchester (UK) and in 1989 earned his PhD in biochemistry (liposome immunobiology) from Charing Cross Hospital Medical School (Imperial College, University of London). He then completed a four-year SERC-funded post-doctoral training programme in Advanced Drug Delivery Research at the School of Pharmaceutical Sciences (University of Nottingham, UK).

Mauro Moglianetti

Mauro Moglianetti is a senior post-doc in Istituto Italiano di tecnologia (IIT), in the center located in Lecce. He has obtained his PhD degree from Oxford university studying the interaction of polymer/surfactant at the interface using Neutron Reflectivity as a main technique. During his postdoc at MIT/ EPFL, he worked on the use of Small Angle Neutron Scattering (SANS) as a tool to investigate the nano-domains present at the surface of nanoparticles covered by different thiols. He has also developed a new method to study the interfacial property of nanoparticles using neutron reflectivity.

He is now dedicated to the study of new nanomaterials for biomedical application, using his phyisco-chemical background to elucidate the structural key aspects dominating the bio-nano interactions.

Sitaramaiah Mokkapati

Dr Sitaramaia Mokkapati is presently Director Research at Kamineni Health care Group, Hyderabad. Dr.S.Mokkapati, Deputy Director-General in the division of Reproductive Health and Nutrition at headquarters of Indian Council of Medical Research,New Delhi. Dr.S.Mokkapati received his Ph.D. in Reproductive Biology in the year 1977 from Banaras Hindu University,Varanasi. He worked in the WHO programme at All India Institute Of Medical Sciences, New Delhi from 1980 to 1984. Dr.S.Mokkapati joined as cadre scientist in Indian Council of Medical Research in the year 1984. He published over 100 scientific papers, authored 12 books and presented 50 papers at both National and International scientific meetings. Dr.S.Mokkapati represented as head of the Indian delegation for ISO meetings on Nanotechnologies and Mechanical Contraceptives; served as International Expert and Chairperson on Condoms, IUDs and Diaphragms in ISO working groups. Dr.S.Mokkapati is Advisor, WHO,Geneva; PATH,USA and Ministry of Health and Family Welfare, Govt of India. He is an expert in ISO Technical Committee 229 on Nanotechnologies and Bureau of Indian Standards, Govt of India. He established Indian Nanomedicine Society and represented at International Society of Nanomedicine and European Society of Nanomedicine. He is invited to deliver lecture on Nanomedicine Applications for HIV/AIDS prevention at International Conference on Nanomedicine. At present, Dr.Mokkapati is Advisor from Asia on Nanotechnology.He is also President of Nanoscience and Nanotechnology and Indian Nanomedicine Society.
Recently he has joined Global organizations such as Reproductive Health Supplies. Coalition (RHSC), Family Planning 2020(FP 2020), Partnership in Maternal Newborn And Child Health(PMNCH).

Jan Mollenhauer

Cologne, Germany, and received his PhD in 1998 from the University of Heidelberg, Germany. In 2003 he received his habilitation in Molecular Medicine from the University Heidelberg, which was mentored by the Nobel laureate in Medicine or Physiology 2008, Prof. Harald zur Hausen. Until 2008 he worked as group leader in the Division of Molecular Genome Analyses (Head: Prof. Annemarie Poustka) at the German Cancer Research Center, Heidelberg. In 2008 he joined the University of Southern Denmark, Odense, as Professor for Molecular Oncology. Since 2010, he is director of the Lundbeckfonden Center of Excellence NanoCAN (Nanomedicine Research Center for Cancer Stem Cell Targeting Therapeutics). Jan Mollenhauer received the Future Award in Health Sciences 2005 and was listed in the 2007 edition of the Who Is Who of Emerging Leaders. In 2009 he was awarded with the Leo og Ingeborg Dannins Fondens Legat, and received the Fyens Stiftstidende Researcher Award in 2010. Since 2013, Prof. Mollenhauer is Editor-in-Chief, together with Prof. Patrick Hunziker, of the European Journal of Nanomedicine and member of the Advisory Board to the European Foundation for Clinical Nanomedicine (CLINAM). In 2015, Prof. Mollenhauer became member of the Board of Trustees of CLINAM. He is further serving as chairman and panel member of several national funding programs in Europe, including the Spanish TV3 Marato Foundation, the Swedish Research Council, and the UK Breakthrough Breast Cancer Research Centers. Research focuses on the role of epithelial protection factors in cancer, on the development and application of novel functional genomics techniques for cancer drug target discovery, and the design of tailored nanodrugs for personalized cancer therapy.

Reinhard Möller

Marketing and Sales Manager,
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Since 2012: Manager Marketing and Sales, TECOmedical AG, Switzerland
2000-2012: Director of European Distribution, Quidel Corporation, USA
1997 - 1999: International Marketing Manager, Nichols Institute Diagnostics BV, The Netherlands
1993 - 1997: Product Manager , Nichols Institute Diagnostics GmbH, Germany
1992: Doctors Degree at the University of Göttingen, Germany
1987-1992: Head of Endocrine Laboratory at Department of "Biotechnology and Reproduction" University Göttingen, Germany
1986: Degree as Dipl.Ing.agr.

Chrit Moonen

Prof. Dr. Chrit Moonen did his Masters in Molecular Sciences and his Ph.D. in biophysics (Wageningen University). He did part of his studies with Nobel Laureate Wüthrich in Zürich, Switzerland. He went for a postdoctoral period to the University of Oxford (Sir Georg Radda). He then worked at the University of California at Davis as a Visiting Research Scientist before becoming head of the NIH *In Vivo* NMR Research Center from 1987–1996. He moved back to Europe (Bordeaux, France) in 1996 where he has been director of the laboratory "Molecular and Functional Imaging: from Physiology to Therapy" until 2011. He is currently professor at the Division of Imaging at the University Medical Center in Utrecht, the Netherlands. He coauthored over 200 scientific papers. H-index is 64 (Google Scholar). He was President of the "International Society of Magnetic Resonance in Medicine" (2006), and of the "Society for Molecular Imaging" (2009). He is chairman of the 20015 meeting of the International Society for Therapeutic Ultrasound, and president of the European Society for Molecular Imaging. He received the European Magnetic Resonance Award 2000, is a Fellow of the International Society of Magnetic Resonance in Medicine, of the European Society of Magnetic Resonance in Medicine and Biology, and of the World Molecular Imaging Society. His recent work is mainly in MRI guided Focused Ultrasound, image guided drug delivery, and molecular and cellular imaging.

Stefan Mühlebach

Professor, PhD (Dr.pharm),
Hospital Pharmacist FPH,
Director Regulatory Sciences

Stefan Mühlebach is Regulatory Science Lead for NBCDs at Vifor Pharm-Fresenius Medical Care Renal Pharma Ltd. in Switzerland (http://www.viforpharma.com/en/About-Vifor/about- home.php.) and chairs the Non-Biological Complex Drugs (NBCDs) Working Group c/o Lygature, a non for profit, private-public partnership in the Netherlands (http://lygature.org/nbcd).
He is a professor and a member of the Medical Faculty and group member of the Clinical Pharmacy & Epidemiology unit at the Dept. of Pharmaceutical Sciences, University Basel. Stefan Mühlebach obtained a MSc Pharm and a Federal pharmacist's diploma in 1975. He did a PhD in pharmacology & toxicology which was faculty-awarded

in1979 (University of Bern, Switzerland). He worked a full and then a part-time research fellow at the Institute of Pharmacology in Bern till 2005. In 1993 he got his ”venia docendi” in Pharmacology from the Medical Faculty of the University of Bern; in 2000 from the Medical Faculty of the University of Basel.
In 2004 he was appointed Professor (Titularprofessor) of Pharmacology and Hospital Pharmacy at the University of Basel. He is an external member of the Clinical Pharmacy and Epidemiology Unit at the Department of Pharmaceutical Sciences. In 2005 Stefan Mühlebach did a Sabbatical with Dr. Driscoll at the Harvard Medical School in Boston MA in the field of clinical nutrition. His research activities cover topics in pharmacology, clinical nutrition, hospital pharmacy, and regulatory sciences (https://pharma.unibas.ch/research-groups/people/profile/person/muehlebach/). Stefan Mühlebach is author of more than 100 peer-reviewed papers, more than 50 indexed in Pubmed, of several book chapters, and of many scientific reports. He presents regularly at national and international scientific conferences on topics of regulatory science (Non- Biological Complex drugs), clinical nutrition, (hospital) pharmacy and pharmacology. He teaches in the graduate & postgraduate university level and directs PhD & Ms theses. He is a member of several (inter-)national societies. He was a founding member of the Swiss Academy of Pharmaceutical Sciences in 2014 and is Vice-President of the Senate’s board (http://www.saphw.ch/en/portrait/structure-mission-tasks).
From 1980-2005 Stefan Mühlebach was Chief hospital pharmacist in Biel and then in Aarau where he was also a member of the hospital board; he initiated and implemented the Swiss postgraduate specialisation curriculum for hospital pharmacy (Foederatio Pharmaceutica Helvetiae) and trained the first Swiss hospital pharmacist FPH fellows in Aarau. He served as president of the Swiss Association of Public Health Administration and Hospital Pharmacists in the 90ties and became a honorary member in 2012.
He was Head of the Pharmacopoeia and Head of the Swiss Delegation at EDQM in Strasbourg 2005-2008 working at that time at Swissmedic, the Swiss Agency for Therapeutic Products.
In 2008 Stefan Mühlebach started his industry carrier first as Chief Scientific Officer. Since 2009 he moved into the international headquarter of Vifor Pharma Ltd in Zürich (Global Regulatory Affairs). Since 2015 as Regulatory Science Lead for NBCDs in the headquarter of the joint company Vifor Pharma-Fresenius Medical Care Renal Pharma in Zürich.
Since 2010 he is also the Chair of the Non-Biological Complex Drugs Working Group c/o Lygature a non-for-profit organization in the Netherlands. The mission of the group is to provide science-based support for appropriate authorization of NBCDs and finally to assist patients’ benefit and safety. These large molecular synthetic products belong mostly to nanomedicines. The WG also presents a platform to facilitate the exchange among the different stakeholders.

Dev Mukhopadhyay

Ph.D.
Florida Department of Health Cancer Research Chair
Associate Director, Mayo Clinic Comprehensive Cancer Center
Professor, Departments of Biochemistry and Molecular Biology and Biomedical Engineering, Mayo Clinic College of Medicine, USA

Dev Mukhopadhyay: Professor of Biochemistry and Molecular Biology, Mayo Clinic, Mayo Clinic College of Medicine, has a joint appointment with the Department of Physiology and Biomedical Engineering and Associate Director of Mayo Clinic Comprehensive Cancer Center for Global Alliances. He has a broad background in tumor microenvironment, with specific training and expertise in key research areas including Cancer, Cardiovascular Diseases, and Diabetes. As a postdoctoral fellow, later as an independent investigator followed by as an Associate Professor at Harvard Medical School, Boston, he carried out angiogenesis and tumor microenvironment related research. After moving to Mayo Clinic as a Professor and also as Directors of both Tumor Microenvironment program and Translational Nanomedicine Center, he has been supervising additional research areas including stellate cell biology, new drug delivery systems and trained more than 50 young investigators and several of them are now independent faculties in different institutions throughout the world. He has been serving as reviewer in federal and also international funding agencies and participating as editorial board members of several distinguish journals. He has published more than 186 peer-reviewed publications including Nature, Nature Medicine, Cancer Cell, Cancer Research, Circulation Research, Journal of Clinical Investigation and several other reputed journals. He is also involved several translational research and clinical trials in both cancer and cardiovascular diseases.

Willem Mulder

Willem Mulder, Professor of Radiology, directs the Nanomedicine Laboratory at the Icahn School of Medicine at Mount Sinai in New York. He also is Professor of Cardiovascular Nanomedicine at the Academic Medical Center of the University of Amsterdam. His laboratory focuses on the development of nanoparticle libraries with differential specificity for processes relevant to disease progression. Nanoparticle materials from these libraries can be loaded with drugs, enabling targeted therapy of a variety of pathophysiological processes, relevant to cancer and atherosclerosis. For imaging purposes, these nanomaterials can also be labeled for highly sensitive and quantitative PET/MR or PET/CT imaging. Dr. Mulder’s program is aimed at applying nanomedicine to better understand, diagnose and treat disease, and translating the technologies from mouse models to large animal models, and ultimately humans.

Bert Müller

Bert Müller received a diploma in mechanical engineering, Berlin 1982, followed by M.Sc. degrees in Physics and English both from the Dresden University of Technology in 1989. In 1994, he obtained a Ph.D. in experimental physics from the University of Hannover, Germa-ny. For his achievements he was granted with the Morton M. Traum Award of the American Vacuum Society in 1994. From 1994 to 2001, he worked as a researcher at the Paderborn University, Germany, as Feodor Lynen Fellow and research associate at the EPF Lausanne, Switzerland and as team leader at the Physics Department, Materials Department and De-partment of Information Technology and Electrical Engineering at ETH Zurich, Switzerland. He became a faculty member of the Physics Department at ETH Zurich in April 2001. After his election as Thomas Straumann-Chair for Materials Science in Medicine at the University of Basel, Switzerland and his appointment at the Surgery Department of the University Hos-pital Basel in September 2006, he founded the Biomaterials Science Center in March 2007. Currently this center hosts more than twenty researchers dealing with nanotechnology-based artificial muscles for incontinence treatment, smart nano-containers to treat cardiovascular diseases, high-resolution X-ray imaging to visualize the human body down to the molecular level, computational sciences of tissues in health and disease and other applications of na-nosciences in medicine. The mission of the research team can be summarized by employing physical principles for human health. Professor Müller teaches physics and materials science at the ETH Zurich and the University of Basel and currently supervises more than a dozen doctoral students from medicine, dentistry, physics, nanosciences, and biomedical engineer-ing. 2014 he was elected as Fellow of SPIE and 2015 as an active member of the European Academy of Sciences and Arts.

Sesha Neervannan

PhD
Sr. Vice President, Pharmaceutical Development

Dr. Sesha Neervannan is currently Sr. Vice President of Pharmaceutical Development at Allergan. In his current role, he is responsible for Biologics Product Development and Small Molecule Drug Substance and Drug Product Development from Discovery to Commercialization that includes Process Chemistry, Physical chemistry, Formulation & Process Development, Packaging engineering, Analytical development, Biomaterials Research and Drug Delivery Research and Development efforts.

Prior to joining Allergan in 2007, Sesha held senior positions at Bristol-Myers Squibb where he helped establish Topical Dermal and Transdermal Delivery groups and advanced various internal molecules to clinical dev via transdermal delivery approach, when oral route was not feasible. He then took on an exciting role at Amgen, where, as part of Pharmaceutics organization, he helped to start, build and grow Small Molecule Pharmaceutics functions as well as contributed to several Biologics products. At Allergan, he oversaw and contributed to several global product filings and approvals in the last few years (Ozurdex™, Latisse™, Acuvail™, Lumigan 0.01%™, Zymaxid™, Trivaris™) as well as development, validation and launch of several OTC artificial tears products (Refresh Optive Advanced™ – Single Use and Multi-use; Optive Fusion™).

Sesha has several research publications and patents and is a well recognized invited speaker at several national and international conferences. He serves on several Advisory Boards including the Scientific Advisory Board for CHDI, a non-profit organization, Customer Advisory Board for NineSigma, an open innovation company, Board member of International Consortium of Innovation and Quality (IQ), and the Editorial Advisory Boards for Expert Opinion on Drug Delivery and Life Sciences Leader, as well as Scientific Advisor to Editors of JPharmSci.

He received his Ph.D. in Pharmaceutical Chemistry at The University of Kansas.

Guangjun Nie

Guangjun Nie, Ph.D, Professor
CAS Key Laboratory for Biomedical Effects of Nanomaterials & Nanosafety
Direct of Project Management & International Collaboration, National Center for Nanoscience and Technology of China, 11 Beiyijie, Zhongguancun
Beijing 100190, China
Tel:86-10-82545529; Niegj@nanoctr.cn

Guangjun Nie is a Professor at the National Center for Nanoscience and Technology, China. He obtained his Ph.D in Biochemistry and Biophysics at the Institute of Biophysics, CAS in 2002. Currently, he is a Chief Scientist of a MoST National Basic Research Program and National Distinguished Youth Scientist. He was also awarded the Hundred Talent Program Scholar of CAS in 2008. He is regarded as one of the leading scientists in nanobiology and nanomedicine in China. He has a long standing interest in cancer biology, blood physiology and pathophysiology of human disorders involving disregulation of redox balance and metal metabolism. Currently, his main interests are design of bio-inspired materials to overcome the current barriers in tumor therapy and nanobiology. In particular, his group is working toward controlling the chemical properties of multi-functional nanoparticles in order to allow specific targeting and regulation of tumor cells and their microenvironment.

His most recent research activities generated a group of interdisciplinary works in nanobiology, nanomedicine and blood physiology fields, including over 95 papers published in Acc Chem Res, Adv Mater, Angew Chem, Adv Funct Mater, Blood, Biomaterials, Br J Haematol, JACS, JBC, Molecular Cancer Therapeutics and Small. He has filled over 32 patents on novel nanomedicines and 16 of them have been granted, with two patents on antitumor drug development have been transferred to a biotechnology firm for pre-clinical investigation. He is an experienced supervisor of postgraduate students and collaborates widely both within China and internationally. The cumulative competitive grants for Prof Nie's group is over 32 Million RBM in the past 6 years, including the major grants from MoST, NSFC, CAS of China and some international collaborative grants. Now he is leading a multidisciplinary team with over 30 people working toward better and safer antitumor nanomedicine for pancreatic cancer and liver cancer. He is also the Affiliated Professor of Northeast University, Shenyang, China and East China University of Science and Technology, Shanghai. He is also an Affiliated Senior Member of Houston Methodist Research Institute, Houston, US since 2010.

FIVE REPRESENTATIVE PUBLICATIONS:

1. Gang Liu, Shanshan Guo, Gregory J Anderson, Clara Camaschella, Bing Han and Guangjun Nie, "Heterozygous missense mutations in the GLRX5 gene cause sideroblastic anemia in a Chinese patient", Blood, 2014, 124, 2750-2751.
2. Hai Wang, Yan Wu, Ruifang Zhao and Guangjun Nie, "Engineering the assemblies of biomaterial nanocarriers for delivery of multiple theranostic agents with enhanced antitumor efficacy", Adv. Mater., 2013, 25, 1616-1622.
3. Tianjiao Ji, Ying Zhao, Yanping Ding and Guangjun Nie, "Using nanotechnology to target and regulate the tumor microenvironment: diagnostic and therapeutic applications", Adv. Mater., 2013, 25, 3508-3525.
2. Yiye Li, Yunlong Zhou, Hai-Yan Wang, Sarah Perrett, Yuliang Zhao, Zhiyong Tang, Guangjun Nie, "Chirality of Glutathione Surface Coating Affects the Toxicity of Quantum Dots", Angew. Chem. Int. Ed., 2011, 50, 5860-5864. (Highlighted by Nature Materials).
1. Cuiji Sun, Hui Yang,Yi Yuan, Xin Tian, Liming Wang, Yi Guo, Li Xu, Jianlin Lei, Ning Gao, Gregory J. Anderson, Xing-jie Liang, Chunying Chen, Yuliang Zhao, Guangjun Nie, "Controlling assembly of paired gold clusters within apoferritin nanoreactor for *in vivo* kidney targeting and biomedical imaging", J. Am. Chem. Soc., 2011, 133, 8617–8624.

Paul Botwev Orhii

JD, MD, PhD (OON)

Dr Paul B. Orhii is the Former Director General, National Agency for Food and Drug Administration and Control (NAFDAC), Nigeria. He holds Doctor of Medicine (MD), Doctor of Philosophy (PhD) and Juris Doctor (JD) degrees in Medicine and Law. He is a Pharmacologist, Physician, Biomedical Scientist and an Attorney & Counsellor at Law. He is also a Medical Expert Witness & Pharmaceutical Litigation Support Specialist and highly experienced and globally recognized in Anti-Counterfeiting/ Brand Protection.

Dr Paul Orhii graduated with honours from the Stavropol State Medical Institute, Russia in 1989. Thereafter he successfully obtained a PhD in Medicine (Emphasis in Chrono-neuro-psychopharmacology) from the same Institute in 1992. After briefly teaching Pharmacology to medical students at the University of Jos, he proceeded to Texas, USA where he underwent Post-Doctoral Fellowship at the University of Texas Health Science Center at San Antonio, Texas. From 1993 he worked as a Biomedical Scientist with the University until 2003 when he moved to Houston to study Law full-time at the Thurgood Marshall School of Law in Houston, Texas. He was called to the Texas Bar in 2007. He founded the Orhii Law Firm in Houston, Texas.

On the 13th January, 2009 the Federal Government of Nigeria appointed Dr Paul Orhii as the Director General of the National Agency for Food and Drug Administration and Control (NAFDAC), an agency charged with the responsibility of regulating and controlling the importation, exportation, manufacture, advertisement, sales and use of food, drugs, cosmetics, chemicals, medical devices and packaged water. During his tenure as the Director-General of NAFDAC, Dr Orhii introduced multi-dimensional and well- coordinated strategies in fighting the menace of counterfeit regulated products. He pioneered the use of cutting-edge technologies by introducing TRUSCAN, a handheld device used in checking the quality of medicines on the spot. This innovation made NAFDAC, the first regulatory Agency in the world to successfully deploy TruScan.

One other innovative strategy brought by Dr. Orhii is the use of Mobile Authentication Service, the use of Short Messaging Service to check the quality of medicines before purchase. This innovation has put the power of detecting counterfeit medicines in the hands of the consumers, thereby recruiting the over eighty million cell phone users in Nigeria as potential NAFDAC informants.

He also initiated the review of NAFDAC laws to have policies in place to fight counterfeit and with a view to make the laws more of a deterrent. Such other strategies as improved National and International Collaboration, Capacity building and the upgrading of NAFDAC facilities nationwide were introduced by Dr. Orhii to reposition NAFDAC as a global leader in the fight against counterfeit medical products. The Agency is rated among the top 18 regulatory agencies in the world, the first in Africa and the only other one apart from South Africa to belong to that elite group.

In recognition of these efforts, Dr. Orhii is frequently sought after to contribute to global discussions on anti-counterfeiting and regulatory issues that directly impact on the global community especially in this era of increased globalization. He was the Vice Chair of the International Medical Products Anti-Counterfeiting Taskforce (IMPACT) with headquarters in Geneva from 2009 to 2012. The taskforce is made up of 193 WHO member countries on voluntary basis and other stakeholders involved in the regulation of medicines such as the World Customs Organization, the INTERPOL, the Organization of Economic Cooperation and Development (OECD) and the World Intellectual Property Organization (WIPO), among others. Thereafter he served as the pioneer Chair of the Steering Committee of the newly created WHO Membership Mechanism on Spurious, Substandard, Falsely-Labelled, Falsified Counterfeit Medical Products. Dr. Orhii was invited by US Council on Foreign Relations to present a position paper that was used at the G8 Summit held in Chicago, USA in 2012.

Following the 2015 Presidential elections Dr. Orhii was one of the 16 distinguished Nigerians appointed by President Goodluck Ebele Johnathan to serve on the Presidential Transition Committee to ensure a smooth transition of power from the PDP Presidency to the APC.

He is fully licensed as an Attorney and Counselor at Law in Texas and is a member of the Texas Bar, The American Bar Association (ABA), The American Society for Bone & Mineral Research (ASBMR), The Medical & Dental Council of Nigeria, the European Pineal Society (EPS) and the American Diabetes Association (ADA).

In recognition of his dynamic, innovative and visionary leadership at transforming NAFDAC, Dr. Orhii has received more than 40 National and International Awards amongst which are:

- African Public Health & Safety Award conferred on him by African Leadership Magazine, Kenya, 2009.
- Blue Ribbon Award by Joint Technical Committee – UNICEF, 2009
- 2010 PharmaVOICE 100 Most Inspiring Personalities in the Life Sciences Industry.
- Key to the City of District Heights, Maryland/honorary citizen of the state, 2013.
- Certificate of Recognition by Senate of State of Georgia, USA for outstanding achievements in the use of cutting-edge technologies to fight drug counterfeiting.

Top among the awards is the prestigious National Honour of the Officer of the Order of the Niger (OON) conferred on him by President Goodluck Ebele Jonathan for his contribution in the development of Science and Medicine in September 2012.

Dr Paul Orhii disengaged from public service on February 14, 2016 after having served meritoriously as the Director General of NAFDAC for more than seven years.

He is now a private consultant on Anti-Counterfeiting and Brand Protection.

Catia Ornelas

Assistant Professor at the Institute of Chemistry, University of Campinas, Brazil

- Researcher ID: H-4606-2011
- h-index: 26
- Author of 51 peer-reviewed articles including seven in the Journal of the American Chemical Society, three in Angewandte Chemie, one in Chemical Reviews.
- Co-Founder and Chair of the Brazil International Chemical Sciences Chapter of the American Chemical Society.

AWARDS

2015: Productivity Award by the National Council for Scientific and Technological Development (CNPQ).

2011: Burgen Scholar Award by the Academy of Europe. This award provides recognition to young European scholars, who are emerging talents and potential future leaders in their field.

EDUCATIONAL BACKGROUND

2002: Graduated in Chemistry, at University of Madeira (Portugal). Undergraduate research was carried out under supervision of Prof. João Rodrigues from University of Madeira (Portugal) and Prof. Kari Rissanen from the University of Jyvaskyla (Finland).

2007: PhD degree in Chemistry at the University of Bordeaux (France), under the supervision of Prof. Didier Astruc.

2008–2010: Postdoctoral fellow at the Molecular Design Institute at New York University with Prof. Marcus Weck

2010–2011: Postdoctoral fellow at University of California Berkeley, in the group of Prof. Jean M. J. Fréchet.

2011–2012: Postdoctoral fellow at the Energy Frontier Research Center at Arizona State University in the group of Prof. Ana Moore, Prof. Thomas Moore and Prof. Devens Gust.

Bekim Osmani

PhD

Bekim Osmani is a PhD student at the Biomaterials Science Center at the University of Basel.

Bekim Osmani did his B.Sc. in Mechanical Engineering and received his M.Sc. in Biomedical Engineering and Robotics at the Swiss Federal Institute of Technology in Zurich (ETHZ) in 2002. He worked from 2002 until 2004 as a scientific assistant at the Institute of Mechatronics at the Zurich University of Applied Sciences in Winterthur. After several years of experience in industry, he is currently working towards his PhD degree in Nanosciences on the fabrication and characterization of dielectric elastomer actuators for medical implants. As a member of the Nano-Tera funded project “Smart Sphincter”, his research interests include molecular beam deposition, Atomic Force Microscopy techniques, polymeric implants and biomedical applications for low-voltage dielectric elastomer actuators.

Andrew Owen

PhD FRSB FBPhS

Andrew Owen is Professor of Molecular and Clinical Pharmacology at the University of Liverpool, UK. He is also affiliated to the MRC Centre for Drug Safety Science and the Wolfson Centre for Personalised Medicine. He is Chair of the British Society for Nanomedicine, a fellow of the Royal Society of Biology, a fellow of the British Pharmacological Society and a member of the steering committee for the APS Nanomedicines Focus Group. His clinical and basic research focuses on understanding mechanisms that underpin inter-patient variability in pharmacokinetics and pharmacodynamics. In recent years a major emphasis has been to employ knowledge of these mechanisms to accelerate the translation of nanomedicine candidates to clinical applications. Work is supported by the US Agency for International Development, US National Institutes for Health, UK Medical Research Council, European Commission, and UK Engineering and Physical Sciences Research Council. Professor Owen also has strong links with the Clinton Health Access Initiative and Medicine Patent Pool. He has published over 140 original publications, is co-inventor of patents relating to the application of nanotechnology to drug delivery and a co-founder of Tandem Nano Ltd. He is a Founder and Editor in Chief for the Journal of Interdisciplinary Nanomedicine.

Marisa Papaluca Amati

Internal Medicine specialists, Marisa joined the EMA in late 1994 and occupied scientific and managerial positions in the EMA Unit for Human Medicines Development and Evaluation.

Deputy Head of Quality up to 2002 and of the Efficacy and Safety Sectors up to 2009, Marisa is currently Head of the Scientific Support office providing scientific support to the Agency core activities in transversal and multidisciplinary areas such as clinical trials statistical methodology, raw data analysis, non-clinical drug development, pharmacogenomics and nanotechnology.

The office is also in charge of the EMA the Innovation Task Force, reference group at EU and international level for innovative pharmaceuticals developments with current increasing activities on novel clinical trials designs, genomic biomarkers, combined products, synthetic biology and nanomedicines.

Marisa has been appointed in March 2015 Senior Scientific Advisor attached to the Division for R&D support.

Wolfgang Parak

Since 2007: Full Professor (chair) for Experimental Physics at the Philipps-University of Marburg, Germany

Since 2013: in addition head of the Biofunctional Nanomaterials Unit at CIC biomaGUNE, San Sebastian, Spain

2009: "Nanoscience" – award 2008 from the Association of Nanotechnology-Centres Germany (AGenNT)

Since 2010: Associate Editor for ACS Nano from the American Chemical Society

2011: ranked #59 in Top Materials Scientists of the past decade by Essential Science Indicators (http://science.thomsonreuters.com/products/esi/)

2012: Awarded Chinese Academy of Sciences Visiting Professorship for Senior International Scientists

2014: highly cited in the category materials sciences (http://highlycited.com/)

2014: listed in "The World's Most Influential Scientific Minds: 2014" (http://www.sciencewatch.com)

2015: highly cited in the category materials sciences (http://highlycited.com/)

2015: listed in "The World's Most Influential Scientific Minds: 2015" (http://www.sciencewatch.com)

2017: Visiting Professor Australian Research Council Centre of Excellence in Convergent Bio-Nano Science & Technology (CBNS) present h-index: 63 WebOfScience; 73 GoogleScholar

ResearcherID: M-3998-2014; ORCID: orcid.org/0000-0003-1672-6650

Wolfgang Parak is/was Associate Editor of ACS Nano **(2010-)**, and Nanotoxicology **(2009-2010)**. He is/was in the advisory board of the following journals: Chemistry of Materials **(2015-)**, Angewandte Chemie **(2014-)**, Theragnostics **(2014-)**, Nanomaterials **(2014-)**, ChemNanoMat **(2014-)**, Colloids and Interface Science Communications **(2014-)**, Particle & Particle Systems Characterization **(2013-)**, Nanotoxicology **(2010-)**, Journal of Colloid and Interface Science **(2009-)**, The All Results Journal **(2008-)**, Journal of Nanobiotechnology **(2011-)**, Recent Patents on Nanotechnology **(2007-2010)**, Journal of Nanobiosensors in Disease Diagnosis **(2011-2013)**. Wolfgang Parak is/was member of the following steering comiittees: National Research Programme NRP 64 "Opportunities and Risks of Nanomaterials" of the Swiss National Foundation **(2010-, Switzerland)**, Andalucian Initiative for Advanced Therapies **(IATA, 2012-, Spain)**, Insitute for medical/pharmaceutical exams (IMPP, Institut für medizinische und pharmazeutische Prüfungsfrage, **2012-**, Germany), Minerva Center for Bio-hybrid Complex Systems at the Hebrew University Jerusalem (Chairperson of the Center's Beirat, **2013-**, Israel), CIBER-BBN (**2013-**, Spain), committee of external evaluators of the Italian Institute of Technology (IIT, **2013-**, Italy). Wolfgang Parak is co-organizer of the following conference series: "Colloidal Nanoparticles for Biomedical Applications" of the SPIE Photonics West meeting **(2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016)**, NANAX **(2003, 2008, 2012, 2016)**, Amercial Chemical Society ACS Spring/Fall meeting **(s2014, s2015, f2015, s2016, f2016)**.

Avinash Patel

PhD
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patelavin@gmail.com

RESEARCH EXPERIENCE

2013–Present: Post-doctoral Research Fellow, Hyman Lab, Max Planck Institute for Cell Biology and Genetics, Dresden, Germany.

2012–2013: Post-doctoral Research Associate, Iain Hagan's Cell Division Group, Cancer Research UK Manchester Institute, University of Manchester, UK.

2006: Masters Dissertation at Structural Genomics Consortium, University of Oxford, UK. (6 months)

EDUCATION

2008–2012: Ph.D. at Manchester Cancer Research UK Institute, School of Medicine, University of Manchester, UK. Thesis: "Exploiting classical and chemical genetics to interrogate the polo kinase phospho-proteome of fission yeast"

Advisor: Prof Iain Hagan

2006–2007: Masters (M.Sc.) in Applied Biomolecular Technology at University of Nottingham, UK. Thesis: "Compound Screening of Protein Tyrosine Phosphatases to identify potent inhibitors for structural studies and drug development"

AWARDS AND ACHIEVEMENTS

2015: Post-doctoral research fellowship from the Alexander von Humboldt foundation, Germany.
2012: EMBO short-term fellowship for a 3 months sabbatical in Prof Boris Maček's Proteome Centre, University of Tübingen, Germany.
2012: Travel scholarship, Keystone Symposia, Stockholm, Sweden, Proteomics, Interactomes, Stockholm.
2008-2012: Cancer Research UK PhD studentship for 4-year PhD Programme.
2006 and 2004: Science Merit Scholarship from Delhi University for being in top 10 ranks (of 2000 students) for B.Sc. Chemistry.
200: Academic Brilliance Award for securing outstanding GCSE grades (91%).

PUBLICATIONS

- Patel A *, Hyun O. Lee *, Louise Jawerth et al., A Liquid-to-Solid Phase Transition of the ALS Protein FUS Accelerated by Disease Mutation, 2015 August, Cell 162, 1066–1077.* Equal contribution.

News features on the article:

1) 04 Sep 2015, "ALS Protein Said to Liquefy, Then Freeze en Route to Disease", http://www.alzforum.org/news/research-news/als-protein-said-liquefy-then-freeze-en-route-disease
2) 22 Oct 2015, "Protein 'drops' may seed brain disease", Ken Garber, Science Magazine News.
3) 25 Oct 2015, "Wehe, wenn sie verklumpen", Süddeutsche Zeitung (german daily), Knowledge section.

- Grallert A *, Patel A * et al. Centrosomal MPF triggers the mitotic and morphogenetic switches of fission yeast, 2013 January, Nature Cell Biology; 15(1): 88-95. * Equal contribution.
- Carpy A*, Patel A*, Tay YD, Hagan I, Macek B, Nic1 inactivation enables SILAC labeling with 13C6 15N4-

Arginine in fission yeast, 2015 January, Molecular Cell Proteomics;14(1):243-50.*Equal contribution

- Tay YD, Patel A, Kaemena D, & Hagan I, Mutation of a conserved residue enhances sensitivity of analogue sensitized kinases to generate a novel approach for mitotic studies in fission yeast, 2013 November, Journal of cell science, 126(Pt 21):5052-61.
- Fennessy D, Grallert A, Krapp A, Cokoja A, Bridge AJ, Petersen J, Patel A, Tallada VA, Boke E, Hodgson B, Simanis V, Hagan IM (2014): Extending the Schizosaccharomyces pombe molecular genetic toolbox, 2014 May, PLOS ONE, 21;9(5)

Anil Patri

Dr. Anil Patri serves as the Director of Nanotechnology Core Facility at the U.S. Food and Drug Administration's National Center for Toxicological Research and conducts regulatory science research. He also serves as the Chairman of the Nanotechnology Task Force at FDA in the Office of the Commissioner. In this position, he coordinates Nanotechnology research between different Centers, reviewer training, grant funding, and interfaces with the National Nanotechnology Initiative for inter-agency coordination.
Prior to joining FDA in August 2014, Dr. Patri served as the Deputy Director of the Nanotechnology Characterization laboratory (NCL) at the Frederick National Laboratory for Cancer Research. In a decade long tenure at NCL, he assisted collaborators from industry and academia towards clinical translation of nanomedicines, many currently in clinical trials. He led a collaborative multi-disciplinary team of scientists at NCL and oversaw 85 projects through preclinical assessment that included proposal review and guidance, material characterization, *in vitro* and *in vivo* studies on different nanomaterial platforms intended for drug delivery, gene delivery and imaging. From 2006-2014, he served as a guest scientist at NIST and helped co-develop the first Nanosized gold reference material standards and developed standard protocols through ASTM. Dr. Patri served at the University of Michigan Medical School, Center for Biologic Nanotechnology, and developed targeted drug delivery and imaging agents until 2005. He serves on many review panels, advisory and editorial boards.
Dr. Patri pursued graduate work on Dendrimers with Prof. George Newkome and earned a Ph.D. degree in Chemistry from the University of South Florida followed by a post-doctoral training with Dr. Don Tomalia at the University of Michigan. He had prior tenure at Astra Zeneca and as a lecturer in Chemistry.

Dan Peer

Prof. Dan Peer is a Professor and the Director of the Laboratory of Precision Nanomedicine at Tel Aviv University (TAU). He is also the Director of the Focal Technology Area (FTA) on Nanomedicines for Personalized Theranostics, a National Nanotechnology Initiative and the Director of the Leona M. and Harry B. Helmsley Nanotechnology Research Fund.
Prof. Peer's work was among the first to demonstrate systemic delivery of RNAi molecules using targeted nanocarriers to the immune system and he pioneered the use of RNA interference (RNAi) for *in vivo* validation of new drug targets within the immune system.
Prof. Peer has more than 45 pending and granted patents. Some of them have been licensed to several pharmaceutical companies and one is under a phase III clinical evaluation. In addition, based on his work, four spin-off companies were generated Leuko Biosciences, Quiet Therapeutics, SEPL Pharma and ART Bioscience aiming to bring nanomedicine into clinical practice. In addition.
Prof. Peer is currently the President of the Israeli Chapter of the Controlled Release Society, and a Member of the Israel Young Academy of Sciences.

Marija Plodinec

I am a cancer biophysicist (B.Sc. in Physics and PhD in Structural Biology and Biophysics) with 10 years experience in conducting research from tissue scale down to single molecules. I have a broad background in cell biology, biochemistry, as well as in animal and human tissue experimentation. My scientific projects have been highly related to cancer research with the focus on understanding the mechanistic relationship between cell and tissue architecture that contribute to cancer growth and initiation. Hence, we have established 1) three-dimensional (3-D) *in vitro* tumor models that closely mimic cancer microenvironment (regulating oxygen and extracellular matrix (ECM) variability)
and 2) developed physical assays and microscopes for studying tumor progression utilizing animal models and human tissue samples. More specifically, I designed and co-developed novel microscopy techniques based on atomic force microscopy (ARTIDIS®, Automated and Reliable Tissue Diagnostics, Nature Nanotech., 2012, several patents), integrated with live cell fluorescence imaging and spinning disk confocal microscopy for studying cell-extracellular matrix interactions in situ. Currently I am working as a project leader/ research associate at the Institute of Pathology, University Hospital Basel where I manage an interdisciplinary team on two major projects: 1) our major goal is to understand cell signaling processes mediated by the cell surface integrin receptors at the cell-ECM interface that are activated by changes in stiffness, chemical composition and architecture of basement membrane (BM) and 2) developing novel cancer diagnostics and prognostics assays using ARTIDIS based tissue profiling that is complemented by the Next Generation Sequencing (NGS) and protein expression assays. Moreover,

I am managing a CTI (Commission for Technology and Inovation) project that involves designing a novel micro – biopsy needle for cancer diagnostics. The scope of my work also includes administrative and in part teaching duties. Through my scientific work, I have also acquired in depth knowledge by coordinating interactions between engineers, scientists and clinicians (pathology and surgery) to manage clinical studies on human cancer specimens.

James E. Polli

PhD
University of Maryland Department of Pharmaceutical Science, Baltimore, MD 21201 USA
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Dr. James E. Polli is Professor and Ralph F. Shangraw/Noxell Endowed Chair in Industrial Pharmacy and Pharmaceutics at the University of Maryland School of Pharmacy. He is also co-Director of the University of Maryland Center of Excellence in Regulatory Science and Innovation (M-CERSI), an FDA-funded collaborative agreement with the Agency, and Director of the online MS in Regulatory Science program (www.pharmacy.umaryland.edu/regulatoryscience). He received a B.S. in Pharmacy from the Philadelphia College of Pharmacy and Science and a Ph.D. (pharmaceutics) from the University of Michigan. Dr. Polli's research interest revolves around the performance and pharmaceutical quality of orally administered medicines. His two main research interests are 1) maximizing oral bioavailability through formulation and chemical approaches and 2) developing public quality standards for oral dosage forms. He has published in the areas of dissolution, drug intestinal permeability, transporter substrate requirements, prodrug design, oral bioavailability, *in vitro - in vivo* correlation, and bioequivalence. Dr. Polli has an active clinical research program in both patients and healthy volunteers. He is a member of the University of Maryland General Clinical Research Center Advisory Committee. Dr. Polli is a fellow and past Member-at-Large of AAPS, an Editorial Board member of several journals, an Associate Editor of Pharmaceutical Research, former Vice-Chair of the USP Expert Committee on Biopharmaceutics, and a member of the FDA Advisory Committee on Pharmaceutical Sciences and Clinical Pharmacology. He teaches professional pharmacy students and graduate students, and has served as advisor to 18 Ph.D. graduates.

Hen Popilski

Ben-Gurion University of the Negev,Beer-Sheva, 84105, Israel
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Fields of inters: Novel drug delivery systems for the treatment of solid tumors. Local administration of chemotherapeutic agents, drug targeting, permeability and disposition.

EDUCATION

2015–: Ben-Gurion University of the Negev
M.MedSc of Clinical Biochemistry and Pharmacology
2012–2014: The College of Management Academic Studies
MSC Business Management - Bio-Medical Management internship
2004–2008: Ben-Gurion University of the Negev
B.Pharm- A Bachelor of Pharmacy

EMPLOYMENT

2013–2014: QC (quality control) of pharmaceuticals and medical devices at the Israel; Defense Forces (IDF) medical corps
2009–2014: Pharmacist at IDF
2008–2009: Pharmacy intern at Asaf Harofe medical center

PUBLICATIONS

- Popilski H, Stepensky D. Mathematical modeling analysis of intratumoral disposition of anticancer agents and drug delivery systems. Expert Opin Drug Metab Toxicol. 2015;11(5):767-84.
- Kozlovskaya L, Popilski H, Gorenbein P, Stepensky D. *In vitro* toxicity of infusion sets depends on their composition, storage time and storage conditions. Int J Pharm. 2015:15;489(1-2):285-93.
- Youngster I, Arcavi L, Schechmaster R, Akayzen Y, Popliski H, Shimonov J, Beig S, Berkovitch M. Medications and glucose-6-phosphate dehydrogenase deficiency: an evidence-based review. Drug Saf. 2010:1;33(9):713-26.

Rachela Popovtzer

PhD
Associate Professor
Faculty of Engineering, Bar-Ilan University, Ramat Gan 52900, Israel
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Rachela Popovtzer is an Associate Professor in the Faculty of Engineering and a member of the Nano-Medicine Center at the Bar-Ilan Institute of Nanotechnology and Advanced Materials (BINA), Israel. She received her B.Sc. degree in physics from BIU and her M.Sc. and PhD in Electrical Engineering from Tel Aviv University. She was a postdoctoral fellow in the University of Michigan with Prof. Raoul Kopelman. Popovtzer joined the faculty of Engineering in 2008, where she is currently the head of the Laboratory for Nano-medicine. Rachela Popovtzer has been chosen as one of the 50 most influential women in Israel for the year 2015 by the Israeli Globes financial magazine. She is a winner of numerous international grants and awards, such as the Intel Prize, the EU Environment and Living foundation Prize and the Atol Charitable Trust Fellow in Nano Medicine. Her current research interest focuses on the development of 'smart' nanoprobes for theranostic applications.

Jai Prakash

Dr. Prakash is Associate Professor at the MIRA Institute of Biomedical Technology and Technical Medicine at the University of Twente in the Netherlands. He obtained his PhD (cum laude) in 2006 from the University of Groningen in the field of targeted (nano)medicine. Thereafter, he worked as a senior scientist at the University of Groningen with a joint position at BiOrion Technologies, Groningen as Vice President, Preclinical Research. In 2011, he joined Karolinska Institutet in Stockholm as Assistant Professor in the Department of Oncology-Pathology, where he received an expertise in biology of the tumor microenvironment. In 2012, he joined University of Twente as tenure-track Assistant Professor at the MIRA institute. His research group is focused on the better understanding of role of stroma in the tumor microenvironment and fibrosis and to design novel targeted nanomedicine against myofibroblasts and macrophages for the imaging and treatment of fibrosis and cancer.

Ümit Pul

Dr. Ümit Pul studied biology at the HHU Düsseldorf and graduated with honors in 2004. In 2008 he received his PhD with summa cum laude and started as post-doc with the investigation of the newly discovered CRISPR-Cas systems in prokaryotes. His CRISPR research projects received financial support from the German Research Foundation (DFG) and the Strategic Research Fund of the HHU, which allowed him to lead a junior research group studying Cas proteins and their application in genome modification. In 2014, he joined BRAIN AG as Project Manager and is responsible for the industrial implementation of CRISPR-based technologies and in house developments and applications of genome editing techniques in different prokaryotic, fungal and mammalian cells. In 2016, he took the additional role of coordinator for the Genome Editing technology platform in order to establish and further develop CRISPR-based molecular techniques and concepts at B.R.A.I.N AG. His is an associate industrial member of the DFG-funded academic research group "FOR 1680: Unravelling the Prokaryotic Immune System"

Martin Rausch

Martin Rausch is global head of the Microscopy and BioPhotonics group at the Novartis Institutes for BioMedical Research.

Martin Rausch received his Ph.D in biophysics from the University Marburg, Germany in 1993. In the following years, he joined different research centers in Germany and Switzerland. Martin Rausch joined Novartis in 2001.

Vikash Reebye

Dr Vikash Reebye is a Senior Scientist at MINA Therapeutics Ltd and the Academic Lead in Professor Habib's research team at Imperial College London. Dr Reebye is involved in the development of therapeutic gain of function studies using novel small activating RNA duplexes synthetically designed. These versatile and powerful new tools for gene activation have shown an impact in experimental regenerative medicine by redirecting adult stem cells towards a surrogate insulin secreting phenotype; in oncology by reducing tumour burden in HCC; and in hepatology by improving *in vivo* liver function on cirrhotic and NAFLD models.

The work of Dr Reebye involves close collaboration with private biotechnology and international academic institutions in the US, Europe and Asia. With this unique foothold of academia merging into the industrial sector, the work carried out has culminated in the beginning of a Phase 1 clinical directed by Prof Habib using small activating RNA for liver regeneration in patients with advanced HCC. As more is learnt about the mechanism of action of saRNAs and new nanoparticle delivery vehicles are being designed, there is an exciting future for targeted gene activation therapy.

Herbert Reitsamer

Prof Reitsamer is a physician scientist at the Department of Ophthalmology, Paracelsus Medical University/SALK in Salzburg, Austria. He was trained in engineering as well as in medicine. He is Associate Professor of Neurophysiology at Vienna medical University, Full research Professor at Paracelsus Medical University, where he holds the Lotte Schwarz Chair and is the Director of the research Program for Experimental Ophthalmology and the Fuchs laboratories. He is also the chairman of the Department of Ophthalmology at the SALK/Paracelsus Medical University. Prof Reitsamer is Secretary of the Austrian Ophthalmological Society and member of many scientific organizations in Ophthalmology. He is incoming president of the international chapter affiliate of ARVO.

After engineering school (Informatics and Electronics), Prof Reitsamer attended technical University of Vienna (Electro technical Engineering) and continued with Medical School at the Medical University of Vienna. He held a position as Associate Professor at the Department of Neurophysiology in Vienna. His expertise was focused on neurobiology and neurodegenerative diseases of the retina. He continued his education as a postdoc in Jeffrey Kiel's Lab and Stuart McKinnon's Lab at the Health Science Center in San Antonio and spent several years in the United States and Canada in different Universities (University of Texas, Northwestern University, Dalhousie University). Ever since his scientific interests were focused on ocular blood flow, retina neurobiology and disease as well as glaucoma research. Prof Reitsamer serves on numerous scientific committees and advisory boards and is appointed as reviewer and editor in various scientific journals.

Clinically, Prof Reitsamer is specialized in glaucoma and surgery of the anterior as well as the posterior segment of the eye. He was involved with the development of the glaucoma implants, the development of, Nano second and Femto second lasers for corneal and intraocular surgery and diagnostic tools based on OCT technology as well as blood flow diagnostics.

Bernd Riebesehl

Dr. Bernd Riebesehl is Principal Fellow & Technical Project Leader in the Pharmaceutical Development Parenteral & Topical Dosage Forms of Novartis Pharma AG, Basel, Switzerland since 2008.

At Novartis he is leading the Parenteral Technology Platform Nanomedicine and the early technical development of parenteral drug products. Externally Dr. Riebesehl has been serving as Advisory Board Member of the European Society of Clinical Nanomedicine, and chaired drug delivery sessions for the Section Drug Delivery of International Association for Pharmaceutical Technology (APV).

He completed his thesis in Pharmaceutical Technology at the Technical University of Braunschweig.

1992 he started his industrial career at Lilly Forschung GmbH in Hamburg leading several teams for preformulation, early phase development and formulation development. In his role as Research Advisor in Pharmaceutical R&D he led several initiatives enabling the formulation of poorly soluble drugs. In 2007 he became Director of Pharmaceutical Development at Speedel Experimenta AG, Basel.

Cristianne Rijcken

Cristianne Rijcken is CSO of Cristal Therapeutics, a pharmaceutical company developing first-in-class nanomedicinal products for the treatment of various diseases on the base of its proprietary polymeric technologies (CriPec®)

Cristianne studied Pharmacy and obtained her PhD at the Department of Pharmaceutics (both at Utrecht University, The Netherlands). Starting during her PhD project, she generated preclinical proof of concept of the improved therapeutic performance of CriPec®-based nanomedicines in various disease areas. This resulted in Cristianne founding Cristal Therapeutics in spring 2011. As CEO, she has assured the fast-forward clinical development of CriPec® docetaxel as lead product and initiated further platform development. Due to this early success, the management team was extended end of 2014, and Cristianne was appointed as CSO. Her ambition is to translate innovative technologies into products that have a clear competitive advantage for current medical needs. Cristianne was awarded multiple grants and prizes including the Simon Stevin Gezel Award in 2008 and the Knowledge for Growth Inspiring Young Scientist Award in 2014. She is (co-) author of 28 scientific publications and co-inventor of 5 patents.

Markus Rimann

Markus Rimann completed his degree in Biology, specialty in Biotechnology, at the ETH Zurich in 2005. As a PhD student he was developing a somatic gene therapy approach to improve cutaneous wound healing. He received his Dr. sc. nat. from the ETH Zurich in 2009. As postdoc at the Center for Applied Biotechnology and Molecular Medicine (CABMM) at the University of Zurich he was focusing on the usage and tracking of mesenchymal stem cells (MSCs) for the treatment of osteoporosis. Since 2011, he works at the Zurich University of Applied Sciences (ZHAW) in the Tissue Engineering team of Prof. Dr. Graf-Hausner. He was involved in initiating the TEDD (Tissue Engineering for Drug Development and Substance Testing) Competence Centre. The TEDD network is a collaborative innovation platform, dedicated to 3D cell culture te¬chnology and organ-like tissue models for drug development, substance testing, personalized and re¬generative medicine. The network pools and transfers knowledge and technologies by combining diverse skills through integrative cooperation among academic, clinical and industrial partners. He works with different 3D cell culture systems with or without scaffolds. His research is mainly application-driven to make organotypic model systems available for industry as well as for the clinics. The main focus is on developing 3D cell models for substance testing including automated production, maintenance and analysis. In an approach for personalized medicine osteosarcoma microtissues from patient-derived material were produced and subjected to drug treatment to determine in the future best cancer treatment options. With a scaffold-based cell culture system the entire process of cell encapsulation, maintenance, drug application and viability measurements was automated on a liquid-handling robot to demonstrate HTS-compatibility. As a group leader, he is interested in the further development of the promising bioprinting technology. In different industry projects several tissue models were printed: i) full-thickness skin model for the cosmetic industry to assess harmlessness of cosmetic ingredients, ii) muscle/tendon tissues in a novel 24 well plate for the pharma industry to find new therapeutic treatments for muscle-related diseases, and a kidney model (proximal tubulus of the nephron) to assess nephrotoxicity of drugs. In order to provide suitable bioinks the group is developing printable and cell compatible materials (hydrogels).

Steve R. Roffler

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Steve Roffler holds a permanent position as a Research Fellow and Division Coordinator of the Cancer Division in the Institute of Biomedical Sciences at Academia Sinica in Taipei, Taiwan. He received his B.S. degree from the University of Washington in Seattle and received a PhD in Chemical Engineering from the University of California, Berkeley in 1986. His research interests include antibody engineering, anti-polyethylene glycol antibodies, directed evolution of human enzymes for the treatment of cancer and rare diseases, targeted nanomedicine and prodrug therapy. He developed the first monoclonal antibodies with specificity for polyethylene glycol, which have been used worldwide to accelerate the clinical translation of PEGylated medicines. He has mentored more than 30 graduate and post-graduate students, published more than 100 papers in peer-reviewed journals, is a co-inventor of more than twenty patents and is responsible for numerous commercial licenses and material transfers.

SELECTED PUBLICATIONS

- WC Huang, PA Burnouf, YC Su, BM Chen, KH Chuang, CW Lee, PK Wei, TL Cheng and SR Roffler. Engineering chimeric receptors to investigate the size and rigidity-dependent interaction of PEGylated nanoparticles with cells. ACS Nano 10:648-662, 2016.
- HY Tung, YC Su, BM Chen, PA Burnouf, WC Huang, KH Chuang, YT Yan, TL Cheng and SR Roffler. Selective delivery of PEGylated compounds to tumor cells by anti-PEG hybrid antibodies. Mol. Cancer Ther. 14:1317-26, 2015.
- CH Kao, JY Wang, KH Chuang, CH Chuang, TC Cheng, YC Hsieh, YL Tseng, BM Chen, SR Roffler, TL Cheng. One-step mixing with humanized anti-mPEG bispecific antibody enhances tumor accumulation and therapeutic efficacy of mPEGylated nanoparticles. Biomaterials 35: 9930-40, 2014.
- YC Su, TS Al-Qaisi, HY Tung, TL Cheng, KH Chuang, BM Chen, and SR Roffler. Mimicking the germinal center reaction in hybridoma cells to isolate temperature-selective anti-PEG antibodies. Mabs 6: 1069-83, 2014.
- KC Chen, K Schmuck, LF Tietze and SR Roffler. Selective cancer therapy by extracellular activation of a highly potent glycosidic duocarmycin

Eder Lilia Romero

Eder Lilia Romero was educated at the National University of La Plata, Faculty of Exact Sciences Buenos Aires, Argentina where she obtained her MD in Biochemistry and PhD in Exact Sciences (1997). She performed her posdoctoral research at the Groningen University (Netherlands). She is currently a member of the scientific career at the National Scientific and Technical Research Council (CONICET), full professor of Chemistry at the Science and Technology Department and Director of the Nanomedicine Research Program-2 at the Universidad Nacional de Quilmes (UNQ). She has supervised 5, co-supervised 2 currently supervising 3 doctoral theses, published more than 50 articles in peer reviewed international journals, 5 book chapters, and given nearly 100 national and international

conferences and invited lectures. She is responsible for Nanomedicine Schools in Latin America and is an Editorial Board Member of the European Journal of Nanomedicine (De Gruyter) between other peer reviewed journals. Her main research interests are:
New and unique materials from sustainable sources: Nanovesicles and solid lipid nanoparticles made of archaeolipids, from hyperhalophile archaea from Patagonia, Cuyo, center and north west Argentine salt ponds.
Polymeric legos: Design of tecto-dendrimers (commercial dendrimers as units) for delivery of antitumoral agents.
Organic-metal hybrids: Design of lipid nano-vesicles /metal oxide nanoparticles (ZnO) or metal nanoparticles (Au) as source of oxidative stress triggered by UVA light, as vaccine adjuvants for topical route.
Focus on injectable avoidance: Design of nanostructures for drug delivery or adjuvancy to be administered by mucosal route (respiratory, oral) or skin: archaeosomes, pHsensitive archaeosomes, ultradeformable archaeosomes.
Design of anti-inflammatory, anti infective and anti atherosclerotic plaque therapeutic strategies

Barbara Rothen-Rutishauser

Prof. Dr. Barbara Rothen-Rutishauser has received her Ph.D. in 1996 in cell biology at the Swiss Federal Institute of Technology (ETH) in Zurich. From 1996 to 2000 she held a post-doctoral position in Biopharmacy at the Institute of Pharmaceutical Sciences at the ETH where she developed and characterised cell culture models for drug transport studies. In 2000 she joined Prof. Peter Gehr's research group at the Institute of Anatomy, University of Bern, Switzerland. During the period of her research B. Rothen-Rutishauser has become an expert in the field of cell-nanoparticle interactions in the lung, with a special focus on the development of alternative lung models. Since 2011 she is the new chair in Bio-Nanomaterials at the Adolphe Merkle Institute, University of Fribourg, Switzerland, the position is shared equally with Prof. Alke Fink. She has published more than 150 peer-reviewed papers and is an associate editor of the Particle and Fibre Toxicology.

Kumiko Sakai-Kato

Ph.D.
Section Head, Division of Drugs, National Institute of Health Sciences, Ministry of Health, Labour and Welfare

Kumiko Sakai-Kato received her B.S. and M.S. degrees from the University of Tokyo. She developed her carrier as a research scientist at a pharmaceutical company. She received her Ph.D. degree in analytical chemistry at the University of Tokyo in 2004. After postdoctoral work of the Japan Society for Promotion of Science, she became an assistant professor at Musashino University. In 2008, she became a section head of Division of Drugs at the National Institute of Health Sciences.
She is responsible for the regulatory science research on the evaluation for highly functional medicines, such as DDS drugs and nonomedicines. Her present major work is the development of an evaluation strategy of nanomedicines from the standpoint of quality, efficacy and safety. She worked as a rapporteur of the Joint MHLW/EMA reflection paper on the development of block copolymer micelle medicinal products, MHLW guideline for the development of liposome drug products, and MHLW reflection paper on nucleic acids (siRNA)-loaded nanotechnology-based drug products. She is also contributing to the expert discussions in the review of drug applications, and the revision of the Japanese Pharmacopoeia.

Kirsten Sandvig

Prof. Kirsten Sandvig is associated with Dept. of Biosciences, University of Oslo, Norway and she is heading a research group at the centre of excellence "Centre for Cancer Biomedicine", Institute for Cancer Research, The Norwegian Radium Hospital, Oslo University Hospital. The Norwegian Radium Hospital is the main cancer hospital in Norway. Sandvig's group, counting ~20 members from different countries, is interested in the mechanisms of endocytosis, intracellular transport and secretion. In some of our studies we are using protein toxins such as ricin and Shiga toxin, which are well established as markers for studies of membrane traffic, and which can be used as agents in cancer diagnosis and therapy. Our expertice is also applied to investigate uptake of nanoparticles, and we have obtained a large 5-year grant (Biodegradable nanoparticles in cancer diagnosis and therapy) from the Norwegian Research Council to build national competence in nanomedicine (running until Sept. 2018). This project involves collaboration between 10 Norwegian research groups covering synthesis of nanoparticles, *in vitro* and *in vivo* biology studies, *in vivo* imaging and clinical studies. In addition, international collaboration is included. The group is also involved in an INNO INDIGO granted project, starting April 2016. INNO INDIGO is an innovation-driven initiative for the development and integration of Indian and European research.We also characterize exosomes from prostate cancer cells and prostate cancer patients with the goal of detecting lipid and protein biomarkers. Our research spans all the way from basic to translational medicine, including innovation. We aim at providing a rational basis for diagnosis, treatment and prevention of disease. The group has extensive national and international collaboration.

EDUCATION:

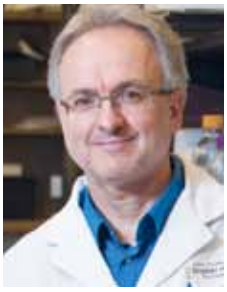
M.Sci. from The Technical University of Norway, Trondheim; Ph.D. from the Medical Faculty, University of Oslo, Norway. Research visits abroad at University of Michigan and at the biological laboratories, Harvard Cambridge, Mass. USA.

SCIENTIFIC ACTIVITY:

Published more than 300 articles and supervised a large number of Ph.D. students and master students. Sandvig has been invited as plenary speaker at more than 100 international meetings, and the work is heavily cited, Hirsch index is 71.

AWARDS AND HONOURS:

Anders Jahres Medical Prize for young researchers, 1989 (first woman to receive this prize); The Norwegian Research Councils research prize, 1990; Member of the Norwegian Academy of Science and Letters, 1993; Stiansens Biomedical Research Prize, 1995; King Olav V´s Cancer Research Prize, 1998; Member of EMBO (European Molecular Biology Organization), 1998; Member of Academia Europea from 2002; Honorary Doctor at the University of Copenhagen, Denmark, 2007; Member of the American Academy of Microbiology, 2010; The Fridjof Nansen Award for outstanding research in science and medicine, 2014.



Pere Santamaria

Dr. Pere Santamaria is a Professor in the Department of Microbiology, Immunology and Infectious Diseases and Chair of the Julia McFarlane Diabetes Research Centre at the University of Calgary. He is also cross appointed as Group Leader at the Institut D'Investigacions Biomèdiques August Pi i Sunyer in Barcelona. A graduate of the University of Barcelona (MD and PhD), he completed his medical specialty in immunology at the University Hospital in Barcelona and also a postdoctoral fellowship at the Institute of Human Genetics at the University of Minnesota.

Dr. Santamaria's research has focused on the cellular mechanisms that cause white blood cells to attack and destroy insulin-producing beta cells in the pancreas, to further our understanding of the mechanisms underlying autoimmunity. His work has culminated in the discovery of a novel immunological circuitry and a novel nanoparticle-based therapeutic approach targeting this circuitry that enables disease-specific therapeutic intervention in different autoimmune disorders.

Dr. Santamaria has written extensively autoimmunity and has published over 160 peer-reviewed articles. He is a reviewer of many journals and national and international granting agencies. He has been the recipient of various honors and awards, including the Canadian Diabetes Association Young Scientist Award, the Juvenile Diabetes Research Foundation (JDRF) Scholar Award, the Alberta Science Technology Leadership Foundation 2013 Outstanding Leadership in Alberta Technology Award and the 2016 Gerold & Kayla Grodsky Award from the JDRF. He is the scientific founder and chief scientist of Parvus Therapeutics, Inc., a biotechnology company as a vehicle to bring his therapeutic platform to the clinic.

Andrea Schenker-Wicki

Born in 1959 Prof. Dr. Dr. h.c. Andrea Schenker-Wicki holds a master degree in Food Engineering from ETH Zurich and a master degree in Business Administration from the University of Zurich. In 1990 she obtained a doctoral degree from the University of Freiburg in the field of Operations Research and Information Technology. She habilitated in 1996 at the University of St. Gallen with a thesis about the measurement of academic performance.

From 1990 to 1997 Andrea Schenker-Wicki worked at the National Emergency Operations Centre in Zurich, as a research associate and from 1993 on she also became its Head of the information office. For the subsequent four years she led the section for higher education at the Federal Office of Education and Science (today: State Secretariat for Education, Research and Innovation).

From 2001 to 2015 she held a full professorship in Business Administration at the University of Zurich and acted as the Director of the Executive MBA as well as the CAS program "Essentials of management". In addition, she was Vice President for Law and Economics at the University of Zurich between 2012 and 2014. On August 1, 2015, she became President of the University of Basel.

Andrea Schenker-Wicki has joined, among others, the Austria Science Board and the Council of the Zürcher Fachhochschule (ZFH). Furthermore she was part of the German Accreditation Council and presided over the Scientific Advisory Board of the Swiss Center of Accreditation and Quality Assurance in Higher Education (OAQ) from 2007 to 2012. From 2012 to 2015, she was a member of the Swiss Science and Innovation Council. In 2013 the University of Natural Resources and Life Sciences in Vienna awarded her an honorary doctoral degree.

Ruth Baumberger Schmid

Vice President Marketing
SINTEF Materials and Chemistry/Biochemistry and Nanomedicine/Polymer Particles and Surface Chemistry

EDUCATION

Diploma (1975) and PhD (1979) in Natural Sciences at ETH Zürich, Switzerland. Teaching physical organic chemistry at the NTNU for several years and supervised several diploma and PhD students.

EXPERIENCE

1980: Postdoctoral research at the Institute of Organic Chemistry, NTH
1981–1994: Research Scientist at SINTEF Applied Chemistry
1989–1991: Lecturer in physical organic chemistry at NTH
1994–1997: Senior Research Scientist at SINTEF Applied Chemistry
1997–2003: Research Director at SINTEF Applied Chemistry
2003–2004: Senior Research Scientist at SINTEF Applied Chemistry
2004–2011: Research manager at SINTEF Materials and Chemistry, Department of Synthesis and Properties, Research Team Polymer Particles and Surface Chemistry
2010–Present: Lecturer in Nanomedicine at NTNU
2011: Senior Research Scientist at SINTEF Materials and Chemistry, Department of Synthesis and Properties, Research Team Polymer Particles and Surface Chemistry
2011–Present: Vice President Marketing at SINTEF Materials and Chemistry

MAIN FIELDS OF COMPETENCE

- Scientific competence: Particle technology, encapsulation of solids and liquids, surface modification of polymers and composites, interactions between polymer surfaces and biological materials, targeted and controlled release, biodegradable polymers, biomaterials, nanomedicine, medical technology, organic chemistry t
- Business Development: Development of SINTEF's strategy in Life Sciences including Biotechnology, SINTEF's strategy in Medical Technology, a technology platform to prepare nano- and microparticles and -capsules based on the miniemulsion process for a broad variety of applications
- Management: Research Management, project management

PROFESSIONAL MEMBERSHIPS

- Member of the American Chemical Society
- Member of the Controlled Release Society (Board of Directors 2009 – present, Secretary 2012-2013, Treasurer-Elect 2013-2014, Treasurer 2014-2015)
- Member of the European Technology Platform in Nanomedicine
- Member of the External Advisory Board of the ERA-Nets EuroNanoMed and EuroNanoMed II
- Vice Chair and member of the Management Committee of the COST Action TD1004

PUBLICATIONS

40 scientific publications, 18 patent and patent applications, 54 oral presentations, 20 poster presentations, 16 webinar, mass media and popular science publications..

SELECTED PUBLICATIONS

- S.E. Borgos, A. Brunsvik, A. Kristiansen, F. Männle, Y. Mørch, R. Schmid, K. Vernstad & K. Zahlsen, 41st Annual Meeting of the Controlled Release Society, 13.-16.7.14, Chicago, IL, USA. "Mass Spectrometry for Comprehensive Characterization of Controlled Release Systems".
- Y.A. Mørch, S. Snipstad, A. Åslund, E. Sulheim, H. Baghirov, C. De Lange Davies, S. Berg, R. Hansen, & R. Schmid, Nordic Polymer Days, 1.-3.6.2015, Copenhagen, Denmark. "Ultrasound-enhanced drug delivery using nanoparticle-stabilized microbubbles."
- Y. Mørch, R. Hansen, S. Berg, A.K.O. Åslund, W.R. Glomm, S. Eggen, R. Schmid, H. Johnsen, S. Kubowicz, S. Snipstad, E. Sulheim, S.

Hak, G. Singh, B.H. McDonagh, H. Blom, C. de Lange Davies, P.M. Stenstad, Contrast Media and Molecular Imaging 10 (5), 356-366 (2015). "Nanoparticle-Stabilized Microbubbles for Multimodal Imaging and Drug Delivery."

- S. Armada, R. Schmid, H. Johnsen & N. Espallargas, in: Future Development of Thermal spray Coatings – Types, designs, Manufacture and Applications, Nuria Espallargas, ed., Elsevier Ltd, 2015, pp. 207-228. "Functionalized Thermal Spray Coatings."
- R. Schmid, XI Spanish-Portuguese Conference on Controlled Drug Delivery, 21.-23.1.2016, Granada, Spain. "Ultrasound-enhanced drug delivery using nanoparticle-stabilized microbubbles." (invited plenary speaker).

Christian Schönenberger

Professor of Experimental Physics,
University of Basel, Department of Physics, Switzerland.
Nanoelectronics group at the Department of Physics and the Swiss Nanoscience Institute, University of Basel, Klingelbergstrasse 82, CH-4056 Basel, Switzerland; E-mail: christian.schoenenberger@unibas.ch; Group web site: www.nanoelectronics.ch; Department web site: www.physik.unibas.ch; Swiss Nanoscience Snstitute (SNI) web site: www.nanoscience.ch

EDUCATION AND THESES

1990: Ph.D. in physics, ETH-Zürich, Switzerland: "Understanding Magnetic Force Microscopy".
1986: Diploma in physics, ETH-Zürich, Switzerland.
1979: Electrical Engineer of Applied Sceinces, HTL Technikum Winterthur

EMPLOYMENT

1995–: Full Chair in Experimental Physics at the University of Basel
1993–1995: Research Staff Member at Philips Research in Eindhoven, The Netherlands
1990–1993: Postdoctoral Fellow at Philips Research in Eindhoven, The Netherlands 1986-1990 PhD candidate in experimental physics, IBM Research laboratory,
Rüschlikon, Switzerland
1979–1980: Research assistant at the Molecular Spectroscopy Group of Prof. K. Dressler at Physical Chemistry, ETH-Zürich, Switzerland

HONORS

2012: Fellow of the American Physical Society **2012:** ERC advanced researcher grant
2010: Life-time member of the Swiss Academy of Technical Sciences (SATW)
1994: Profil-II award of the Swiss National Science Foundation
1991: Swiss Physical Society Price
1990: PhD medal from the ETH-Zürich

INTERESTS

Nano-electronics, charge- and spin-transport in low-dimensional systems, molecular electronics, spintronics, nanowire and quantum-dot physics, carbon nanotubes and graphene, shot-noise and charge-fluctuation phenomena, nanodevice based sensors.

PUBLICATIONS

- Role of hexagonal boron nitride in protecting ferromagnetic anostructures from oxidation, S. Zihlmann, P. Makk, C. A. F. Vaz, C. Schönenberger, 2D Materials (2016), 3, 011008.
- Gate tuneable beamsplitter in ballistic graphene, P. Rickhaus, P. Makk, M.-H. Liu, K. Richter, C. Schönenberger, Appl. Phys. Lett. (2015), 107, 251901.
- Shot noise of a quantum dot measured with GHz stub impedance matching, T. Hasler, M. Jung, V. Ranjan, G. Puebla-Hellmann, A. Wallraff, C. Schönenberger, Phys. Rev. Applied (2015) 4, 054002.
- Point contacts in encapsulated graphene, C. Handschin, B. Fülöp, P. Makk,S. Blanter, M. Weiss, K. Watanabe, T Taniguchi, S. Csonka, C. Schönenberger, Appl. Phys. Lett. (2015) 107, 183108.
- Resonant and inelastic Andreev tunneling observed on a carbon nanotube quantum dot , J. Gramich, A. Baumgartner, C. Schönenberger, Phys. Rev. Lett. (2015) 115, 216801.

PATENTS

- C. Schönenberger, M.A.M. Gijs, J.B. Giesbers, and J.A. Pals, Patent Number(s): WO9526547-A ; EP705474-A ; WO9526547-A2 ; EP705474-A1 ; WO9526547-A3 ; JP8511378-W
- M. Calame, M. Gräber, M.L.Perrin and C.Schönenberger, Patent Number(s): WO2012019819-A1 ; TW201215701-A

Avi Schroeder

PhD.
Assistant Professor of Chemical Engineering, Technion – Israel Institute of Technology.
E-mail: avids@technion.ac.il

Avi Schroeder is an Assistant Professor of Chemical Engineering at the Technion – Israel Institute of Technology where he heads the Laboratory for Targeted Drug Delivery and Personalized Medicine Technologies.
Dr. Schroeder conducted his Postdoctoral research at the Massachusetts Institute of Technology, after receiving his PhD from the Hebrew and Ben Gurion Universities.
Avi's laboratory focuses on the interface between nanotechnology and health, aiming to study and develop new tools for improving patient care.
He is the author of more than 30 research papers, an inventor on 14 patents, and was granted 20 national and international awards for his research achievements and innovation in the field of nanomedicine.

Felix Schumacher

Felix Schumacher holds a BSc and MSc in Biochemistry from the Technical University of Munich and a PhD in Chemical Biology from the University College London. He is co-founder of Thiologics, a small London-based biotech company that works in the field of bioconjugation. He joined Roche pRED/LMR in 2013 where his lab supports early phase development projects from EiH to PhI with molecular assessment and biochemical method development. Felix lives in Munich/Germany and works at the Roche Innovation Center Penzberg/Germany.

Simó Schwartz

Dr Simó Schwartz Jr (1967th, Barcelona) is the Director and Board member of the CIBBIM-Nanomedicine, which is focused on the research of new biomedical nanotechnology-based applications. In particular, new drug delivery systems, image based diagnostic systems and preclinical validation of therapeutic conjugates and bio-nanosensors, mainly in the areas of oncology and rare diseases. He is also member of the Science Advisory Board of the Vall d'Hebron Research Institute (VHIR) and member of the Science Ad-

visory Board of the European Nanotechnology Characterization Laboratory (EU-NCL). He also leads the "drug delivery and targeting group" at the CIBBIM-Nanomedicine. He helds 13 patents, most transfered to leading companies of the biotech and pharma sectors and coauthors more than 70 papers in high impact factor journals. Dr Schwartz Jr is coordinator and collaborator of several research projects directly related with the obtention and validation of therapeutic drug delivery systems. Among them are international and EU projects involving SME's in which animal models are being used for preclinical validation of new therapies directed against tumor cells. Dr Schwartz Jr is also member of the Nanomedicine Spanish Platform (NanomedSpain) and of the "European Platform for Nanomedicine". His research group is also a group member of the "CIBER de Bioingeniería, Biomateriales y Nanomedicina" (CIBER-BBN) of the Spanish Health Institute CarlosIII (ISCIII) which gathers a total of 45 research groups of national excellence in the field of nanotechnology and nanomedicine. Dr Schwartz Jr was the Nanomedicine Coordinador of CIBER-BBN at the national level and later appointed as Deputy Director and technology transfer coordinator. Dr Schwartz was also Co-founder and Science Advisor of ARGON Pharma SL (2008-2015), a Spin-Off company established at the Barcelona Science Park with the mission to develop new innovative therapies to provide solutions to unmet medical needs in the oncology field, and also to develop new technologies for drug delivery and diagnosis to improve current therapies. Dr Schwartz Jr is also member of the editorial Board of the journals Nanomedicine-NBM and the Eur. J. Nanomedicine. He is currently Science Advisor of SOM BIOTECH and CELGENE and member of the Advisory Board of NANOCAN, Southern Denmark University.

Giacinto Scoles

Adjunct professor – University of Udine (Fac. of Medicine), Distinguished scientist at ELETTRA Synchrotron Light Source Laboratory in Trieste P.I. of an Advanced Grant from the ERC Donner Professor of Science, Emeritus, Princeton University Distinguished Adj. Prof. of Physics and Biology, Temple Univ. Philadelphia

PERSONAL STATEMENT

GIACINTO SCOLES' scientific career has spanned an unusually long length of time and an equally unusually broad range of subjects. The general philosophy was to exploit new physical ideas and novel instrumentation to solve outstanding problems in chemistry and materials science before, and biology and medicine now. Much before the coming of age of Nanotechnology and Nanoscience, particular emphasis was given to the behavior of nano systems and materials

POSITIONS AND LEADERSHIP

2011 to date: Adjunct Professor, University of Udine, Faculty of Medicine, Department of Biological and Medical Sciences, Ospedale Universitario Santa Maria della Misericordia Building #13, Udine , Italy.
2011 to date: Distinguished scientist at ELETTRA Synchrotron Light Source Laboratory in Trieste (It).
2011 to date: Holder of an ERC Advanced Grant within the Program IDEAS at the Univ. of Udine
2008 to date: Donner Professor of Science, Emeritus, Princeton University, Princeton, NJ 08544, USA and Distinguished Adjunct Prof. of Biology, Temple University, Philadelphia, PA, (USA).
2003–2010: Professor of Biophysics at SISSA Miramare (Trieeste) Italy;
2009: Senior Consultant to the Inter. Center for Science and High Technology of the United Nations Industrial Development Organization (ICS-UNIDO) responsible for Nanotechnology & Nano Drug Delivery
2005–2009: Scientific Coordinator of LANADA the Laboratory for NAno Diagnostic, Drug Delivery and Analysis of CBM The Consortium for Biomolecular Medicine in Trieste (Italy).
2003–2009: Collaborator of ELETTRA, Sincrotrone Trieste S.C.p.A. Basovizza (Trieste), Italy;
1987–2008: Donner Professor of Science at Princeton University and Princeton Materials Institute;
1971–1986: Prof. of Chemistry and Physics Univ. of Waterloo, Waterloo, Canada;
1982–1985: Director of the Center for Mol. Beams and Laser Chemistry, University of Waterloo (Ca)
1977–1979: Professor of Solid State Physics, University of Trento, Italy;
1974–1975: Acting Director, of the Guelph- Waterloo Centre for Graduate Work in Chemistry.
1968–1971: Assoc. Prof., Physics Dept., University of Genova, Genova, Italy;
1964–1968: Assist. Prof., Physics Dept., University of Genova, Genova, Italy;
1961–1964: Research Associate, Kamerlingh-Onnes Lab., University of Leiden, The Netherlands
1960–1961: Assist. Prof., Physics Dept., University of Genova, Genova, Italy.

HONORS AND AWARDS

2013: Herschbach Medal for Chemical Dynamics; **2006**: Benjamin Franklin Medal in Physics (with J.P.Toennies) from the Franklin Institute; **2003**: Creativity Award from the NSF 2003-5 and Earle K. Plyler Prize for Molecular Spectroscopy from the American Physical Society (with Kevin K. Lehmann). **2002:** Peter Debye Award in Physical Chemistry from the American Chemical Soc.; **2000:** Elected Foreign Member of The Royal Netherlands Academy of Arts and Sciences and Honorary Science Doctorate from the University of Waterloo; **1996:** Recipient of an Honorary Doctorate in Physics from the University of Genoa; **1995:** Recipient of a Senior Fellowship of the Alexander von Humboldt Foundation and Recipient of the 1995 Lippincott Award of the Optical Society of America, the Coblentz Society, and the Society for Applied Spectroscopy; **1986:** Senior Killam Fellowship.

RESEARCH SUPPORT

Giacinto Scoles has been recently granted an advanced ERC grant (2011, MONALISA QUIDPROQUO, MOlecular NAnotechnology for LIfe Science Applications: QUantitative Interactomics for Diagnostics, PROteomics and QUantitative Oncology) of nearly 3M€ over 5 years for an ambitious collaborative research project. The focus and the goal of his research is to introduce innovative devices and protocols (based on micro/nano-fluidics and on the nano-mechanical response of bio-molecular nano-strucures) to carry out precise, quantitative and low cost measurements on large, predetermined diagnostically relevant, subsets of the proteome obtained from very small samples in samples produced by a very small number of cells or within single cells with potential capability of measuring its interactions (Interactomics). By means of these measurements he hopes to make new inroads into quantitative diagnostics and disease monitoring.

Hripsime Shahbazian

Mrs. Hripsime Shahbazian holds a MSc. in Medical Physics and a BSc in Molecular Physics. She joined Health Canada in 1988 as a Technology Assessor at the Medical Devices Bureau (MDB) and from 1991 to 1998 she acted in different managerial roles within the Bureau. In 1998 Mrs. Shahbazian joined the Office of Science within the Therapeutic Products Directorate (TPD) as an Associate Manager. She is currently a Senior Science Advisor in the Office of

Science. Her duties include management of the activities of Scientific/Expert Advisory Committees and Panels that are established to obtain medical/technical/scientific advice and recommendations on regulatory issues for drugs and medical devices in specific therapeutic areas/classes or on specific drug and medical device issues. She is responsible for reviewing Opportunity to be Heard Requests and Second Level of Appeals for medical devices and making recommendations for resolution of outstanding issues.
Mrs. Shahbazian is one of the key members working on nanotechnology related activities at Health Canada. Currently Mrs. Shahbazian chairs the Branch (HPFB) Working Group on Nanotechnology and coordinates Nanotechnology related international activities for regulated health products for the Branch. She is a member of the Health Portfolio Nanotechnology Working Group composed of key officials across the department, coordinating departmental approach to science, policy and research needs for nanotechnology. She is a member of the Ad Hoc Interdepartmental Discussion Group on Nanotechnology that was established in June, 2011 to provide an overview of departmental roles and interests in nanotechnology and update on departmental activities.
She serves as HC representative on the International Regulators on Nanotechnology Working Group that was established in summer of 2009 to discuss nanotechnology related issues relevant to regulated products that may contain nanoscale materials and currently represents HC on the Nanomedicines WG, that was established in 2015 within International Pharmaceutical Regulators Forum (IPRF) for the exchange of non-confidential information.

Sunil Shaunak

Studied medicine in London, Edinburgh and at Duke University, and received his PhD from Imperial College London. He became a Clinician-Scientist at the RPMS at Hammersmith Hospital London in 1991 and was awarded the first Personal Chair in Infectious Diseases at Imperial in 2004. He is a Fellow of the Royal Colleges of Medicine, Pathology and Tropical Medicine. Over the last 25 years, his research has focused on the discovery and clinical development of cost-effective new medicines for infection and inflammation. He has published 6 Nature journal papers and is the inventor of 42 patents. In 2001, he Founded PolyTherics to enable better biopharmaceuticals; this company was listed as Abzena plc on the London AIM stock market in 2014.

Amotz Shemi

Dr. Amotz Shemi is the Chief Executive Officer and a co-founder in Silenseed. Prior to Silenseed, Dr. Shemi served as a Senior VP Technologies in Medinol LTD, a leading medical-stent company, and beforehand as the CEO of Color Chip, a leader in Ion-exchange based Planar Lightwave Circuits (PLC); Dr. Shemi brings with him 25 years of experience in end-to-end management from concept level via development, regulatory approvals to actual sales. Shemi received his PhD degree in Physics and Astrophysics from the Tel Aviv University in Israel. Dr. Shemi is a lead inventor of more than dozen patents in the RNAi-delivery field, and an author and co-author of about 40 scientific papers.

Christina G. Siontorou

Assist. Prof. Christina G. Siontorou holds a BS (Hons) degree in Biomedical Science from the University of Sutherland (UK) and a PhD in Analytical Chemistry from the University of Athens (Greece). Her PhD had focused on the design and development of lipid membrane biosensors platforms. Subsequently she has spent 5 years working in the pharmaceutical industry as a drug developer consultant and for about 9 years held various academic appointments within the Department of Industrial Management and Technology at the University of Piraeus (Greece), from affiliated lecturer to lecturer and assistant professor. Her research interests involve the design of (bio)chemical technology products in laboratory scale. She has 40 publications in ISI Journals, 547 ISI citations (self-citations excluded) and an ISI h-index of 16. She has developed an expertise and line of research which takes an integrated view to assess the complete biosensor pipe line from beginning to end, namely, from design, to scale-up, deployment, robust operation and transition from the lab to the field, pursuing the entire spectrum of questions ranging from identification and characterization of materials appropriate for the design of sensors, to fault detection and diagnosis.

Tore Skotland

Tore Skotland is a biochemist by training and received his PhD from the University of Bergen, Norway in 1980. After 11 years at the university studying protein chemistry and enzymology, he moved to pharmaceutical R&D (Nycomed AS, Oslo, Norway) in 1983. He stayed within the same field of research for 26 years in one of the world leading companies developing contrast agents for medical imaging; Nycomed was bought by Amersham in 1997 and Amersham was bought by GE Healthcare in 2003. During the last 20 years in pharmaceutical R&D he was heading work to describe the biodistribution, metabolism and excretion of all types of contrast agents (water soluble as well as particle based) for CT, MRI, ultrasound, SPECT, PET and optical imaging. He has been involved in bringing 5 products to the marked (including 2 particle-based) and another 5 products into clinical trials (also including 2 particle-based). Skotland is the first or last author of publications related to all these 10 products. He is co-author of approx.100 publications and is used as referee for many journals in the field of bioanalysis, metabolism, biochemistry, nanomedicine and contrast agents for medical imaging.
Skotland is since 2009 a senior researcher at the Centre for Cancer Biomedicine (one out of three Centres of Excellence in biomedicine in Norway) at The Norwegian Radium Hospital, the main cancer hospital in Norway, being part of Oslo University Hospital. He is there a member of a group studying exosomes and endocytosis and intracellular transport of protein toxins and nanoparticles. This group is heading a 5-year national competence building project in Norway going up to autumn 2018. The project title is “Biodegradable nanoparticles for cancer diagnosis and therapy”. Skotland is co-ordinating the *in vivo* studies in this project, which has members from academia, university hospitals, research institutes and pharmaceutical industry. The 10 groups involved have expertise in nanoparticle syntheses and characterization, *in vitro* studies of cellular uptake and intracellular transport, immunology studies, and studies using small animals with xenograft models, including use of different *in vivo* imaging modalities such as MRI, PET/CT and fluorescence. Clinicians are also involved. Our group is also partner in an INNO INDIGO granted project starting April 2016. INNO INDIGO is an innovation-driven initiative for the development and integration of Indian and European research.

Most important publications in the field of nanoparticle research:

- Skotland T, Iversen TG, Sandvig K: New metal-based nanoparticles for intravenous use: requirements for clinical success with focus on medical imaging. Nanomedicine: NBM 6 (2010) 730-737.
- Iversen TG, Skotland T, Sandvig K: Endocytosis and intracellular transport of nanoparticles: Present knowledge and need for future studies. Nano Today 6 (2011) 176-185.
- Skotland T, Iversen TG, Sandvig K: Development of nanoparticles for clinical use. Nanomedicine (Future Medicine) 9 (2014) 1295-1299.

Alejandro Sosnik

Prof. Alejandro Sosnik received his Pharmacy degree from the Faculty of Pharmacy and Biochemistry of the University of Buenos Aires in 1994. After two years as junior research scholar of the University of Buenos Aires in the field of organic chemistry (1993-5), he worked as research pharmacist in the Department of Chemistry of the Argentine regulatory agency (equivalent to the US-FDA), a dependency of the Ministry of Health of Argentina (1996). In early 1997, he emigrated to Israel where after obtaining the pharmacist license, he continued his graduate studies, receiving M.Sc. (equivalency, 1998) and Ph.D. degrees in applied chemistry (polymeric biomaterials) from the Casali Institute of Applied Chemistry (The Hebrew University of Jerusalem, Israel, 2003) under the supervision of Prof. Daniel Cohn. In 2003-6, Prof. Sosnik spent a postdoctoral in the laboratory of Professor Michael Sefton (Institute of Chemical Engineering and Applied Chemistry/Institute of Biomaterials and Biomedical Engineering, University of Toronto, Canada) working in the development of hybrid matrices for cell culture and tissue engineering. Between 2006 and 2013, Prof. Sosnik was Assistant Professor (tenure) of Pharmaceutical Technology at the Faculty of Pharmacy and Biochemistry (University of Buenos Aires) and Investigator of the National Science Research Council of Argentina (CONICET, tenure). In this period, he established a research group that worked at the interface of drug crystallization and processing, biomaterials science, nanotechnology and microtechnology, drug delivery and therapeutics. In this context, he supervised three junior staff scientists (CONICET), five postdocs (CONICET) and four Ph.D. theses at the Faculty of Pharmacy and Biochemistry of the University of Buenos Aires. Prof. Sosnik established the "Iberoamerican Network of New Materials for the Design of Advanced Drug Delivery Systems in Diseases of High Socioeconomic Impact" (RIMADEL) of the CYTED Program that gathered eleven research groups and companies of Spain, Portugal, Mexico, Cuba, Colombia, Brazil and Argentina and over 75 scientists and served as its international coordinator in the period 2011-2013. He also served as advisor of several Argentine pharmaceutical companies in scientific, technical and intellectual property issues. In late 2013, Prof. Sosnik was appointed Associate Professor of the Department of Materials Science and Engineering of Technion-Israel Institute of Technology where he founded the Laboratory of Pharmaceutical Nanomaterials Science. He currently supervises one postdoc, nine graduate students and six undergraduate students. His current research lines comprise drug self-assembly and crystallization phenomena and processing, polymer and macromolecular chemistry, biomaterials science, colloidal chemistry (drug and polymer self-assembly), mucoadhesive drug delivery systems, nanomedicine (drug encapsulation, release and targeting), therapy of poverty-related diseases (HIV, tuberculosis), pediatric cancer, intestinal diseases and pharmacokinetics (oral, inhalatory and intranasal administration routes) in both preclinical and clinical trials. He was Visiting Professor and Scientist at the National University of Colombia (Colombia), the University of Santiago de Compostela (Spain), the Council for Scientific and Industrial Research (South Africa), North-West University at Potchefstroom (South Africa), the National Autonomous University of Mexico (Mexico), the Hospital Sant Joan de Déu (Spain), the Free University of Berlin (Germany), the National Institute of Materials Science (Japan) and the University of Helsinki (Finland) where he taught graduate courses and presented invited conferences. In addition, he has served and serves as evaluator for more than twenty national and international research funding agencies and universities. Prof. Sosnik is co-author of over 110 peer-reviewed articles, reviews, editorials and book chapters in areas of pharmaceutical research and development and innovation, and co-inventor in three patents and patent applications related to biomedical and pharmaceutical innovation.

Scott Steele

Ph.D.

Scott Steele serves as the Director of Government and Academic Research Alliances at the University of Rochester (USA), where he facilitates strategic research and educational partnerships between the University and government agencies and laboratories, industry, and other academic institutions. He is actively involved in developing regulatory science educational programs at their Clinical and Translational Science Institute, serving as Program Director for a new Certificate in Regulatory Science and as Core Director of the Regulatory Science to Advance Precision Medicine Function. Dr. Steele also coordinates national Clinical and Translational Science Award affiliated initiatives, including co-leading the development of a set of Regulatory Science competences to guide training and education in this area. He is an associate professor in the Department of Public Health Sciences, where his academic interests are focused on a range of science and technology policy issues including translational research and regulatory science, public health preparedness, and national security. He also serves as the Deputy Director of the Goergen Institute for Data Science. Dr. Steele recently chaired a subcommittee of the FDA Science Board evaluating the FDA Centers of Excellence in Regulatory Science and Innovation.

Prior to joining the University of Rochester, Dr. Steele served in the U.S. White House Office of Science and Technology Policy (OSTP), initially as a policy analyst and later as the Executive Director of the President's Council of Advisors on Science and Technology (PCAST). Dr. Steele coordinated PCAST studies addressing issues in personalized medicine, nanotechnology, information technology, energy technologies, and approaches to enhance university-private sector research partnerships. At OSTP, he also led several programs related to biosecurity, medical countermeasures development, biotechnology, and science education. Dr. Steele received his BS with Honors in Biology from Union College in Schenectady, NY. Following this, he performed research at the General Electric Center for Research and Development (NY), was a fellow at the National Institutes of Health (Bethesda, MD) and performed research at the University of Geneva (Switzerland). Dr. Steele completed his MA and PhD in Molecular Biology at Princeton University.

David Stepensky

Dr. David Stepensky is an Assistant Professor from the Department of Clinical Biochemistry and Pharmacology, Ben-Gurion University of the Negev, Beer-Sheva, Israel. He is a pharmacist with expertise in a field of drug delivery and targeting, pharmacokinetics, and pharmacodynamics. He completed his B.Sc.Pharm., M.Sc.Pharm., and Ph. D. studies at the Dept. of Pharmaceutics, School of Pharmacy, The Hebrew University of Jerusalem, Israel. After military service as a pharmacist in the Israeli Defense Forces, he performed post-

doctoral studies at the Dept. of Immunology, Weizmann Institute of Sciences, Rehovot, Israel and at the Dept. of Immunobiology, Yale University, New Haven, CT, USA. His primary research interests are analysis of pharmacokinetic and pharmacodynamic properties of pharmacological agents (small molecular weight drugs and biopharmaceuticals) and development of nanovesicle- and nanoparticle-based systems that can efficiently deliver these agents to their site of action. He has authored over 50 papers and book chapters in these and related research areas.

PUBLICATIONS

- Kozlovskaya L, Popilski H, Gorenbein P, Stepensky D. *In vitro* toxicity of infusion sets depends on their composition, storage time and storage conditions. Int J Pharm. 2015, 489(1-2):285-93.
- Maity AR, Stepensky D. Delivery of drugs to intracellular organelles using drug delivery systems: analysis of research trends and targeting efficiencies. Int J Pharm. 2015, 496(2):268-74.
- Ruzov M, Rimon G, Pikovsky O, Stepensky D. Celecoxib interferes to a limited extent with aspirin-mediated inhibition of platelets aggregation. Br J Clin Pharmacol. 2016, 81(2):316-26.
- Maity AR, Stepensky D. Limited efficiency of drug delivery to specific intracellular organelles using subcellularly "targeted" drug delivery systems. Molec Pharmaceutics, 2016, 13(1):1-7.
- Maity AR, Stepensky D. Efficient subcellular targeting to the cell nucleus of quantum dots densely decorated with nuclear localization sequence peptide. ACS Applied Materials & Interfaces, 2016, 8(3):2001-9.

Gert Storm

contact: g.storm@uu.nl

Prof. Dr. Gert Storm is a (bio)pharmaceutical scientist at Utrecht University. His research interests are in the fields of biopharmaceutics and advanced drug delivery/drug targeting. Since 2009 he has been Honorary Professor in Biomacromolecular Drug Delivery at the University of Copenhagen. In 2000 he was appointed as a professor (Targeted Nanomedicine) at Utrecht University. Since 2012 he is also professor (Targeted Therapeutics) at the MIRA institute of the University of Twente (Netherlands). Besides, he keeps a position (Imaging-Guided Drug Delivery) at the University Medical Center Utrecht (UMCU) within the Centre for Image-Guided Oncological Interventions, and is Visiting Professor at the Department of Pharmacy at the National University of Singapore. He is the (co-) author of about 500 original articles, reviews and book chapters. His H-index is >75 (Google Scholar), and he is included in the 2014 and 2015 lists of The World's Most Influential Scientific Minds of Thomson Reuters (Highly Cited Researchers, period 2002-2013).

Erik Stroes

Professor Erik Stroes is Professor of Medicine and Chairman of the department of Vascular Medicine at the Academic Medical Center (AMC), Amsterdam, the Netherlands. He received his Internal Medicine degree from UMCU, Utrecht in the Netherlands and conducted a fellowship in Nephrology.Professor Stroes has a particular interest in lipid disorders in relation to atherogenesis and has participated in numerous lipid lowering trials. He is a member of the American Heart Association - Atherosclerosis council and the Advisory board Dutch Heart foundation and Chair of the Dutch Atherosclerosis Society. Prof. Stroes has authored or co-authored over 300 articles in international peer review publications such as NEJM, JACC, ATVB, Diabetes and the JLR.

Janos Szebeni

Janos Szebeni, M.D., Ph.D., D.Sc., Med. Habil., immunologist, director of the Nanomedicine Research and Education Center at Semmelweis University, Budapest, Hungary. He is also founder and CEO of a contract research company, SeroScience Ltd., and professor of immune biology at the University of Miskolc, Hungary. During his 40-years professional career he has held various scientific positions in Hungary and abroad, mainly in the United States, where he lived for 21 years. Among others, he worked for 6 years at NCI (NIH) in Maryland, 2 years at Harvard University in Boston, and 11 years at the Walter Reed Army Institute of Research in Washington DC. His research on various themes in hematology, membrane biology and immunology has resulted in over 130 papers and chapters (with citations: ~5000), two granted patents, and a book entitled "The Complement System: Novel Roles in Health and Disease" (Kluwer, 2004). Three fields stand out where he has been most active: artificial blood, liposomes and the complement system. His original works from the late 1990s led to the "CARPA" concept, i.e., that complement activation underlies numerous drug-induced pseudoallergic infusion reactions

Ennio Tasciotti

Ph.D.

Dr. Tasciotti received a PhD in Molecular Medicine and trained as a postdoctoral fellow in Nanomedicine at the University of Texas Health Science Center. In 2010, he joined the Houston Methodist Research Institute as co-chair of the department of Nanomedicine. Currently he serves as Director of the Center for Biomimetic Medicine, and Director of the Surgical Advanced Technology Laboratory. Dr. Tasciotti directs a large research operation, with 30 research staff and trainees involved in the development of nano- and bio-materials to target inflammation, modulate immune response, and improve musculoskeletal tissue regeneration. The overarching principles of Dr. Tasciotti's research revolve around the creation of biomedical and surgical technologies inspired to biological processes and structures. His bio-inspired delivery platforms and scaffolds mimic the architecture, composition, and function of native tissues and live cells.

Dr. Tasciotti's areas of expertise are: Nanotechnology, Regenerative medicine, Bioengineering, Drug delivery, and Cancer therapy. The main research projects currently being carried on in his laboratory are on: Biomimetic materials to improve tissue regeneration and modulate the immune response; Bio-inspired delivery platforms to target tissue inflammation; Nanostructured injectable hydrogels for the localized release of clinically approved drugs; and Development of new surgical procedures based on nanomaterials and nanodevices. He is an inventor on 8 U.S. patents, authored 90 scientific papers, and was invited as a speaker at more than 50 international meetings. Dr. Tasciotti serves as reviewer for more than 30 scientific journals, and is a stable member of NIH and DoD study sections.

John Thornback

Dr Thornback was educated at Imperial College London where he graduated with 1st Class Honours in Chemistry and followed up with a Ph.D, in Inorganic Chemistry with Prof. Sir Geoffrey Wilkinson, Nobel Laureate at the same institution. He then held several academic posts at University of London and Loughborough University in UK he joined industry as Head of Radiopharmaceutical R&D for Medgenix SA, in Belgium, one of Europe's first biotech companies. He subsequently took senior positions within sales and marketing and general management which has led to
28 years of diagnostic industry experience in executive management, including CEO positions in Canada, UK and Singapore. He has raised start up funding for a number of companies and changed large public research centres to successful private enterprises by maintaining academic excellence but delivering commercial targets. He has created senior management teams in both N.America and Europe and managed companies through to exit, including trade sale and JV. Commercially, he has identified, negotiated and implemented key commercial partnerships and technology licencing deals that radically improved the businesses and developed healthy pipelines of products and established route to market through global commercial networks.
He has been Non Executive director of commercial and government enterprises since 2004 as well as a member of Scientific Advisory Boards and professional organisations maintaining strong links and networks to academic and scientific communities.
He is currently Managing Director of Apta Biosciences Pte Ltd based in UK and Singapore, an advisor to A*Star, the Singapore Biotechnology hub and a Director of the Centre of Probe Development and Commercialisation(Hamilton, Canada) and Sestria Ltd(UK).
He has published more than 100 original scientific articles, more than 20 patents and has co-written one book along with numerous presentations and abstracts at scientific conferences.

Donald A. Tomalia

Ph.D.
CEO/Founder
NanoSynthons LLC, National Dendrimer & Nanotechnology Center, 1200 N. Fancher Avenue,Mt. Pleasant, MI 48858 USA

Dr. Tomalia is the CEO/Founder of NanoSynthons LLC and National Dendrimer & Nanotechnology Center, Distinguished Visiting Professor (Chemistry Department) Columbia University, NY; Adjunct Professor (Department of Chemistry) University of Pennsylvania, PA and Affiliate Professor (Department of Physics) Virginia Commonwealth University, VA. He received his B.A. in Chemistry from the University of Michigan and Ph.D. in Physical–Organic Chemistry from Michigan State University while working at The Dow Chemical Company (1962-1990). He has founded three dendrimer-based nanotechnology companies; namely: NanoSynthons LLC (2010), Dendritic Nanotechnologies, Inc. (2001) and Dendritech, Inc. (1992). Other positions currently held by Tomalia include: Advisory Board CLINAM, European Foundation for Clinical Nanomedicine; Sr. Scientific Advisor to the European Union CosmoPHOS Nano Project (2012-present). Dr. Tomalia also serves as Faculty Member, Faculty 1000 Biology; Associate Editor, Journal of Nanoparticle Research (Springer); Editorial Advisory Board, Nanomedicine (Elsevier) and Current Bionanotechnology.
He is the pioneering scientist/inventor associated with the discovery of poly(oxazolines) (Industrial Research-100 Awards in 1978 & 1986) and dendrimers. His 1979 discovery of dendrimers (dendritic polymer architecture) led to a third R&D-100 Award in 1991 and the Leonardo da Vinci Award (Paris, France) in 1996. He received the International Award of The Society of Polymer Science Japan (SPSJ) (2003) which recognized his discovery of the fourth major macromolecular architectural class; namely, dendritic polymers. He was the invited "Linus Pauling Memorial Lecturer" (2010) Portland, OR and recipient of the Wallace H. Carothers Award (American Chemical Society) (2012).
He has authored/co-authored over 265 peer-reviewed publications with more than >20,300 citations and granted >128 U.S. patents. Over 170 papers are focused in the dendrimer/dendritic polymer field including two monographs entitled: Dendrimers and Other Dendritic Polymers (J. Wiley) co-edited with J.M.J. Fréchet (2001) and more recently Dendrons, Dendrimers, Dendritic Polymers (Cambridge University Press (2012)). His review article entitled: "Starburst Dendrimers: Molecular Level Control of Size, Shape, Surface Chemistry, Topology and Flexibility from Atoms to Macroscopic Matter," D.A. Tomalia, A.M. Naylor W.A. Goddard III, Angew. Chem. Int. Ed. Engl., 29(2), 138 (1990) has > 2,820 citations. Tomalia was inducted into the Thomson Reuters Hall of Citation Laureates in Chemistry (2011) (i.e., top 40 most highly cited scientists in the field of chemistry).
Tomalia is recognized as a pioneer in dendritic polymers and international focal point for activities related to dendrimer-based nanotechnology and nanomedicine. His extensive studies on dendrimers provided a conceptual window to his recent development of a systematic framework for defining and unifying nanoscience. This concept is now accepted by both chemists and physicists as cited in "Developing Superatom Science" (Chemical & Eng. News (USA), April 15, 2013) and "In Quest of a Systematic Framework for Unifying and Defining Nanoscience" (Modern Physics Letters B, 28, (3), 1430002, 2014). This paradigm proposes the application of traditional first principles to discrete nano-building blocks (i.e., nanoelement categories) which are found to behave much like picoscale atoms by exhibiting stoichiometries, heuristic surface chemistries and nanoperiodic property patterns/relationships associated with traditional atoms ("A Systematic Framework and Nanoperiodic Concept for Unifying Nanoscience: Hard/Soft Nanoelements, Superatoms, Meta-Atoms, New Emerging Properties, Periodic Property Patterns and Predictive Mendeleev-like Nanoperiodic Tables," Chem. Rev., 16, 2705-2774, 2016. Tomalia is now applying this nanoperiodic paradigm and many of these principles to nanomedicine (J. Intern. Med., 276, 579-617, 2014).

Chris Torrance

Chief Executive Officer, PhoreMost Ltd.
www.phoremost.com

Dr Chris Torrance is a cancer researcher and entrepreneur. In 2007 he founded Horizon Discovery to translate advances in human genome editing into a range of research tools and services to accelerate the discovery of new and improved 'Personalized Medicines', including the identification of novel drug targets for pharmaceutical development. By 2014 Dr Torrance and his colleagues had built Horizon into the fastest growing Biotech company in the UK. In the same year, the company listed on the London Stock Exchange with over 100 commercial and scientific staff and significant deal-flow with the pharmaceutical Industry, for which Horizon received the Queens Award for International Trade in 2012.
Previously, Dr Torrance was Head of Oncology and Biology at the UK Biotechnology company Vernalis PLC (LSE: VER), where he was responsible for progressing several novel kinase oncology programs.
Dr Torrance has a bachelor's degree in Biomedical Technology from Sheffield Polytechnic; a PhD in Biochemistry from East Carolina University (U.S.A) and completed Post-Doctoral training in the laboratory of Professor Bert Vogelstein at the Johns Hopkins University (U.S.A), where he pioneered the use of 'X-MAN' isogenic

disease models in high-throughput screening and drug discovery. In 2014, Dr Torrance and Dr Venkitaraman (Cambridge University) founded PhoreMost Ltd to develop a new technology platform solution to the next big issue in delivering Personalized Medicine, which is developing a toolbox of therapies big enough and affordable enough to impact the diverse array of key targets in cancer and other complex diseases, most of which are intractable to current drug discovery technologies.

Luisa Torsi

Luisa Torsi is full professor of Chemistry since 2005 and is the President of the European Material Research Society (E-MRS), being the first women to hold both these roles. She received her laurea degree in Physics from the University of Bari in 1989 and the PhD in Chemical Sciences from the same institution in 1993. She was post-doctoral fellow at Bell Labs from 1994 to 1996. In 2005 and 2006 she was invited professor at the University of Anger and Paris 7, respectively and has been coordinator of the council for the degree courses in Materials Science and Technology at the University of Bari from 2011 to 2015.

In 2010 she has been awarded with the Heinrich Emanuel Merck prize for analytical sciences, this marking the first time the prestigious award is given to a woman. She is also the recipient of the 2013 "Best Italian Inventor Women" prize of the Italian Women Inventors & Innovators Network (IT–WIIN). This award made her eligible for the Global-WIIN competition where she has been awarded with the main overall platinum prize for 2015. She has also served as Chair of the E-MRS 2012 (Strasbourg) and of the MRS 2015 Fall Meeting (Boston). Her principal scientific contributions are in the fields of advanced materials and electronic devices mostly employed for sensing applications. Recently she co-investigated interfacial electronic effects in functional biological systems integrated into organic field-effect transistors. The devices shows exceptional sensitivities (down to pM detection limits) and selectivity.

Torsi has authored more than 150 scientific contributions, including papers published in Science, Nature Materials, Nature Communications, PNAS and is co-inventor of several awarded international patents. Her works gathered over 7800 citations resulting in an h-index of 43 (Google scholar). She has given more than 130 invited lectures, including plenary and key notes to international conferences. Awarded research funding comprises several European contracts as well as national and regional projects.

Panagiotis N. Trohopoulos

Dr med Panagiotis (Panos) N. Trohopoulos is a Distinction of Excellence Greek (Ellin) Medical Doctor, his Specialty is Cardiologist, and he is based in Thessaloniki, Greece (Ellas).

Dr med Trohopoulos is the Founder (12 years ago, since 2004) and the Scientific/Exploitation/Strategic Coordinator of the CosmoPHOS-nano Project (GA 310337) which is a Large-scale EU FP7 NMP Funded Translational Nanomedicine R&D Project in Cardiovascular Diseases, and more specifically in Atherosclerotic Heart Disease. The Project co-funded by the European Union under the FP7 Programme/NMP Theme (Nanosciences, Nanotechnologies, Materials and New Production Technologies) with 8,5 Million Euros, and additionally co-funded by All Project Beneficiaries with 4,5 Million Euros, having a total project budget of 13 Million Euros. The EU FP7 NMP Funded Large-Scale CosmoPHOS-nano Project (GA 310337) is a Multidisciplinary Five-year R&D Project started on March 1, 2013 and will be concluded on February 28, 2018, and consists of 19 World-Class Participants, including 13 Universities and Research Foundations and 6 Companies, from 11 European Countries, Japan, and USA, with a wide variety of complementary and cutting-edge scientific, technological and manufacturing expertise and know-how. The EU FP7 NMP Funded Large-Scale CosmoPHOS-nano Project (GA 310337) is the World's Largest R&D Project of Nanomedicine in Cardiology aiming to develop a Radical Innovative Theranostic (Diagnostic and Therapeutic) "Smart" Nanomedicine Product, the CosmoPHOS System, to enable: a) Near-Infrared Fluorescence-based Molecular In Vivo Imaging (NIRF-based Molecular In Vivo Imaging), b) Targeted Near-Infrared nanoPhotodynamic Therapy (Targeted NIR nanoPDT), and c) Real-time and Follow-up Therapy Monitoring of Atherosclerotic Coronary Artery Disease (CAD) of the Heart, which is the number one cause of human death and morbidity in Europe and worldwide. The CosmoPHOS-nano Project (GA 310337) is the First EU FP7 NMP Funded Large-scale R&D Project planning to apply Nanomedicine for Cardiac Patients. It foresees conducting during the final Project-year, a First-in-man Phase-I Clinical Trial in CAD Patients, to evaluate the safety and feasibility of the novel CosmoPHOS System for human use.

Dr med Trohopoulos is also the Founder/Owner/Managing Director of the CosmoPHOS Ltd, which is an Innovative European SME established in Thessaloniki, Greece (Ellas). CosmoPHOS Ltd is focused on the Translational Research & Development of Novel Nanomedicine Products for Early Diagnosis, Targeted Therapy, and Therapy Monitoring of Diseases, with main focus in Cardiovascular Diseases, and especially in Atherosclerotic Heart Disease which causes the myocardial infarctions (heart attacks) and in Atherosclerosis in general.

Dr med Trohopoulos is also an Executive Board Member and Vice-chair of the Working Group Business of ETPN (European Technology Platform Nanomedicine), which is an Initiative led by the Industry and set up together with the European Commission, addressing the application of nanotechnology to achieve breakthroughs in healthcare.

Additionally, Dr med Trohopoulos is an Advisory Board Member and Fellow Member of CLINAM (European Foundation for Clinical Nanomedicine) which is a non-profit institution based in Basel Switzerland aiming at advancing medicine to the benefit of individuals and society through the application of nanoscience.

Finally, Dr med Trohopoulos is Founding Member and Steering Board Member of the International Society for Nanomedicine, Fellow Member of the European Society for Nanomedicine, Member of the Hellenic Cardiological Society, and Member of the European Society of Cardiology.

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Prabitha Urwyler

Prabitha Urwyler is a senior researcher at Gerontechnology and Rehabilitation group of the University of Bern.

Prabitha Urwyler was born in Mangalore, India on March 3rd, 1974. After receiving a Bachelor of Technology (B.Tech) in Computer Engineering from Mangalore University, India in 1995, she worked as a software developer at Melstar Information Technologies Ltd, Mumbai, India until 1997. In 1997, she joined the Swiss News Agency (SDA – ATS) in Bern, Switzerland as a software engineer and continued working there until the end of master studies.

She earned her M.Sc in Biomedical Engineering from the University of Bern in 2008. She joined the Laboratory of Micro and Nanotechnology at the Paul Scherrer Institut to work on her doctoral project focused on fabrication, characterization and application of disposable micro-cantilevers for biomedical applications. Her doctoral project was a collaborative project between the Biomaterial

Science Center, University of Basel, Paul Scherrer Institut and University of Applied Sciences Nord-West Schweiz. She completed her PhD degree in Biomedical Engineering from the University of Basel in January 2012 and continued to work at the Biomaterials Science Center, University of Basel as a Postdoc until 2013.
She joined the Gerontechnology and Rehabilitation group as a Postdoctoral Researcher in May 2013 and is working on projects related to visual hallucinations and activity recognition. Owing to interdisciplinary background, her research interests include biomaterials, implant surfaces and characterization, micro/nanofabrication, Gerontechnology, tele-rehabilitation, ambient assisted living and neurodegenerative diseases.

Gooitzen M. van Dam

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University Medical Center Groningen (UMCG), Department of Surgery, Division of Abdominal and Surgical Oncology Department of Nuclear Medicine and Molecular Imaging Department of Intensive Care.P.O. Box 30.001
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Dr. van Dam attended the University of Groningen, Faculty of Medical Sciences, from 1995–1992. As a Fulbright fellow he initiated the foundation for his PhD-thesis on prognostic models in primary biliary cirrhosis (1998). During a research fellowship at the NIH/NCI at the Radiation Biology Branch, his interest was awakened by the initiation of Molecular Imaging Programs in the US. After his return to the University Medical Center in Groningen in 2002, he became staff member and certified surgical oncologist and gastrointestinal surgeon since 2004. He has been the founder of the Small Animal Bio-Optical Imaging Center Groningen, which has paved the way towards clinical applications since 2007 and co-founder of the European Society of Molecular Imaging and more recently the International Society of Image Guided Surgery. The main focus of the Optical Molecular Imaging Group Groningen lead by dr van Dam and dr Nagengast (gastroenterology) is translational bio-optical imaging and therapy in cancer and infectious diseases, i.e. image-guided surgery, pathology and endoscopy for the detection of tumor cells and locoregional metastases including so-called photo-immunotherapy and bacterial detection in lung or implant infections.
Bio-optical imaging may provide the surgeon and pathologist with light-beacons for radical excision of tumor tissue and in case of irradical resection a tool for guidance for intraoperative radiotherapy or targeted therapeutic modalities like targeted radiotherapy, photodynamic therapy or plasmonic nanobubbles. Translational research in bio-optical imaging relates to various fields of medicine and in particular surgery and endoscopy in solid tumors. For preclinical applications (transgenic) animal models, fluorescent probes and instrumentation is available at the UMCG. Moreover, emphasis is aimed at in-house GMP synthesis of fluorescent tracers, with different characteristics such as antibody-based, nanobody-based, small-peptides, smart-activatable probes etc. Clinical camera prototypes for intraoperative imaging have been developed, tested and approved by the Investigational Research Board within the consortium UMCG/TUM (Technical University of Munich, prof Ntziachristos)[1]. Prof van Dam has published over more than 110 peer reviewed papers (h-index 30) and his research on translational optical imaging expanding towards theranostics in the field of oncology (www.betacure.eu), infectious diseases/inflammation[2] and cardiovascular diseases.

[1] van Dam GM, Themelis G, Crane LMA, Harlaar NJ, Pleijhuis RG, Kelder W, Sarantopoulos A, de Jong JS, Arts HJG, van der Zee AGJ, Bart J, Low PS, Ntziachristos V Intraoperative tumor-specific fluorescent imaging in ovarian cancer by folate-receptor-alpha targeting: first in-human results. Nat Med. 17:1315-9

[2] van Oosten M, Schäfer T, Gazendam JA, Ohlsen K, Tsompanidou E, de Goffau MC, Harmsen HJ, Crane LM, Lim E, Francis KP, Cheung L, Olive M, Ntziachristos V, van Dijl JM, van Dam GM. Real-time *in vivo* imaging of invasive- and biomaterial-associated bacterial infections using fluorescently labelled vancomycin. Nat Commun. 2013;4:2584. doi: 10.1038/ncomms3584.

Hans van der Voorn

Hans van der Voorn is the Executive Chairman and CEO for Izon Science Ltd, based in Oxford, UK. He originally trained as an engineer in New Zealand. Hans was one of the founders of Izon and became its full-time CEO in 2007. He has been the inventor on several Izon patents and has a particular interest in developing high quality and reliable nano-measurement capabilities for biomedical use.
In particular, he is interested in Tunable Resistive Pulse Sensing (TRPS) and its applications to nanomedicine development and extracellular vesicle research for therapeutics and clinical diagnostics.

Peter van Hoogevest

Peter van Hoogevest, is a pharmacist by training (Utrecht University in The Netherlands), who got his PhD degree in biochemistry 1984 at the Utrecht University in The Netherlands. In 1994 he received the degree of Privat Dozent in pharmacy at the University of Basel, Switzerland.
His industrial career started at the Biovet Group of the Animal Health Division of Ciba-Geigy Ltd. (Basel) in 1994. Shortly thereafter he obtained a position at the Novel Dosage Form Department of Pharmaceutical Development of the Pharmaceuticals Division of Ciba-Geigy Ltd. After having several positions at this department at Ciba Ltd. and Novartis Ltd. he founded in 1998 together with colleagues of the Pharmaceutical Development Department and reputed industrial managers and scientists the company ADD Advanced Drug Delivery Technologies (Muttenz, CH) and became CEO of this company and was member of the Board of Directors. In 2000 he joined Phares Drug Delivery AG (Muttenz, CH), a company specialized in the delivery of poorly water soluble drug substances, as Managing Director and COO and member of the Board of Directors. Since 2012 he is Managing Director of the Phospholipid Research Center, Heidelberg and Head of the Scientific Department (including the Development Department) of Lipoid GmbH, Ludwigshafen am Rhein, Germany.
Because of this work experience and scientific background, he obtained a very broad experience in the pharmaceutical industry covering, business development and pharmaceutical technology development aspects of small and big Pharma industries and the pharmaceutical/cosmetic/dietetic excipient industry. His drug delivery expertise especially in the (phospho)lipid research and development area is underscored by 59 scientific publications, including 7 book chapters, 30 symposium posters, co-promotion of 47 PhD Theses, 13 patents and 44 patent applications.

Subbu Venkatraman

Subbu Venkatraman obtained his Bachelor's and Master's degrees from India, then a PhD in Polymer Science from Carnegie-Mellon University, Pittsburgh. Following a post-doctoral fellowship at University of Pittsburgh, he was hired into the R&D organization of Raychem Corporation in California. He then went on to specialize in drug delivery research at two leading biotech companies, ALZA Corporation and Cygnus Therapeutics, before joining NTU in 2000. He is the author of over 200 manuscripts, 25 patents and another 15 patent applications worldwide. From these publications, he has a citation count of over 3500, with an h-index of 30. He is also the co-founder of three companies, Amaranth Medical, Peregrine Ophthalmic and AdComp Therapeutics, specializing in therapeutic medical devices and drug delivery products. Recently, he was awarded the Singapore President's Technology Award for his work on Nanomedicine for glaucoma, Singapore's highest award. He also won the Nanyang Award for Innovation in 2012, on the strength of his patented work on cardiovascular implants.

- Chair, School of Materials Science and Engineering, NTU, Singapore;
- Director, NTU-Northwestern, University Nanomedicine Institute;
- Dy Director, Nanyang Institute of Technology in Health & Medicine.

- http://media.ntu.edu.sg/MediaReports/Pages/newsdetail.aspx?news=c6393d19-9864-4b0f-996f-e18c578ef70d
- http://www.peregrineophthalmic.com/theteam/index.html
- http://amaranthmedical.com/

Sandra Vranic

Sandra obtained her BSc Degree in Molecular Biology and Physiology in the Belgrade University, Serbia in 2007.
After graduation she completed her MSc Degree in Toxicology at University Paris Diderot – Paris 7, France. She pursued her PhD in Toxicology in the Laboratory of Molecular and Cellular Responses to Xenobiotics at University Paris Diderot – Paris 7, supervised by Professor Armelle Baeza and Dr Sonja Boland. She focused on interactions of manufactured engineered nanoparticles with cells, especially on mechanisms of their internalization and subsequent cellular effects.
After her PhD, Sandra worked on a short postdoctoral project at Nagoya University and Tokyo University of Science in Japan supervised by Professor Gaku Ichihara, where she focused on the effects of silica nanoparticles on mice and Zebra fish. Sandra joined Nanomedicine Lab in the University of Manchester in January 2015 as a Marie Curie Research Fellow under the RADDEL ITN project. She has been working under the supervision of Professor Kostas Kostarelos and is currently engaged in Graphene Flagship project. Her current project is focussed on intracellular trafficking of graphene based 2D materials and their biomedical applications in the field of siRNA and drug delivery.

AWARDS AND HONORS

- « Prix de Thèse » DIM du Nano-K (award for the best Thesis), NanoSciences, region Ile de France, France, October 2013.
- Best poster at NANOIMPACTNET conference, Lausanne, Switzerland, March 2010.
- Best graduation work "Interaction of the muscle cell protein alpha-actinin with the transcriptional factor YB-1", Foundation "Goran Ljubjankic", Institute of Molecular Genetics and Genetic Engineering, Belgrade, Serbia, December 2007.

PUBLISHED ARTICLES

- S. Vranic, I. Gosens, R. Schins, N. R. Jacobson, B. Bokkers, A. Kermanizadeh, V. Stone, A. Baeza Squiban, F. R. Cassee, L. Tran, S. Boland - Impact of serum as a dispersion agent for *in vitro* and *in vivo* toxicological assessments of TiO2 nanoparticles, Arch Toxicol. 2016 Feb 12. doi:10.1007/ s00204-016-1673-3.
- S. Vranic, I. George, S. Boland, Courtois A, A. Baeza Squiban - Comparison of different cellular models to study translocation of NPs *in vitro*, Toxicol *In Vitro*. 2014 Sep 6. pii: S0887- 2333(14)00156-8. doi: 10.1016/j.tiv.2014.08.003.
- S. Vranic, N. Boggetto, S. Mornet, N. Reinhardt, F. Marano, A. Baeza-Squiban, S. Boland - Deciphering the mechanisms of cellular uptake of engineered nanoparticles by accurate evaluation of internalization using imaging flow cytometry; Part Fibre Toxicol. 2013 Feb 6;10:2. doi: 10.1186/1743-8977-10-2.
- S. Vranic, I. Garcia Verdugo, C. Darnis, JM. Sallenave, N. Boggetto, F. Marano, S. Boland, A. Baeza Squiban - Internalization of SiO2 nanoparticles by alveolar macrophages and lung epithelial cells and its modulation by the lung surfactant substitute Curosurf®, Environ Sci Pollut Res Int. 2013 May;20(5):2761-70. doi: 10.1007/ s11356-012-1436-5. Epub 2013 Jan 5.
- I. George, S. Vranic, S. Boland, MC Borot, F. Marano, A. Baeza-Squiban - Translocation of SiO2- NPs across *in vitro* human bronchial epithelial monolayer, Journal of Physics: Conference Series.

ORAL COMMUNICATIONS

- S. Vranic, F. Marano, A. Baeza, S. Boland - Internalization of SiO_2 nanoparticles by lung epithelial cells, NanOEH conference, Nagoya, Japan, October 2013.
- S. Vranic, S. Ichihara, Y. Shimada, T. Tanaka, W. Wu, S. Boland, L. Tran, G. Ichihara – Bio-distribution and effects of SiO_2 NPs in a transgenic model of Zebra fish (Danio rerio) and in mice, Japanese Society for Hygiene, 84th Annual Meeting, Okayama, Japan, May 2014.
- S. Vranic, M. Martincic, G. Tobias, K. Kostarelos - Metal salts filled multi-walled carbon nanotubes internalization and toxicity in MCF-7 and Beas-2B cell lines, NANOBIOAPP2015, Barcelona, Spain, September 2015.

Julie T.-W. Wang

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ACADEMIC QUALIFICATIONS

2006 -2010: MPhil/PhD in Photobiology in Cancer Therapeutics (Supervisors: Prof Stephen G Bown & Prof Alexander J MacRobert); Division of Surgery&Interventional Science, University College London, UK
2002 -2004: MSc in Biomedical Engineering; Division of Biomedical Engineering, National Taiwan University, Taiwan
1998 -2002: BSc Clinical Laboratory Sciences and Medical Biotechnology, National Taiwan University, Taiwan

PROFESSIONAL EXPERIENCE

2012 -present: Post-Doctoral Research Fellow, Drug delivery group, Institute of Pharmaceutical Science, King's College London, UK
2011 -present: Honorary Post-Doctoral Research Scientist, Cancer Imaging Laboratory, Barts Cancer Institute, Queen Mary University London, UK
2011 -2012: Post-Doctoral Research Fellow, Department of Pharmaceutics, UCL School of Pharmacy, University College London, London, UK
2010 -2011: Post-Doctoral Teaching and Research Fellow, National Medical Laser Centre, University College London, London, UK (Awarded research fellowship funded by PCI Biotech, a Norwegian pharmaceutical company)

2001 -2002: Clinical Laboratory Scientist Internship, Department of Laboratory Medicine, National Taiwan University Hospital, Taiwan

PUBLICATIONS (SELECTED FIRST AUTHORED PUBLICATIONS)

1. J. T.-W. Wang, H. Kafa, N. Rubio, R. Klippstein, P.M. Costa, H.A. Hassan, J. K. Sosabowski, S. S. Bansal, J. E. Preston, N. J. Abbott and K. T. Al-Jamal. (2016) Translocation of LRP1 targeted carbon nanotubes of different diameters across the blood-brain barrier *in vitro* and *in vivo*. Journal of Controlled Release. 225:217-229.
2. J. T.-W. Wang, N. Rubio, H. Kafa, E. Venturelli, C. Fabbro, C. Ménard-Moyon, T. Da Ros, J. K. Sosabowski, M. Prato, A. Bianco, F. Festy, J. E. Preston, K. Kostarelos and K. T. Al-Jamal. (2016) Brain-targeting multi-walled carbon nanotubes: revolution in systemic brain delivery. Journal of Controlled Release. 224:22-32.
3. J. T.-W. Wang and K. T. Al-Jamal. (2015) Functionalized carbon nanotubes: revolution in brain delivery. Nanomedicine (Lond). 10(17):2639-42.
4. J. T.-W. Wang, C. Fabbro, E. Venturelli, C. Ménard-Moyon, O. Chaloin, T. Da Ros, L. Methven, A. Nunes, J. K. Sosabowski, S.J. Mather, M.K. Robinson, J. Amadou, M. Prato, A. Bianco, K. Kostarelos, K.T. Al-Jamal. (2014) The relationship between the diameter of chemically-functionalized multi-walled carbon nanotubes and their organ biodistribution profiles *in vivo*. Biomaterials. 35(35):9517-9528.
5. J. T.-W. Wang, L. Cabana, M. Bourgognon, H. Kafa, A. Protti, K. Venner, A. M. Shah, J. K. Sosabowski, S. J. Mather, A. Roig, X. Ke, G. Van Tendeloo, R. T. M. de Rosales, G. Tobias, and K. T. Al-Jamal (2014) Magnetically decorated multiwalled carbon nanotubes as dual MRI and SPECT contrast agents. Advanced Functional Materials. 24(13):1880-1894.

Frank F. Weichold

M.D., Ph.

Dr. Weichold is director for the Office of Regulatory Science and Innovation (ORSI) as well as the Office of Critical Path and Regulatory Science Initiatives at the FDA in the office of the Chief Scientist and the Office of the Commissioner for the Food and Drug Administration. The expertise he brings to the FDA builds on his ability to advance, coordinate, and integrate the scientific resources of the Agency addressing mission critical regulatory responsibilities in a global environment.

Dr. Weichold's experience includes execution of strategic and operational initiatives across the sciences' value chain. Dr. Weichold has led the development of international collaborations and public private partnerships for discovery and early development, implemented global operating and development models, and executed large scale business model transformations. He has accumulated more than a decade of industrial research and medical product development experience while leading teams in Clinical Pharmacology, DMPK, as a Director at MedImmune LLC, Gaithersburg, Maryland. Prior, he directed research and clinical development of vaccines at the Aeras Foundation (founded by The Bill and Melinda Gates Foundation).

Barbara Weiser

Program Manager Therapeutic Modalities
Roche Pharma Research and Early Development (pRED) Roche Innovation Center Munich, Germany

I am Program Manager within Therapeutic Modalities, Roche Pharma Research and Early Development (pRED). In this role I am supporting the build-up, coordination and strategic development of our early biologics research portfolio in the indications of neuroscience, ophthalmology and rare diseases.

I have joined Roche in 2007 as a postdoc and took over responsibility as a laboratory head in 2009 in the fermentation department of Large Molecule Research, the unit within pRED responsible for discovering, engineering and developing therapeutic proteins from target assessment phase up to GLPTox and entry into clinical testing. During that time I was especially accountable for evaluation of CHO production clones as well as development and optimization of fermentation processes. In 2011 I moved to the discovery department of LMR, heading a lab responsible for high throughput screening, lead identification and characterization of novel antibodies. During this time, I was furthermore leading a technical project team developing one of the bispecific antibodies in our oncology portfolio, which is now in phase 1 clinical testing.

I am an Engineer of Biotechnology by training with a background especially in cell biology and cell culture technologies. I acquired my PhD at the Department of Pharmaceutical Technology, University of Regensburg, Germany working in the field of 3-dimensional cell cultures and regenerative cartilage and adipose tissue engineering.

Wolfgang Wenzel

Prof. Wolfgang Wenzel, Ph.D. (* 1963) obtained his Ph.D. in physics 1989 at the Ohio State University in Columbus, Ohio (USA) and then moved as a research associate to the University of Dortmund, where he obtained the viena legend for physics in 1997. In 2001 he joined the Karlsruhe Institute of Technology (KIT), where he works as a group leader of the bio/nano simulation group at the Institute of Nanotechnology and as an associate professor of physics. He is author of over 200 scientific publication, obtained a number of fellowships and prizes and served as speaker of the competence field Nanoscience at KIT (ca. 600 scientists). His work focuses on nanoscale simulations on long time-scales, structural biology: protein folding, docking and structure prediction, rational drug design, high-throughput in-silico screening, molecular electronics with particular emphasis on biomolecules and disordered systems

Marieluise Wippermann

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Since 2000: CEO, TECOmedical AG Switzerland
1997–2000: Managing Director, CH-Werfen Group, Spain
1988–1997: Vice President International, Nichols Institute, USA
1983–1988: Head of development and production, Eurodiagnostics, The Netherlands
1983: School of economics, Basel, Switzerland

1979–1983: Head of development and production, Bühlmann Laboratories AG, Switzerland
1976–1978: Research scientists, Institute of Biochemistry, University of Hamburg, Germany
1973–1976: Research scientists, Dep. of Internal Medicine, University of Zurich, Switzerland
1973: Degree as Chemistry Engineer

Joy Wolfram

Joy Wolfram is a Research Fellow at the Department of Nanomedicine at the Houston Methodist Research Institute in the United States. She received her bachelor's and master's degrees in biology from the University of Helsinki in Finland. She received her Ph.D. in Nanoscience and Technology in 2016 from the National Center for Nanoscience and Technology at the University of Chinese Academy of Sciences in China under the mentorship of Professor Mauro Ferrari (United States) and Professor Yuliang Zhao (China). She has published 30 scientific articles and has received more than 20 scientific awards from seven different countries. She is also an alumna of the Amgen Scholars Program funded by the Amgen Foundation in the United States. Her research focus involves the development of novel, effective, and clinically applicable nanotherapeutics for the treatment or cancer. In particular, her goal is to increase the accumulation of cancer therapeutics in tumor tissue through the use of drug delivery systems and through modulation of the biological environment. She also aspires to generate fruitful inter-institutional research collaborations around the world.

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EDUCATION

01/07/2008: PhD, Biochemistry and Molecular Biology, Peking University, China
01/07/2001: BSc, Biochemistry, Sichuan University, China

CURRENT POSITION

18/05/2015–Present: Marie Curie Fellow, School of Chemistry and Chemical Biology, University College Dublin, Ireland

SELECTED AWARDS AND SCHOLARSHIPS

2015: Dyason Fellowship, University of Melbourne, Australia
2013: Eureka Prize for Excellence in Interdisciplinary Scientific Research, The Australian Museum, Australia
2013: Discovery Early Career Researcher Award, Australian Research Council, Australia
2012: CASS Travel Grant, CASS Foundation, Australia
2010: Early Career Researcher Award (Best Poster Presentation), 1st Sydney International Nanomedicine Conference, Australia
2004: Kwang-Hua Scholarship, Kwang-Hua Education Foundation, China
2002: DuPont Scholarship, Peking University, China
2001: Postgraduate Scholarship (5 years), Ministry of Education of the P. R. of China

PUBLICATIONS

- 43 peer-reviewed publications (33 journal articles, 8 journal reviews, 1 conference article, and 1 book chapter)
- Web of Science H-index = 25

Selected Research Articles:

1. Wan, S.; Kelly, P. M.; Mahon, E.; Stöckmann, H.; Rudd, P. M.; Caruso, F.; Dawson, K. A.; Yan, Y.+; Monopoli, M. P.+ "The "Sweet" Side of the Protein Corona: Effects of Glycosylation on Nanoparticle-Cell Interactions." ACS Nano 2015, 9, 2157. (+Co-correspondence)
2. Yan, Y.; Gause, K. T.; Kamphuis, M. M. J.; Ang, C. S.; O'Brien-Simpson, N. M.; Lenzo, J. C.; Reynolds, E. C.; Nice, E. C.; Caruso, F. "Differential Roles of the Protein Corona in the Cellular Uptake of Nanoporous Polymer Particles by Monocyte and Macrophage Cell Lines." ACS Nano 2013, 7, 10960.
3. Yan, Y.; Lai, Z. W.; Goode, R. J. A.; Cui, J.; Bacic, T.; Kamphuis, M. M. J.; Nice, E. C.; Caruso, F. "Particles on the Move: Intracellular Trafficking and Asymmetric Mitotic Partitioning of Nanoporous Polymer Particles." ACS Nano 2013, 7, 5558.
4. Shimoni, O.+; Yan, Y.+; Wang, Y.; Caruso, F. "Shape-Dependent Cellular Processing of Polyelectrolyte Capsules." ACS Nano 2013, 7, 522. (+Equal contribution)
5. Yan, Y.; Wang, Y.; Heath, J. K.; Nice, E. C.; Caruso. F. "Cellular Association and Cargo Release of Redox-Responsive Polymer Capsules Mediated by Exofacial Thiols." Adv. Mater. 2011, 23, 3916.
6. McGuinness, L. P.; Yan, Y.; Stacey, A.; Simpson, D. A.; Hall, L. T.; Maclaurin, D.; Prawer, S.; Mulvaney, P.; Wrachtrup, J.; Caruso, F.; Scholten, R. E.; Hollenberg, L. C. L. "Quantum Measurement and Orientation Tracking of Fluorescent Nanodiamonds Inside Living Cells." Nat. Nanotechnol. 2011, 6, 358.

Xiao Zhao

Xiao Zhao received B.S. from Henan University and M.S. from Tianjin medical university with Profs. Jihui Hao. Now he was a Ph.D. candidate major in oncology at the Tianjin medical university with Profs. Jihui Hao. Since August 2013, he has been a joint student with Profs. Guangjun Nie in CAS key laboratory for biomedical effects of nanomaterials and nanosafety at the Chinese National center for nanoscience and technology. His research interests focus on using nanotechnology to improve clinical treatment in pancreatic cancer patients, such as employing a multiple layer-by-layer lipid-polymer hybrid nanoparticle to encapsulate FOLFIRINOX chemotherapeutic regimen which can effectively improve anti-tumor effect and decrease the side effects. Due to this work, he was invited to give a oral presentation and awarded as B-level CSPAC-Celgene Award at 19th International Association of Pancreatology (IAP) Annual Meeting. He has published about 10 papers (4 papers as first or co-first author).

Alfred Zippelius

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2007: Habilitation, venia legendi for Experimental Oncology: 'Dissecting anti-tumor immune responses in cancer patients'

University of Zurich, Switzerland
2006: Swiss Board in Medical Oncology
1999: Doctor of Medical Sciences (Dr. med.):
'Morphological and molecular characterization of minimal residual disease in cancer patients'; summa cum laude; Institute of Immunology, Ludwig-Maximilians-University Munich, Germany

CLINICAL POSITIONS

From 7/2011: Deputy Head, Medical Oncology
University Hospital Basel, Switzerland
From 1/2010 : Chief Oncologist in the Lung and Skin Cancer Competence Centers; University Hospital Basel, Switzerland
From 1/2010: Senior Attending, Medical Oncology
University Hospital Basel, Switzerland (Dir.: Prof. R. Herrmann)

RESEARCH POSITIONS

From 2013: Full Professor of Translational Oncology, Research Group Leader, Laboratory of Cancer Immunology & Biology, Department Biomedicine, University Hospital Basel
2010 - 2013: Research Group Leader, Swiss National Science Foundation Professorship Programme, Laboratory of Cancer Immunology, Department Biomedicine, University Hospital Basel
2003–2008: Research Group Leader, Emmy-Noether Programme (Phase II) of the German Research Foundation (DFG), Tumor Immunology Lab, Medical Oncology, University Hospital Zurich
2000–2003: Postdoctoral training in experimental tumor immunology, funded by the Emmy- Noether Programme (Phase I) of the German Research Foundation (DFG); Ludwig Institute for Cancer Research, Division of Clinical Onco-Immunology, Lausanne, Switzerland (Dir.: Prof. J-C. Cerrottini, Prof. P. Romero)

FELLOWSHIPS/GRANTS/HONOURS

2015–2016: Innovative Fund–Basel Translational Medicine Hub (principal investigator)
2014–2016: Wilhelm-Sander-Stiftung (principal investigator)
2014–2015: Roche Postdoctoral Fellowship Programme (principal investigator)
2014–2015: Huggenberg-Stiftung für Krebsforschung (co-investigator)
2013–2014: Cancer League Basel (co-investigator)

OTHER POSITIONS/EXPERIENCE

- Head, Immunotherapy Program, Cancer Cancer of the University Hospital
- Member, Immunology Program, University Basel
- Member, Faculty of Medicine, University Basel
- Member of scientific boards of national funding bodies and companies
- Section Editor, Journal of Immunotherapy of Cancer

Jing Zou

Associate Professor, Head of Hearing and Balance Research Unit, School of Medicine, University of Tampere. Tampere (UTA), Finland; Professor, Chairman of Otology, Department of Otolaryngology-Head & Neck Surgery, Center for Otolaryngology-Head & Neck Surgery of Chinese PLA, Changhai Hospital, Second Military Medical University (SMMU), Shganghai, China. Supervised 3 PhD students in UTA and 6 Master's Degree students in China. Supervising 1 PhD student in UTA and 2 PhD students in SMMU. Published 4 book chapters and over 100 peer-reviewed articles.

EDUCATION AND DEGREES AWARDED:

- Post-doc, Experimental MRI, MR Center, Department of Clinical Neuroscience, Karolinska Hospital, Karolinska Institutet, Stockholm, Sweden, **1/9/2002-28/2/2003**
- Post-doc, Hearing Research, Department of Otolaryngology, Department of Clinical Neuroscience, Karolinska Hospital, Karolinska Institutet, Stockholm, Sweden, **14/10/1999-31/8/2002**
- Doctor's Degree of Scientific Medicine (=PhD in western countries), Advanced Doctor Study College of Chinese PLA (General Hospital of Chinese People's Liberation Army), Beijing, China. Otolaryngology, **26/7/1994**
- Master's Degree of Scientific Medicine (Chinese system), Third Military Medical University of Chinese PLA, Chongqing, China, Otolaryngology, **13/7/1989**
- Bachelor's Degree of Scientific Medicine (=MD in western countries), Third Military Medical University of Chinese PLA, Chongqing, China, Medicine, **30/7/1986**

RESEARCH EXPERIENCE AND MAJOR SCIENTIFIC CONTRIBUTION:

- Nanotechnology based cochlear implant with gapless interface to auditory neurons (NANOCI): EU FP7, **01/09/2012-31/8/2015**
- Development of reference methods for hazard identification, risk assessment and LCA of engineered nanomaterials (NanoValid): EU FP7, **01/11/2011-30/10/2015**
- Nanotechnology-based Targeted Drug Delivery (NanoEar): (Coordinator: UTA) EU FP6, **1/10/2006-30/9/2011**
- Quality of life and management of living resources (BioEar): (Coordinator: UTA), **1/10/2001-30/9/2005**
- Molecular mechanism of Ras GTPases modulating skeletal protein and p-glycoprotein in the blood-endolymph barrier of cochlea, granted by National Natural Science Foundation of China (contract no. 81170914), **1/1/2012/-31/12/2016**

Nicola Stingelin

Dr. Nicola Stingelin has held various research and lecturing posts at the University of Basel and other institutions in different practical ethics fields, specialising in research ethics in medical, public health, business and environmental fields, (especially in the emerging sciences and technologies, including genomics).
Prior to entering academia, she held positions in the pharmaceutical industry, specializing in intellectual property. The change in career direction was achieved after completing a Master of Advanced Studies in Applied Ethics at the Ethics Centre, University of Zürich, followed by her PhD in Medical and Health Ethics at the University of Basel, Medical Faculty; a Master of Advanced Studies in Intercultural Communication at the University of Luzern, and the Program MGU in Sustainability Studies at the University of Basel.
Dr. Stingelin regularly serves as ethics expert and reviewer to the European Commission; advises on a range of international projects, as well as maintaining contacts with the life sciences by working as independent Consultant in the pharmaceutical sector.

CLINAM

European Foundation for Clinical Nanomedicine

9/2016

Basel, June 26–29

CURRICULA VITAE POSTERS

Maxim A Abakumov

My name is Maxim A Abakumov. I have graduated from Moscow State University at 2009 and have defended my Ph.D. thesis in biochemistry at 2012. I am working on a research fellow position at Russian National Research Medical University. My current research work is devoted to synthesis of biocompatible iron oxide nanoparticles and their application for tumor diagnostics and treatment. During my work I have published 12 articles in international journals and presented them in different Russian and International conferences. My work was supported by 3 grants of Russian Federation. I have worked in collaboration with laboratories in Europe in Karolinska Institute in Sweden and University of North Carolina at Chapel Hill and University of Nebraska Medical center at USA during my career. During this collaboration I have worked with prof A.V. Kabanov (Univercity of Chapel Hill) and prof. Maria Issanguliantis (Karolinska Institute). Results of my work are published in international journals such as Nanomedecine, Colloids and Surfaces B: biointerfaces, Contrast media and Molecular imaging. I have an experience in field of bioimagnig such as fluorescent, bioluminescent, CT and MRI.

Tatiana Abakumova

Tatiana Abakumova was born in May 10, 1988. In 2012 she graduated from Pirogov Russian National Research Medical University. From December 2012 to May 2015 she worked as a Junior Researcher in the laboratory of neurochemistry in the department of fundamental and applied neurobiology in the Serbsky Medical Research Center of Psychiatry and Narcology, since May, 2015 she works as a Researcher in immunochemistry laboratory in the same department (Head of laboratory - Prof. Vladimir P. Chekhonin). Research interests of Tatiana Abakumova linked with targeted delivery of therapeutic and diagnostic agents to the brain (glioma, multiple sclerosis), synthesis of nanocontrainers (nanogels, liposomes) and Gd-contrast agents. In January 2016 she got a Ph.D. in biochemistry with thesis «Targeted systems for MRI visualization of brain pathologies». Tatiana Abakumova has a number of awards for her oral and poster presentations on the international conferences, including NANO2014 award for the best poster presentation among young scientists. She got scholarships from the Government of Russian Federation, funds and grants from different funds to support own research. She published 7 papers and 30 conference abstracts.

Ibane Abasolo

Ibane Abasolo,obtained the degrees in Biochemistry and Biology from the University of Navarra (Spain) in 1997 and 1998, respectively. During her PhD in the labs of Dr. Alfonso Calvo (CIMA, Pamplona, Spain) and Prof. Zhou Wang (Northwestern University, Chicago, USA), she studied the role of a peptidic hormone, adrenomedullin, in prostate cancer. Afterwards, Dr. Abasolo continued her post-doctoral training in the group of Prof. F.X. Real (IMIM, Barcelona), where she focused on the study of key factors on the progression of pancreatic cancer and cerebellar development. During this time, she gained extensive experience in experimental mouse models, including all the steps from the generation of new transgenic models, to the molecular and cellular characterization of previously existing ones. In 2006 Dr. Abasolo moved to the Institut d'Alta Tecnologia (PRBB, Barcelona), where she got trained in molecular imaging techniques such as the microPET, SPECT and CT. Since 2007, Dr. Abasolo is the coordinator of the Area of Functional Validation & Preclinical Research (FVPR) of the CIBBIM-Nanomedicine. Within this area, she is in charge of developing standardized assays for testing the activity and function of candidate genes, target molecules and therapeutic compounds, and providing the industry and other research groups with an optimum technological platform for testing new biomedical applications based on nanotechnology. This technological platform participates in several national and international research infrastructure programs such Nanbiosis (Spanish ICTS), CIBER-BBN and EATRIS and is also in involved in multiple Spanish and European research projects. Dr. Ibane Abasolo has participated in several international R&D projects (1 Euronanomed project, 1 ERA-IB–IMAPROT-, 1 Capacitation Program in Nanotechnology –OncoNanoTarget-) and is currently leading one Euronanomed project (DiamESTar) and directly participates in a NMP11 H2020 project (NoCanTher) aimed at bringing into clinical trials magnetic nanoparticles for hyperthermia treatment of pancreatic cancer. She has also leaded as a principal investigator a project within the national industry-public research cooperation program (INNPACTO-Polysfera) that included the *in vitro* and *in vivo* testing of nanomaterials for cancer treatment. Moreover, she currently the principal investigator of a project for targeting breast cancer stem cell using nanotechnology (Marató TV3- Pentri).

Aracely Angulo-Molina

Full Time Professor, Health Department.
Universidad de las Américas, Puebla (UDLAP). Ex-Hda. Sta. Catarina Mártir, San Andrés, Cholula 72820, Puebla, México,
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aracelyam@hotmail.com

EDUCATION/TRAINING

2013: PhD, Food and Development Research Center (CIAD), Hermosillo, Sonora, Mexico; Functional nanofood in cancer
2005: Specialization in immune-hematology, University of Sonora (UNISON), 2005, Hermosillo, Sonora, México.; Development of drugs for cancer from bee products
2000: Master of Science (Nutritional sciences area), Food and Development Research Center (CIAD), Hermosillo, Sonora, Mexico; Physiology and nutrition
1994: Bachelor in Clinical Chemistry, University of Sonora (UNISON) Hermosillo, Sonora, México; Immunology and nutrition, routine clinical lab test.

ACTIVITIES

2015 to date: Full time professor University of Sonora (UNISON)
2008 to date: Full time professor University of las Américas Puebla, Mexico (UDLAP)
2000–2006: Full time associate professor University of Sonora (UNISON)-south campus, Mexico

RESEARCH STATEMENT:

In summer 2014 I got and award from Swiss National Science Foundation as a foreign research visitor invited by Dr. Marcus Textor (ETH) and the group of Dr. Uwe Pieles (FHNW). In 2003 I have been a member of the research group who reported the first human case of Gnathostomosis, a foodborne zoonotic disease caused by several species of nematode Gnathostoma sp, in Sonora, Mexico. I have a broad background in biomedicine and experimental biology, with specific training and expertise in key research areas for those applications. Furthermore I have expertise in the areas of nutritional assessment and diet treatment for chronic diseases such as diabetes, obesity, and cancer and sport performance.

Dietmar Appelhans

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www.ipfdd.de/Appelhans.69.0.html?L=1

STUDIES

10/84–08/90: Chemistry – Diploma study; Philipps-University Marburg
09/90–02/94: PhD study in organic chemistry; Philipps-University Marburg

SCIENTIFIC CAREER

10/1990–10/1993: Scientific co-worker at the department of Chemistry at the Philipps-University Marburg
09/1994–12/1998: Scientific co-worker in the working group of Prof. H.-J. Adler at the Institute of Macromolecular Chemistry and Textile Chemistry – TU Dresden
01/1999–12/2004: Scientific co-worker under the supervision of Mrs. Prof. B. Voit at the Leibniz Institute for Polymer Research Dresden (IPF)
01/2005–12/2011: Principal investigator and leader of the research group "Bioactive and biofunctionalized Polymers" in the group of Mrs. Prof. B. Voit at the IPF
Since 01/2012: Department head of "Bioactive and Responsive Polymers" at the IPF

RESEARCH INTEREST

- synthesis, modification and characterization of dendritic polymers consisting of perfectly-branched and hyperbranched structures;
- investigation of complexing properties of dendritic polymers, especially towards metal ions and metal clusters in aqueous phase;
- molecular modelling of dendritic and linear structures and theoretical calculations of dendritic metal complexes;
- realization of water-soluble dendritic and linear polymers for biohybrid structures and bio-active polymers, but also polymeric vesicles for synthetic biology, drug delivery and material sciene;
- the interactions of dendritic and linear polymers with proteins and enzymes;
- development of polymeric therapeutics and diagnostics, including the fundamental understanding of this topic.

PUBLICATIONS

More than 130 peer-reviewed papers

Amina Arslanagic

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arslanagic@bmb.sdu.dk

2009-2012: University of Southern Denmark, Odense, Denmark, B.Sc. Biomedicine
Bachelor's Thesis awarded 12(A), entitled "Effect of microglia-derived factors on dopaminergic differentiation of human neural stem cells''. Supervisor: Associate Professor Morten Meyer, Department of Neurobiology Research, Institute of Molecular Medicine, Faculty of Health Sciences, University of Southern Denmark, Odense (Manuscript in progress)

2012-2014: University of Southern Denmark, Odense, Denmark
Cand. Scient Biomedicine **Master's Thesis** awarded 12(A), entitled "LDL Receptor-Based Drug Delivery to Breast Cancer Cells''. Supervisor: Professor David Needham, SPSE, Department of Physics, Chemistry and Pharmacy, SDU, Odense in collaboration with Professor Jan Mollenhauer, Lundbeckfonden Center of Excellence NanoCAN, Department of Cancer and Inflammation, Institute for Molecular Medicine, Faculty of Health Sciences, University of Southern Denmark.

WORK EXPERIENCE

2012 Aug. – 2014 Nov.: Student representative for the NAT-SUND board at University of Southern Denmark, Odense
2012 Mar. – 2014 Nov.: Student assistant at Clinical Institute, University of Southern Denmark, Odense, Denmark
2012 Feb. – 2014 Nov.: Student Mentor, University of Southern Denmark, Odense, Denmark
2012 Sept. – 2013 Dec.: Instructor at Southern University of Denmark, Odense, Denmark
2011 Nov. – 2012 Apr.: Student Assistant, Sygehus Apotek Fyn, OUH, Odense, Denmark

POSTERS PRESENTED:

Danish Chemical Society Annual Meeting June 2014: '' Characterization of LDL Receptor (LDLR) Expression level in Human Mammary Epithelial cell line'', Amina Arslanagic, Barbara Korzeniowska, Jan Mollenhauer, David Needham

EXPERIMENTAL QUALIFICATIONS

Experience in a wide variety of experimental techniques, the main once are listed below:
• Cell culturing (Neural, Breast, Lung and Prostate cancer cells) • 3D culturing • *In vivo* studies • PCR and Real-time qPCR •Western Blotting • Immuno-cytochemical and Immunohistochemical staining • ELISA • FACS • HPLC • Gene-cloning and cell manipulation • Mouse behavioural studies • Fruit fly experiments • Tissue Collection and Vibratome Sectioning of whole mouse brain • Confocal spinning disk microscopy • Nanoparticle uptake studies • Cytotoxicity studies

Mohamed Fathy Attia

Faculty of Pharmacy
74 route du Rhin, CS 60024
67401 ILLKIRCH CEDEX, FRANCE.
E-mail: mattianrc@hotmail.fr

CONFERENCES (ORAL COMMUNICATIONS):

- Mohamed F. Attia, Nicolas Anton, and Thierry F. Vandamme. Contrast agents for targeted biomedical imaging. Journées du campus d'Illkirch (JCI), Strasbourg, France April 13,14, 2015.
- Mohamed F. Attia, Nicolas Anton, Thierry F. Vandamme. Journée des doctorants en chemie. Novel contrast agents for targeted X-ray micro computed tomography. 13th November 2015.
- Mohamed F. Attia, Nicolas Anton, Thierry F. Vandamme. Nanoemulsions for Targeted Biomedical Imaging. "5th International Conference on Nanotek and Expo" November 16-18, 2015 at Hilton San Antonio Airport, San Antonio, Texas, USA.
- Mohamed F. Attia, Nicolas Anton, Thierry F. Vandamme. Contrast agents based on nano-emulsions for targeted Biomedical Imaging. International Conference on Nanotechnology in Medicine (NANOMED) at the Manchester Conference Centre in Manchester, UK during 23-25 November 2015.

LIST OF PUBLICATIONS:

- Mohamed F. Attia, Ikram Ullah Khan, Nicolas Anton, Nadia Messaddeq, Anshuman Jakhmola, Thierry F. Vandamme. One-step synthesis of iron oxide polypyrrole nanoparticles encapsulating

ketoprofen as model of hydrophobic drug. International journal of pharmaceutics. In progress.
- Mohamed F. Attia, Nicolas Anton, Roman Akasov, Manuela Chiper, Elena Markvicheva, Thierry F. Vandamme. Biodistribution and toxicity of X-ray iodinated contrast agent in nano-emulsions in function of their size. Journal of Pharmaceutical Research. 33(3), 603-614, 2015.
- Mohamed F. Attia, Nicolas Anton, Redouane Bouchaala, Pascal Didier, Youri Arntz, Nadia Messaddeq, Andrey S. Klymchenko, Yves Mély and Thierry F. Vandamme. Functionalization of nano-emulsions with an amino-silica shell at the oil-water interface. RSC Advances journal. Vol. 5, 74353–74361, 2015.
- Mohamed F. Attia, Nicolas Anton, Manuela Chiper, Roman Akasov, Halina Anton, Nadia Messaddeq, Sylvie Fournel, Andrey S. Klymchenko, Yves Mély, and Thierry F. Vandamme. Biodistribution of X-Ray Iodinated Contrast Agent in Nano-Emulsions Is Controlled by the Chemical Nature of the Oily Core. ACS Nano journal. Vol. 8, No. 10, 10537-10550, 2014.
- Mohamed F. Attia, Tahar Azib, Zakaria Salmi, Ajay Singh, Philippe Decorse, Nicolas Battaglini, Hélène Lecoq, Mária Omastová, Asha A. Higazy, Amira M. Elshafei, Mohamed M. Hashema, Mohamed M. Chehimi. One-step UV-induced modification of cellulose fabrics by polypyrrole/silver nanocomposite films. Journal of Colloid and Interface Science. Vol. 393, 130-137, 2013.

Azinopyridine Derivatives. Phosphorus, Sulfur, and Silicon and the Related Elements, Vol. 185, 1346-1357, 2010.

FIELDS OF INTEREST:
- Synthesis, fabrication, characterization and pharmacokinetic studies of: Organic molecules - Nano-emulsions - Polymeric nanoparticles – Liposomes - Inorganic nanoparticles
- Drug delivery systems - Surface chemistry of nanocarriers (functionalisation) - Contrast agents for active and/or passive targeted X-ray CT imaging – Nanomedicines.

Ignacio Baanante

National Institute of Health Carlos III, Spain
E-mail: ibaanante@isciii.es

Veterinarian by training works as Project Officer at EU and Internationalization Division (Office of the Deputy Director General for International Programmes and Institutional Relations) of the National Institute of Health Carlos III, Spain.

Since 2008 has been involved in different European research funding programmes in the Health area such as ERA-Nets (PRIOMED-CHILD, NEURON, E-Rare, EuroNanoMed, ERA-CVD, TRANSCAN, Infect-ERA, etc), Art. 185 TFEU (AAL and EDCTP), European Research Infrastructures (ECRIN, EATRIS), Joint Programming Initiatives (JPI on Antimicrobial Resistance and JPI on Demographic Change and Wellbeing) and other projects funded by the European Commission (e.g. CSA on Personalized Medicine).

During 2010 and 2011 he worked at the European Office of the Spanish Ministry of Science and Innovation based in Brussels. Throughout his time there, he participated in the evaluation and the monitorization of scientific policies at national, European and International level as well as participated in European Projects on Impact Assessment.

Furthermore, Mr Baanante is also collaborator of the Spanish Ministry of Economy and Competitiveness in different European Programmes.

Africa G. Barrientos

Ph.D.

Africa G. Barrientos is Head of Tech Transfer and Nanoparticle & Analytical Development at Midatech Pharma España S.L.U. (Bizkaia, Spain). She obtained Chemistry Degree at University of Granada in 1997 and the PhD Degree in Sciences (glyconanoparticles) in 2003 at University of Sevilla. She has a Master Degree in Carbohydrate Chemistry in 1998 at University of Granada.

Her PhD thesis was related to the development of a new multivalent system, gold glyconanoparticles, for the study of the carbohydrate interactions. After that, she has been in different laboratories in Germany (University of Luebeck) and Spain (CSIC) as a research associate developing new methods in glyconanotechnology. In 2005, she moved to London to hold a postdoctoral position at UCL (Marie Curie fellow grant). During her stage (2005-2007), her work was involved in the synthesis of siRNA nanoparticles for intervening silencing process and radioactive nanoparticles. In 2007, she joined to Midatech Pharma team in Spain, leading Research and Development department and later the Laboratory of Technology Transfer, where she has been working in the design and preparation of nanoparticles for therapeutical applications, the manufacturing of nanoparticles with GMP grade and analytical development. Currently, she is participating as principal investigator of international projects as NANOFACTURING and IMNUNOSHAPE, both EU H2020 grants.

Through her work experience, she became proficient in a variety of tools and techniques from diverse areas including organic, inorganic, radiochemistry, and medicinal chemistry. She has been involved in binding assays and metabolite studies as well. She has 13 publications in high-impact scientific journals, 4 patents (related to nanoparticles) and she has contributed to more than 50 international congresses and workshops.

Maike Baues

I am passionate about applied science ever since early schooldays and for me biotechnology is the perfect combination of chemistry, biology, medicine and mechanical engineering. I had already gathered extensive research experience in this field during two internships before I began my studies. This was what led me to study molecular biotechnology at the RWTH Aachen University.

The highlight of my undergraduate years was my bachelor thesis, where I was able to apply the knowledge I had gained. I worked under the guidance of Dr. Rolf Fendel in Prof. Dr. Dr. Stefan Barth's department for Pharmaceutical Product Development which is part of the Fraunhofer Institute for Molecular Biology and Applied Ecology (IME). The project I was involved in aims at the development of a nanoparticle-based multiplex immuno-assay for the detection of Plasmodium falciparum antigens. The greatest part of my work was devoted to antigen production by stress expression, generation of murine monoclonal antibodies, antibody coupling to nanoparticles, and proof of concept in form of immunofluorescent assay. After completing my bachelor studies in the summer of 2012, I have taken up my postgraduate studies with medical biotechnology as area of specialization. Additionally I had the incredible chance to broaden my knowledge and practical skills in the field of pharmaceutical biotechnology during a 6 month research internship at the Fraunhofer USA Center for Molecular Biotechnology (FhCMB) in 2014.

After the stay abroad I finished my master studies in 2015 by performing my thesis at the Helmholtz-Institute for Biomedical Engineering at the department for Experimental Molecular Imaging,

under the supervision of Prof. Dr. Dr. Twan Lammers, where I analyzed several pharmaceutical and mechanical EPR enhancing strategies. In this context I evaluated neoadjuvant therapies that induce vascular normalization and ultrasound mediated sonoporation for their ability of improving the accumulation, macro- and microdistribution of different nanomedicines.
Currently, as a first year PhD student I continued with the research of drug targeting to tumors and modulation of tumor blood vessels (with both pharmacological and physical strategies), and gradually also started working on molecular imaging.
I am really looking forward to being a part of the CLINAM in Basel.

Filippo Bertoli

Dr. Filippo Bertoli is currently working as a Post-Doctoral scientist in the Centre for BioNano Interactions (CBNI) under the supervision of Prof. Kenneth A. Dawson at University College Dublin. After graduating with a B.S.c in Biotechnology from the University of Padova (Italy) in 2006, Dr. Bertoli went on to take a Masters in Medical Biotechnology with a specialisation in NanoBiotechnology at the University of Trieste (Italy). He did his thesis on the possible use of carbon nanotubes as a tool for drug delivery across the blood brain barrier. He then joined the Centre for Bionano Interactions in April 2010 as a PhD student, where he obtained his PhD under the school of Chemistry and Chemical Biology in 2014. Within his PhD Dr. Bertoli focused on how the layer of molecules adsorbed on nanoparticles evolves as nanoparticles enter cells and reach different intracellular locations and to achieve this he has developed new methods to fractionate cells and recover and characterise subcellular compartments containing the nanoparticles. He has applied to his research several different methods, from fluorescence imaging and flow cytometry to mass spectrometry and several methods in molecular biology, always complemented with careful nanoparticle physico-chemical characterisation. Currently, Dr. Bertoli is working on different aspects on cellular processing of nanomaterials and associated corona, applying the methods he developed to different platforms, in order to unveil the possible effects nanomaterials have on living systems.

Christos Bikis

Christos Bikis is an MD-PhD student at the Biomaterials Science Center of Basel University
Christos Bikis has obtained his Medical Degree from the National and Kapodistrian University of Athens in 2011. During the last three years of his studies he was also a research fellow at the Department of Forensic Medicine and Toxicology at the Athens Medical School. Subsequently he enrolled at the Federal Institute of Technology in Zurich (ETHZ), for his second degree a B.Sc. in Physics that he obtained in 2014. He then joined the Biomaterials Science Center as part of the MD-PhD Program at the University of Basel. Currently, he is working towards his PhD degree in Experimental Physics on the subject of X-ray computed micro-tomography of nervous tissue. His research interests include microtomography by using synchrotron radiation or laboratory sources, data treatment, image analysis, histology, optical microscopy, animal model studies, as well as possible clinical applications based on this interdisciplinary background.

Débora Bonvin

Débora Bonvin received her MSc in Bioengineering from the Ecole Polytechnique Fédérale de Lausanne (EPFL) in 2013. She did her Master's thesis on the improvement of the photodynamic therapy with anti-angiogenic drugs as anti-tumor treatments (The Medical Photonics Group, EPFL). She is currently doing her PhD in Materials Science with Prof. Heinrich Hofmann (The Powder Technology Laboratory, EPFL), developing iron-oxide-based nanoparticles for theranostic applications, i.e. detection of tumors by magnetic resonance imaging (MRI) combined with their treatment by hyperthermia.

Luca Boselli

Centre for BioNano Interactions,
University College Dublin, Ireland
E-mail: luca.boselli@cbni.ucd.ie

Dr. Luca Boselli, is currently a postdoctoral fellow at the Centre for BioNano interactions (CBNI) in the University College of Dublin (Ireland) working under the supervision of Prof. Kenneth Dawson, since 2015. His main research focus at CBNI involve the synthesis of a broad library of gold nanoparticles (GNPs) of different sizes and shapes and the study of their interactions with the biological milieu. His work is supported by the H2020 Nanofacturing project.
Dr. Luca Boselli graduated in chemistry in 2007 and obtained his M.Sc. in Photochemistry and Molecular Chemistry in 2011 for the University of Bologna. After an internship in the "Photochemical Nanosciences Laboratory" at the University of Bologna, under the supervision of Prof. Paola Ceroni, he obtained his PhD working on the synthesis of bioactive N-heterocyclic carbene gold complexes in the group "New antiplasmodial molecules and pharmacological approaches" at the Laboratoire de Chimie de Coordination (LCC, CNRS, Toulouse) under the supervision of Prof. Heinz Gortnitzka.
In 2013 he was awarded the mobility grant "ATUPS" for the University "Paul Sabatier" of Toulouse, that allowed him to visit the "Photochemical Nanosciences Laboratory" at the University of Bologna, in the context of a collaboration project. During the PhD he also spent several months working at the Institute of Pharmacology and Structural Biology (IPBS, CNRS, Toulouse) in the context of a collaboration with the "Sphingolipids and Cancer" group under the supervision Prof. Olivier Couvilier. Following the three main publications: Boselli, Luca, et al. "Synthesis, Structures, and Biological Studies of Heterobimetallic Au (I)–Ru (II) Complexes Involving N-Heterocyclic Carbene-Based Multidentate Ligands." Organometallics 34.6 (2015): 1046-1055; Boselli, Luca, et al. "Synthesis, structures, and selective toxicity to cancer cells of gold (I) complexes involving N-heterocyclic carbene ligands." European journal of medicinal chemistry 85 (2014): 87-94; Zarschler, Kristof, et al. "Ultrasmall inorganic nanoparticles: State-of-the-art and perspectives for biomedical applications." Nanomedicine: Nanotechnology, Biology and Medicine (2016).

Rikke Yding Brogaard

M.Sc.
E-mail: rydbr@nanotech.dtu.dk

EDUCATION

2013–present: Ph.D. student at the Colloids and Biological Interfaces Group, Department of Micro- and Nanotechnology, Technical University of Denmark.
2013: M.Sc. in Pharmaceutical Design and Engineering, Technical University of Denmark and Rensselaer Polytechnic Institute (RPI), Troy, NY, United States.
2010: B.Sc. in Human Life Science Engineering, Technical University of Denmark.

SCIENTIFIC CAREER AND POSITIONS:

2015–present: Visiting scientist at MD Anderson Cancer Center, Houston, TX, United States.
2013–2013: Research Scientist at Immudex in Copenhagen, Denmark.
2011–2013: Student assistant at Immudex in Copenhagen, Denmark.
2011–2013: Laboratory assistant at Hagedorn Research Institute in Gentofte, Denmark.
2010: Assistant Teacher in "Nutrition" at the Technical University of Denmark, Denmark.

ABSTRACTS

2016: Participated and presented a poster at 2016 Annual meeting of American Association for Cancer Research Annual, New Orleans, LA. "Enhanced Chemotherapeutic Effect with Matrix Metalloproteinase Sensitive-Liposomes" Rikke Y. Brogaard, Rasmus Eliasen, Fredrik Melander, Anders E. Hansen, Andreas Kjær, Thomas L. Andresen.
2014: Participated and presented posters at the Liposome Research Days 2014, Copenhagen, Denmark. "Comparing Matrix Metalloproteinase (MMPs) expression *in vitro* and in human cancer xenografts". Rikke Y. Brogaard, Fredrik Melander, Anders E Hansen, Thomas L. Andresen. "A Matrix Metalloproteinase-dependent drug delivery system for hemotherapeutics" Fredrik Melander, Rasmus Eliasen, Rikke Y. Brogaard, Torben jetting, Jonas Henriksen and Thomas L.Andresen.

AWARDS AND SCHOLARSHIPS

2016: Trainee Recognition Program, MD Anderson Cancer Center, Houston, TX, United States.
2015:
- Otto Mønsteds Fond, Denmark.
- Civilingeniør Frants Allings Legat, Denmark.
- Margrete Møller Fonden, Denmark.

2014:
- Reinholdt W. Jorck og Hustrus Fond, Denmark.
- Clara Hansens Mindelegat, Denmark.

2010:
- Nordea Fonden, Denmark.
- Reinholdt W. Jorck og Hustrus Fond, Denmark.
- nud Højgaards Fonden, Denmark.
- Oticon, Denmark.

Marzia Buscema

Marzia Buscema got her M.Sc. in Physics at the University of Catania (Italy). In 2013 she joined the research group of Prof. Dr. Bert Müller as PhD student. She is currently working on the optimization of shear-sensitive phospholipid liposomes to be used as nano-containers for local drug release in diseased human coronary arteries. Her research includes the study of liposome stability (dynamic light scattering), the fabrication of microfluidic devices used as window to spatially resolve morphological changes of liposomes undergoing to shear stress (microfluidics combined with X-ray scattering-based technique) and the study of the morphology of normal and blocked human coronary arteries (X-ray tomography-based technique).

Camila Cánepa

I am Camila Cánepa, BS in Biotechnology, graduated in March 2014 from the Universidad Argentina de la Empresa-UADE (Argentinean University of the Company) with a qualification of 8.2/10 (diploma of honor). I started working in MD. PhD. Mirna Biglione´s group in 2013 at the Instituto de Investigaciones Biomédicas en Retrovirus y SIDA-INBIRS (Institute of Biomedical Investigation on Retroviruses and AIDS), School of Medicine, University of Buenos Aires, Argentina. She heads the National Reference Group on Human T Cell Lymphotropic Virus (HTLV) in Argentina. I have learned molecular and serological techniques such as DNA and RNA extraction, n-PCR, gel electrophoresis, ELISA and Western Blot assays. I have also learned cell culture techniques and acquired basic knowledge in cytometry. I have attended several courses in order to deepen knowledge in this regard. As part of my thesis, I set up a qPCR technique for clinical application. My thesis results were published as "Low proviral load is associated with seroindeterminate Western Blot patterns in Human T-Cell Lymphotropic Virus Type 1 infected individuals: could punctual mutations be related?" (Viruses, 2015; 7:5643-58/ 1st author). I have also collaborated in epidemiological projects in HTLV and Hepatitis B Virus resulting in the publication of two papers : "Contributions and considerations on Human T Lymphotropic virus type 1 and 2 infection (HTLV- 1/2) in Argentina" and "Hepatitis B virus and hepatitis D virus in blood donors from Argentina: circulation of HBsAg and reverse transcriptase mutants" (BioReview, 2014; 39: 6-16/ 3rd author; Archives of Virology, 2014 159(5):1109-17/ 6th author). Recently, a rare HTLV infection case report has been accepted for publication at the International Journal of Cancer Therapy and Treatment ("Chronic Adult T-Cell Leukemia in a young male after blood transfusion as a newborn" /2nd author). Furthermore, I have co-authored five posters and one oral presentation presented in international conferences between 2013 and 2015, as well as twelve posters in national conferences.
On April 2014, I started a Doctoral Fellow at the National Council for Scientific and Technological Research, being Dr. Biglione my thesis director and Dr. Julieta Imperiale and Dr. Alejandro Sosnik my co-director and assistant director, respectively. Dr. Imperiale is a member of the Chair of Pharmaceutical Technology II, School of Pharmacy and Biochemistry (UBA). Dr. Sosnik is an Associate Professor at the Laboratory of Pharmaceutical Nanomaterials Science (Technion- Israel). The abstract sent to CLINAM is part of my thesis entitled: "Development of nanotechnological strategies for controlled drug release: new approach on interferon alpha (IFNα) based therapy". Furthermore, a review titled "Micro and nanotechnological strategies for optimizing the delivery of interferon" is under preparation (2nd author).

Valentina Castagnola

Centre for BioNano Interactions, University College Dublin, Ireland
valentina.castagnola@cbni.ucd.ie

Dr. Valentina Castagnola joined the group of Prof. Kenneth Dawson as a postdoctoral research fellow at the Centre for BioNano Interactions (CBNI) at the University College of Dublin (UCD) in 2015. Her research, supported by the FP7 Graphene Flagship and H2020 Graphene Core 1, involves carbon-based nanomaterial synthesis and study of the interactions with biological fluids, in order to elucidate mechanisms of cell internalization.

Dr. Valentina Castagnola graduated in Chemistry in 2008 and obtained the M.Sc. in Photochemistry and molecular Chemistry in 2011 from the University of Bologna with a final internship focussed on the development of biosensors for toxins detection based on electrochemiluminescence transduction carried on under the supervision of Prof. Francesco Paolucci in the group of Electrochemistry of molecular and functional materials (EMFM).

After that, she obtained the Ph.D. in Micro and Nanosystems from the GEET doctoral school (Electrical and electronic engineering and telecommunications) working at the institute LAAS-CNRS in Toulouse, France, under the supervision of Dr. Christian Bergaud in the NanoBioSystem (NBS) group. Her doctoral thesis concerned the conceiving and implementation of flexible implantable microelectrodes with nanostructured active surface for stimulation and recording of the brain activity. During this period she has been engaged in scientific research in a broad range of topics, ranging from surface science, electrochemistry and microfabrication to neurophysiology and cell biology and her work resulted in several peer reviewed articles (ACS nano 6.9 (2012): 7989-7997; Synthetic metals 189 (2014): 7-16, Biosensors and Bioelectronics 67 (2015): 450-457; Journal of Micromechanics and Microengineering 25.12 (2015): 125003; Sensors and Actuators B: Chemical 214 (2015): 1-9). The main Ph. D. work has also been selected for 4 international conferences and 4 national French conferences and she was awarded the prize “Best thesis 2014” from the GEET doctoral school.

Vivek Chandra

Dr.Vivek Chandra, MBBS, MD was born in Gorakhpur,India. After his graduation (MBBS) study in India, he completed his Postgraduate MD in Clinical Internal Medicine in London,UK.He has Postgraduate Certificate in Diabetology from the Boston University School of Medicine. He served as a Lecturer of Medicine in India for a short duration before joining as a Clinical Fellow to NHS, London for two years from January 2009 to December 2010. He also was an editorial board Sub-Editor to a London based medical Journal, the WLMJ. He presently works as a Consultant Physician in India and is a visiting Consultant to the Board at West London Postgraduate Medical School, London, UK. He sits on the Board for few Indian Start-ups also and is working on exploiting cognition as a technicaly available resourse. He presented at the ISN,Singapore in 2003, a paper on the Renal Assistance machine- a implantable dialysis unit-using nanotechnology. He has presented at several prestigious conferences worldwide and has attended several postgraduate courses. Being a keen learner, he joined the Royal society of Medicine,UK, Association of Physicians of India, British Society of Nanotechnology.UK amongst others. Several patent applications/ patent pending/FTo approvals are in process.

Bing-Mae Chen

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Bing-Mae received her B.S. degree from the National Taiwan Ocean University and M.S. Degree in Microbiology and Immunology from the Soo-Chow University in Taipei, Taiwan. Bing-Mae has worked as a research assistant and since 1995 as the Senior Research Technician and lab manager with Dr. Steve Roffler in the Institute of Biomedical Sciences, Academia Sinica in Taipei, Taiwan. Bing-Mae is an expert in hybridoma technology and has generated more than one hundred monoclonal antibodies, including a panel of monoclonal IgG and IgM antibodies against polyethylene glycol, which are used world-wide in the development of PEGylated drugs and nanomedicines. Bing-Mae is co-author of more than 30 manuscripts and co-inventor of several patents.

SELECTED PUBLICATIONS

- WC Huang, PA Burnouf, YC Su, BM Chen, KH Chuang, CW Lee, PK Wei, TL Cheng and SR Roffler. Engineering chimeric receptors to investigate the size and rigidity-dependent interaction of PEGylated nanoparticles with cells. ACS Nano 10:648-662, 2016.
- Y Tung, YC Su, BM Chen, PA Burnouf, WC Huang, KH Chuang,YT Yan, TL Cheng and SR Roffler. Selective delivery of PEGylated compounds to tumor cells by anti-PEG hybrid antibodies. Mol Cancer Ther. 14(6):1317-26, 2015.
- CH Kao, JY Wang, KH Chuang, CH Chuang, TC Cheng, YC Hsieh, YL Tseng, BM Chen, SR Roffler, TL Cheng. One-step mixing with humanized anti-mPEG bispecific antibody enhances tumor accumulation and therapeutic efficacy of mPEGylated nanoparticles. Biomaterials 35: 9930-40, 2014.
- YC Su, TS Al-Qaisi, HY Tung, TL Cheng, KH Chuang, BM Chen, and SR Roffler. Mimicking the germinal center reaction in hybridoma cells to isolate temperature-selective anti-PEG antibodies. Mabs 6: 1069-83, 2014.

Jianhai Chen

Dr. Jianhai Chen is a Professor and PhD supervisor of pharmaceutical Science, who works in the Southern Medical University, Guangzhou city, P R CHINA. He is also a Professor in the School of Pharmaceutical Science, at SunYat-sen University. He is a Director of Council, Biomaterial Committee of China and Director of National Biochemistry and Molecular Biology Society in industry. He also is Member of National foundation evaluation committee on high- tech. project (863), Member of National foundation evaluation committee on Nanomaterials and Nanotechnology in China. He held the positions of following the member of Editorial Board: ACTA Pharmaceutics Sinica, Asian J. of Pharmaceutical Science, J. of Functional materials, Central South Pharmacy in China and so on. He got his BSc degree in chemistry, M.Sc degree in biomaterials in Peking University of China. Then He move to Belgium to got Ph D degree of pharmaceutical science in the Liege University. And he accepted Prof. S S Davis’ invitation, working in his lab.at the areas of nano-medicine research at the Nottingham University of UK. at 1996. When he came back to China, he got and finished about 15 items research projects of National and local government grant, including one national hi-tech project 863 and 9 items of National nature and science foundations. He also got a National Science and Technology Second Grade Award in China. He has published the book “Polymeric biomaterials and pharmaceutics” and “Biomaterials”, “Modern tropical medicine” and 188 research papers. Now his researches focus on gene delivery system and nano-medicine.

Iwona Cicha

Iwona Cicha studied Biology at the Jagiellonian University, Cracow, Poland. After obtaining her PhD in medical sciences at the Ehime Medical School, Ehime University, Japan, she moved to University of Erlangen. She was a postdoctoral fellow in the Department of Nephrology in 2003, before joining the Department of Cardiology, where she obtained her habilitation in Experimental Medicine in 2012. She has an extensive research experience in the field of atherosclerosis, with focus on the role of inflammation and blood flow dynamics in plaque development and destabilization. Since July 2013, she has been leading the Cardiovascular Nanomedicine Unit at the Section of Experimental Oncology and Nanomedicine (SEON), University Hospital Erlangen, focusing on the projects involving the application of nanomedical strategies for the treatment of cardiovascular disease.

Gaëlle Corne

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EDUCATION

2013-2016: Luxembourg Institute of Science and Technology Belvaux, Luxembourg LIST, PhD Student, Nano-Enabled Medicine and Cosmetics Group (NEMC), Materials Research and Technology (MRT), Subject: "Small and Smart Theragnostic protocells (SSTPs)"
2012–2013: INSTITUT SUPERIEUR OF HEALTH AND BIOPRODUCTS OF ANGERS Angers, France; 2nd year of Master Degree "Innovative FormulationTechnology»; Physicochemistry, chemistry, physics, formulation of drug
2012: FACULTY OF PHARMACY- UNIVERSITE PARIS SUD XI Paris, France, 1rst year of Master Degree "Drugs and Other products of Health"; Formulation and production of drugs, nanotechnologies, physicochemistry, galenic
2009-2011: FACULTY OF MEDICINE Nancy, France; Three years university degree of "Health Engineering»; Sciences of drugs, physiology, biology, pharmacology, and biochemistry

WORK EXPERIENCES

March–August 2013: CRP Gabriel Lippmann Belvaux, Luxembourg; Internship of Master Degree (2nd years); Departement of "Sciences and Analysis of Materials"; Subject: "Mesoporous Silica Nanoparticles"
July 2012: Faculty of Pharmacy, Laboratory CTFEPM, Nancy, France Laboratoire de Galénique Pharmaceutique et Biopharmacie, EA 3452 – CTFEPM, "CibleThérapeutique, Formulation et Expertise Pré Clinique du Médicament»; Additional training Master (1rst years); Subject: "Encapsulation of S-nitrosoglutathion in liposomes"
Febrary-April 2012: Faculty of Pharmacy, UMR CNRS 8612 Paris, France; Training of Master Degree (1rst year); Subject: "Formulation and characterisation of polymeric bioadhesive nanoparticles able to promote the oral absorption of Resveratrol"`
March–May 2011: Faculty of Pharmacy, Laboratory CTFEPM Nancy, France; Laboratoire de Galénique Pharmaceutique et Biopharmacie, EA 3452 – CTFEPM, "Cible Thérapeutique, Formulation et Expertise Pré Clinique du Médicament"; Internship of licence's study; Subject: "Encapsulation of S-nitrosoglutathion in polymeric nanoparticles and in liposomes."

Jean-Baptiste Coty

Jean-Baptiste Coty received his diploma of french engineer in chemistry from Ecole Nationale Supérieure de Chimie de Rennes (ENSCR, France) in 2014. He is currently a PhD student at the Institut Galien Paris Sud at the University Paris-Saclay, studying under the supervision of Dr. Christine Vauthier. His project is supported by BPI France as part of the project NICE. His research is focused on the development of methods for the characterization of interactions between nanomedicines and blood proteins to be applicable at a clinical level.

EDUCATION

Oct 2014 – now: PhD student at Institut Galien Paris Sud – UMR CNRS 8612 – University Paris-Saclay; Developing analytical tools for a better characterization of nanomedicine/biological components interactions (protein adsorption, complement and coagulation, protein corona investigation)

2011 – 2014: Graduate Student at the Engineering College of Chemistry in Rennes (ENSCR). Specialization: Analysis and Formulation; Engineering degree in chemistry equivalent to M Sc plus one year. Research projects: IPSEN Innovation: Researches of new formulation as suitable vehicles for therapeutic compunds - Stability studies of formulated compounds; University of Pennsylvania - Pr. Gary A. Molander research group : Development of new synthetic methods (organic synthesis)

COMMUNICATIONS

January 2016: Poster at the 5th annual meeting of nanometrology (Paris, France): Development of a high throughput method for characterization of interactions between nanomaterials and the complement system. (J-B. Coty, F. Varenne, J-J. Vachon, C. Vauthier)
July 2015: Poster at ULLA Summer School (Chatenay-Malabry, France): Characterization of polymer nanoparticles nanomedicines: which properties and which methods ? (J-B. Coty, F. Varenne, M. Noiray, C. Vauthier)
June 2015: Poster at PhD/Post-Doc day of Institut Galien Paris Sud (Orsay, France): Biological interactions of polymer nanomedicines with blood components – Development of methods for a routine characterization. (J-B. Coty, F. Varenne, M. Noiray, C. Vauthier)
Coauthor Vauthier Christine

Seyed Mohammadali Dadfar

Doctoral Candidate at Department of Nanomedicine and Theranostics
Institute for Experimental Molecular Imaging (ExMI), RWTH Aachen University Clinic; Pauwelsstrasse 30; 52074 Aachen, Germany
Supervisor: Prof. Dr. Dr. Twan Lammers

I have had a great interest in science from my childhood. I stood among the top 1% scorers in a nationwide entrance exam for aspirants seeking admission in engineering for getting Bachelor's degree, among more than 500,000 participants to enter universities in Iran. Given the choice, I took up Chemical Engineering as my undergraduate major in Tehran University, a pioneer university of technology in my country. Studying Chemical Engineering not only imparts me knowledge but also lays emphasis on the overall development of the individual. Besides broadening the range of my knowledge, it gave me valuable insight into practical aspects of study.
Having decided to continue my study, I participated in Iran National University Exam for graduate study and I was ranked 35th among

more than 10000 participants in chemical engineering nationwide entrance exam. I chose Sharif University of Technology (SUT), known as the best university of technology in the country in the field of Chemical Engineering with main focus on polymer science and nanotechnology, for my graduate study. During Master's and after that I could publish 5 papers in ISI journals, which one is among the top 25 hottest articles. Also, I have published 2 patents and submitted 4 more papers. I have nine national an international conference papers.

I was a member of Iran's National Elites Foundation (INEF) and I had all the conditions for elite student (published papers in ISI journals, patents, high university level and scores). After graduation, I worked for 2 years on a research project on drug delivery for cancer therapy instead of military service at Sharif University of Technology in Iran. After that I came across the work of Dr. Lammers and colleagues at RWTH Aachen University, who have been developing materials and methods to improve drug delivery to tumors. I believe that my background in nanotechnology and my experience in drug delivery will help me to improve my knowledge and experience under supervision of Dr. Lammers. My future plans are to get the highest academic level and position at university and also to establish a pharmaceutical company in my field.

Dominyka Dapkute

Biomedical Physics Laboratory, National Cancer Institute, Vilnius, Lithuania.
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EDUCATION

2014–2016: MSc degree in molecular biology, Faculty of Natural Sciences, Vilnius University

2010–2014: BSc degree in molecular biology, Faculty of Natural Sciences, Vilnius University

RESEARCH GRANTS

2014–2016: Joint Lithuanian-Latvian-Taiwanese research project "Mesenchymal stem cell and cancer stem-like cell response to nanoparticle treatment" (TAP LLT 03/2014), specialist.

CONFERENCES

- Dapkute D, Steponkiene S, Rotomskis R. Selective Accumulation of Quantum Dots in Breast Cancer Stem Cells. Open Readings 2014. March 19-21 Vilnius, Lithuania. Abstract book 47 p.
- Dapkute D, Steponkiene S. Selective Accumulation of Quantum Dots in CD44+ breast cancer cells. Nanotechnology: Research and Development. May 15-16 Vilnius, Lithuania. Abstract book 85 p.
- Dapkute D, Steponkiene S, Rotomskis R. Pharmacokinetics of Quantum Dots in Different Types of Breast Cancer Cells. NanoCon 2014. 6th International Conference. November 5-7 2014, Brno, Czech Republic. Abstract Book 137 p.
- Steponkiene S, Dapkute D, Rotomskis R. Application of Functionalized Quantum Dots in Targeted Cancer Therapy. NanoCon 2014. 6th International Conference. November 5-7 2014, Brno, Czech Republic. Abstract Book 64 p.
- Dapkute D, Steponkiene S. Accumulation, Distribution and Elimination of Quantum Dots in Different Types of Human Breast Cancer Cells. LTF 2015. January 2-3 2015 Vilnius, Lithuania. Abstract Book 13 p.
- Dapkute D, Steponkiene S, Kaseta V, Riekstina U, Rotomskis R. Quantum Dot-loaded Mesenchymal Stem Cells for Tumor-Tropic Therapy. Open Readings 2015. March 24-27 2015 Vilnius, Lithuania. Abstract book 77 p.
- Dapkute D, Steponkiene S, Rotomskis R. Antibody-Conjugated Quantum Dots for Targeting of Breast Cancer Stem-Like Cells. Current Trends in Cancer Theranostics 2015. June 1-3 2015 Jena, Germany. Abstract book 47 p.
- Budenaite L, Matulionyte-Safine M, Dapkute D, Rotomskis R. Cellular Uptake and Biological Effect of Photoluminescent Gold Nanoclusters on Cancer Cells. Vita Scientia 2016. January 4 2016 Vilnius, Lithuania. Abstract book 29 p.
- Dapkute D, Steponkiene S, Kisonas J, Bulotiene D, Saulite L, Riekstina U, Rotomskis R. Tumor Tropic Delivery Of Quantum Dots Using Mesenchymal Stem Cells. Open Readings 2016. March 15-18 2016 Vilnius, Lithuania. Abstract book 21 p.
- Budenaite L, Matulionyte-Safine M, Jarockyte G, Dapkute D, Rotomskis R. Cellular Uptake and Biological Effect of MES- and BSA-Coated Photoluminescent Gold Nanoclusters *in vitro*. Open Readings 2016. March 15-18 2016 Vilnius, Lithuania. Abstract book 118 p.

SCIENTIFIC PUBLICATIONS

Steponkiene S, Dapkute D, Riekstina U, Rotomskis R. Accumulation and Distribution of Non-targeted and Anti-CD44-conjugated Quantum Dots in Distinct Phenotypes of Breast Cancer. J Nanomed Nanotechnol 2015, 6:6. http://dx.doi.org/10.4172/2157-7439.1000341

Rafael T. M. de Rosales

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CURRENT POSITION

- Lecturer in Imaging Chemistry (3/2011- date), Division of Imaging Sciences & Biomedical Engineering, King's College London (UK)

EDUCATION AND POSTDOCTORAL TRAINING

- Postdoctoral Research Fellow in imaging chemistry (6/2007-2/2011), Division of Imaging Sciences and Biomedical Engineering, King's College London (UK)
- Postdoctoral Research Associate in synthetic inorganic chemistry (2/2006 - 3/2007), Department of Chemistry, Imperial College London (UK)
- Marie Curie Postdoctoral Fellow in bioinorganic chemistry (1/2005-2/2006), Department of Chemistry, University of Naples "Federico II" (Italy)
- PhD in Bioinorganic Chemistry (10/2001-1/2005), Department of Chemistry, University of Edinburgh (UK)

SELECTED RECENT PUBLICATIONS

1. A. Woods, A. Patel, D. Spina, R.-V. Yanira, A. Babin-Morgan, R. T. M. de Rosales, K. Sunassee, S. J Clark, H. Collins, K. D Bruce, L. A. Dailey, B. Forbes*, Lung targeting and *in vivo* safety of albumin nanoparticles for pulmonary drug delivery, Journal of Controlled Release, 2015, IN PRESS
2. X. Cui, S. Belo, D. Krüger, Y. Yan, R. T. M. de Rosales, M. Jauregui-Osoro, H. Ye, S, Su, D. Mathe, N. Kovács, I. Horváth, M, Semjeni, K. Sunassee, K. Szigeti, M. Green, PJ Blower*, Aluminium hydroxide stabilised MnFe2O4 and Fe3O4 nanoparticles as dual-modality contrasts agent for MRI and PET imaging, Biomaterials, 2014, 35, 5840-5846.
3. J. Tzu-Wen Wang, L. Cabana, M. Bourgognon, H. Kafa, A. Protti, K. Venner, A. M. Shah, J. Sosabowski, S. J. Mather, A. Roig, X. Ke, G. Van Tendeloo, R. T. M. de Rosales, Gerard Tobias*, and Khuloud T. Al-Jamal*. Magnetically Decorated Multi-Walled Carbon Nanotubes as Dual MRI and SPECT Contrast Agents, Adv. Funct. Mater. 2014, 24,1880-1894.
4. R. T. M. de Rosales*, Potential Clinical Applications of Bimodal PET-MR or SPECT-MR Imaging Agents, J. Label Compd. Radiopharm. 2013, 57, 4, 298-303.
5. M. Ahmed, R. T. M. de Rosales, M Douek*, Preclinical studies of the role of iron oxide magnetic nanoparticles for non palpable lesion localization in breast cancer, J. Surgical Res. 2013, 185, 1, 27-35.

Tapas K De

Dr. Tapas K De has extensive industrial experience with both proteins/enzymes and small molecules in formulation and process development, including scale-up/technology transfer. His scientific specializations are in parenteral and ocular drug delivery and release kinetics, nanoparticle (ceramic, polymeric, bioadhesive, hydrogel) formulation development, reverse micellar enzymology, enzyme kinetics and reaction mechanism. He is an expert on nab formulation development and characterization. He has developed Abraxane nanoparticle formulation at Abraxis Bioscience, as well as nab-17-AAG, nab-docetaxael, nab-rapamycin, nab-ABI011 etc. He was the lead formulation scientist for the nab business venture with National and international pharmaceuticals companies.

He has authored and co-authored more than 10 USA and world patents, 20 research articles and written chapters in CRC Handbook of Surface and Colloid Chemistry, second Edition and Encyclopedia of Surface and Colloid Sciences.

He is one of the co-founders of the lipid-based nanoparticle formulation company LipoMedics Inc, Fort Worth, TX, USA and currently serving as Chief Executive Officer of the Company.

Tiziana Di Francesco

Tiziana Di Francesco is a 3rd year Ph.D. student at the School of Pharmacy at the University of Geneva, Switzerland. Her research is supervised by Prof. Gerrit Borchard and is focused on Non-Biological Complex Drugs (NBCDs). In particular, she performs detailed physico-chemical characterization of these compounds in order to evaluate their effectiveness as well as possible differences in terms of efficacy and safety between originator products and so-called similars.

Tiziana holds a Bachelor and a Master's degree from the Department of Chemistry and Pharmaceutical Technology at Università degli Studi "G. d'Annunzio" Chieti-Pescara, Italy. During her studies in Italy, she took part in the European Program LifeLong Learning Erasmus at the University of Geneva. She also joined the Biopharmacy Department in Geneva to undertake research for her Masters' thesis, investigating polyelectrolyte nanocomplexes as tools for wound healing.

Marco Diego Dominietto

Marco Diego Dominietto is post-doc at the Biomaterials Science Center of the University of Basel.

Marco Dominietto is a medical physicist. He received his PhD degree in Biomedical Engineering in 2011 at the Institute for Biomedical Engineering (ETH Zurich).

His research focuses on the following area: physiology of tumor growth and image analysis both in mouse models and human beings, artificial muscle development, and energy harvesting from human body to power implantable medical devices.

Among other sites, he worked at University of Zurich (Zurich, Switzerland), Kantonsspitäler Schaffhausen (Switzerland), CERN (Geneva, Switzerland), European Institute of Oncology (Milano, Italy) and University Hospital of Novara (Novara, Italy).

Simona Dostálová

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EDUCATION AND TRAINING

9/2014–onwards: Principal subjects/occupational: Postgradual Student, branch: Chemistry, Department of Chemistry and Biochemistry, Thesis: Specific targeting of nanocarriers for theranostics

9/2012–6/2014: Title of qualification awarded Engineer (Ing.)
Principal subjects/occupational: Biomedical Engineering and Bioinformatics, Thesis: Nanocarriers for theranostics
Name of organization: Faculty of Electrical Engineering and Communication, Brno University of Technology, Czech Republic

PROFESSIONAL PRACTICE

2011–onwards: Laboratory of Metallomics and Nanotechnologies, Department of Chemistry and Biochemistry, Mendel University in Brno – scientific and technical worker, Research activities on analysis of nucleic acids and nanomedicine in theranostics

AWARDS

- **11/2015:** 2nd instead, The Conference competition MendelNET 2015. S. Dostalova, V. Milosavljevic, R. Guran, M. Kominkova, K. Cihalova, P. Kopel, M. Vaculovicova, V. Adam a R. Kizek, Antiviral activity of fullerenes modified with maximin H5 derivatives.
- **06/2014:** Dean's award for final thesis, Faculty of Electrical Engineering and Communication, Brno University of Technology
- **11/2013:** Rector's award for outstanding academic and research results, Brno University of Technology
- **11/2013**: 1st instead, The Conference competition MendelNET 2013. S. Dostalova, R. Konecna, I. Blazkova, M. Vaculovicova, P. Kopel, S. Krizkova, T. Vaculovic, V. Adam a R. Kizek, Apoferritin as a targeted drug delivery system, Section: Applied chemistry and biochemistry
- **11/2012:** 3rd instead, The Conference competition MendelNET 2012. S. Dostalova, E. Jilkova, S. Krizkova, M. Masarik, K. Smerkova, D. Hynek, B. Ruttkay-Nedecky, L. Krejcova, V. Adam a R. Kizek, Automated zinc protein extraction from prostatic cancer cell using magnetic particles, Section: Applied chemistry and biochemistry
- **06/2012:** Dean's award for final thesis, Faculty of Electrical Engineering and Communication, Brno University of Technology

RESEARCH

Author or coauthor of 22 conference papers and 20 original scientific papers in ISI-indexed journals with a total of 48 citations, H=4 according to Web of Science.

Stella-Saphira Ehrenberger

Stella-Saphira Ehrenberger started her Ph.D. in October 2013 in Biopharmacy within the School of Pharmaceutical Sciences at the University of Geneva, Switzerland. She graduated in Pharmacy at the Albert Ludwigs University of Freiburg im Breisgau, Germany. During her studies she was intern in the pharmacy department of the Khoo Teck Puat Hospital in Singapore as well as at Novartis sterile production of biopharmaceuticals in Stein, Switzerland. Her research is focused on active targeting of superparamagnetic iron oxide nanoparticles for detection of early prostate cancer metastases in lymph nodes. In detail, she is developing, characterizing and comparing ligand-nanoparticle constructs (small molecule-, ap-

tamer- and antibody-functionalized iron oxide nanoparticles). She is also working on the development of an injectable, in-situ forming nanocomposite for application of local hyperthermia in solid tumors. Her work, supervised by Prof. Gerrit Borchard and Dr. Olivier Jordan, is part of the Nano-Tera project MagnetoTheranostics.

Valentina Francia

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EDUCATION AND TRAINING

Aug 2014–Present: PhD student in Pharmacology, University of Groningen, Antonius Deusinglaan 1 - 9713 AV Groningen - The Netherlands, Research topic: Nanoparticle-cell interaction, endocytosis, cellular uptake mechanisms of nanoscale objects
Jan 2011–Dec 2013: Master's Degree in Molecular Biology of the Cell; University of Milan "Università degli Studi di Milano"
Via Celoria 26–20133 Milano–Italy, Final score: 110/110 cum Laude
Oct 2012–Dec 2013: Title of degree thesis: "The roles of the kinase Cdk1 and of the phosphatase PP2A in the adaptation to the mitotic checkpoint in Saccharomyces cerevisiae"; IFOM (FIRC Institute of Molecular Oncology Foundation); Via Adamello 16 - 20139 Milano - Italy
Oct 2006–Oct 2010: Bachelor's Degree in Biological Sciences, University of Milan "Università degli Studi di Milano"; Via Celoria 26–20133 Milano–Italy; Final score: 108/110
Apr 2010–Sep 2010: Title of degree thesis: "Study of the interactive potential between alternative sigma factors in Pseudomonas putida"; Department of Biomolecular Sciences–Giovanni Bertoni's Lab;

PUBLICATIONS

Sep 2013: Vernieri C, Chiroli E, Francia V, Gross F, Ciliberto A.; Adaptation to the spindle checkpoint is regulated by the interplay between Cdc28/Clbs and PP2ACdc55; J Cell Biol. 2013 Sep 2;202(5):765-78

Tamás Fülöp

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EDUCATION:

- Medical and Pharmaceutical Biotechnology Bachelor program, IMC University of Applied Sciences Krems, Austria 2008-2011
- Medical and Pharmaceutical Biotechnology Master program, IMC University of Applied Sciences Krems, Austria 2011-2013
- PhD program in Nanomedicine, Semmelweis Medical University Budapest, Hungary 2014-

TRAININGS:

- Practical training semester at Academic Medical Center (AMC) Amsterdam, the Netherlands, Laboratory of experimental oncology and radiobiology 01.10.10-19.02.11
- Research training semester at Centro de Investigationes Biologicas (CSIC) Madrid, Spain, TGF-beta and endothelial cells research group 06.09.12-06.02.13

PUBLICATIONS:

- Dezsi, L. et al. Features of complement activation-related pseudoallergy to liposomes with different surface charge and PEGylation: Comparison of the porcine and rat responses. Journal of Controlled Release 195, 2-10, doi:10.1016/j.jconrel.2014.08.009 (2014).
- Fülöp, T. G. et al. The possible role of factor H in complement activation-related pseudoallergy (CARPA): A failed attempt to correlate blood levels of FH with liposome-induced hypersensitivity reactions in patients with autoimmune disease. European Journal of Nanomedicine 7, 7-14, doi:10.1515/ejnm-2015-0004 (2015).
- Meszaros, T. et al. Factor H inhibits complement activation induced by liposomal and micellar drugs and the therapeutic antibody rituximab *in vitro*. Nanomedicine, doi:10.1016/j.nano.2015.11.019 (2015).
- Jackman, J. A. et al. Comparison of complement activation-related pseudoallergy in miniature and domestic pigs: foundation of a validatable immune toxicity model. Nanomedicine, doi:10.1016/j.nano.2015.12.377 (2016).
- Szebeni, J., Fulop, T., Dezsi, L., Metselaar, B. & Storm, G. Liposomal doxorubicin: the good, the bad and the not-so-ugly. J Drug Target, 1-7, doi:10.3109/1061186x.2016.1172591 (2016).

Doris Gabriel

Doris Gabriel is R&D manager at Apidel SA, a pharmaceutical startup company developing innovative (nano)-medicines mainly for applications in Dermatology and Opthalmology. Doris studied Pharmaceutical Sciences at the Universities of Bern and Basel, Switzerland. During her PhD project from 2004 – 2008, she developed protease-activated nanomedicines at the University of Geneva, Switzerland. This was followed by post-doctoral training at the Swiss Federal Institute of Technology Lausanne, Switzerland (2009) and the Massachusetts Institute of Technology/Harvard Medical School, USA (2010-2012), where she further focused on drug delivery and the development of light-triggered biomaterials. Driven by a motivation to translate innovative technologies into products, Doris joined Apidel, a University of Geneva spin-off company, end of 2012 as first full-time employee. She is currently heading Apidel's R&D lab.

Ragnhild Garborg Østrem

WORK EXPERIENCE

Jan 14 –: PhD student, Colloids and Biological Interfaces Group, Department of Micro- and Nanotechnology, Technical University of Denmark, DK
June 12–Jan 14: Private tutor of English, mathematics and natural sciences
Jan 08–May 08: Sous-chef, Le café francaise, Stavanger, Norway
Aug 07–Dec 07: Horse groom, Private stable, Normandy, France

EDUCATION

Jan 14–: PhD student, Colloids and Biological Interfaces Group, Department of Micro- and Nanotechnology, Technical University of Denmark, DK

- Subject: Tuning liposomes for controlled release of chemotherapeutics, and evaluating their potential *in vitro* and *in vivo*.
- External stay, Section of Environmental Health, Department of Health and Medical Sciences, University of Copenhagen

Sep 11–Sep 13: Master (MSc) in Nanoscience, University of Copenhagen

- Master Thesis, Center for Pharmaceutical Nanotechnology and

Nanotoxicology, University of Copenhagen, "Engineering and characterization of peptide-coupled liposomal nanoparticles for targeting of human brain capillary endothelial cells".

Sep 08–June 11: Bachelor (BSc) in Nanoscience, University of Copenhagen DK

Eduard Gatin

Lecturer, Ph.D
University of Bucharest, Faculty of Physics, Department Science Materials, P.O.Box, MG 11, Bucharest-Magurele, Romania; University of Medicine 'Carol Davila', Faculty of Dentistry, Dental Materials Department, Calea Plevnei 19, Sect.5, Bucharest, Romania.

Me Eduard Gatin, Physicist Education, beginning with 2008 I am dedicated to material science related to Medical Field (dentistry, as: dental restoration materials, corrosion, dental enamel quality, tissue regeneration – dentine). From 2010 – present, Lecturer – Biophyscs Department University of Medicine "Carol Davila", Faculty of Dentistry. Techniques skills: RAMAN spectroscopy (improved by SERS), SEM, EDX. Between 2010 – 2013, I was postdoctoral student in EU Program PostDoc (to improve research work, EU finance support). In 2013 I succeded to propose a method for quality evaluation of dental enamel by Raman method, to be applied " *in vivo*". On 30th October 2015, patent registration certificate was issued. It was started a study " Introducing RAMAN technique to Periodontology" (*in vivo* application), according bioethical approval from January 2016, with Semmelweis University Budapest – Faculty of Dentistry.

Robert Geertsma

Senior Scientist, Centre for Health Protection, National Institute for Public Health and the Environment (RIVM)
E-mail: Robert.Geertsma@rivm.nl

Robert Geertsma has worked at the Dutch National Institute for Public Health and the Environment (RIVM) for almost twentytwo years. As a senior scientist and project leader he is responsible for the provision of scientific advice to regulators on quality and safety of medical technology and nanomedicine. He works on multiple research projects on opportunities as well as risks of nanotechnologies and nanomaterials in medical applications, performing both desk research and experimental research. He participated in FP7-projects ObservatoryNano and NanoMedRoundTable. He is also one of the experts of the Risks of Nanotechnology Knowledge and Information Centre (KIR nano), a Dutch government-supported observation organisation based at RIVM. His areas of expertise include risk management, biological safety, nanotechnology and emerging medical technologies. He participates actively in international ISO/CEN Standards Committees on these subjects and he is chairman of the joint CEN/CENELEC/TC3 responsible for horizontal standards on topics like quality and risk management systems. He was a member of the SCENIHR WG that wrote the Scientific Opinion "Guidance on the Determination of Potential Health Effects of Nanomaterials Used in Medical Devices". He also co-chairing the ISO/TC194/WG17 on Biological Evaluation of Medical Devices – Nanomaterials and he is a member of the Nanomedicines WG of the International Pharmaceutical Regulators Forum. Furthermore, he frequently represents the Dutch competent authority in European Commission's working groups such as the New & Emerging Technologies WG, of which he was appointed co-Chair in 2009. He is a member of the European Society for Nanomedicine and the European Technology Platform Nanomedicine. Since 2011, he coordinates the National Platform Nanomedicine in the Netherlands.

Balázs Györffy

M.Sc.
Eötvös Loránd University, Budapest
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My name is Balázs Györffy and I obtained my Bachelor's degree in biology at Eötvös Loránd University, Budapest, Hungary in 2010 and my Master's degree in biology with specialization to neurobiology in 2012 at the same university. I started my PhD studies in 2012 at Eötvös Loránd University in the Laboratory of Proteomics, Institute of Biology. In 2014, I started working in the MTA-ELTE NAP B Neuroimmunology Research Group, Department of Biochemistry, Institute of Biology, Eötvös Loránd University as an assistant research fellow. My research focus is on neuroimmunology using neuroproteomics tools.

Luciana-Maria Herda

Marie Curie Early Stage Researcher
Centre for BioNano Interactions,
University College Dublin, Ireland
luciana-maria.herda@cbni.ucd.ie

EDUCATION

2014–present: University College Dublin, Ireland, Centre for BioNano Interactions Marie Curie Early Stage Researcher, PhD student
2012–2013: University College London (London, UK), MSc in Drug Discovery and Development
2007–2012: Carol Davila University of Medicine and Pharmacy (Bucharest, Romania); MSc in Pharmacy

EXPERIENCE

2014–present: University College Dublin, Ireland; Graduate Teaching Assistant
Feb–Aug 2012 MCM Delphi Community Pharmacy (Bucharest, Romania); Pre-Registration Pharmacist
July-Aug 2012 St. Constantin Hospital (Brasov, Romania); Hospital Pharmacy Intern
July-Aug 2012 Zentiva (Sanofi Company); Pharmaceutical Industry Intern

PUBLICATIONS

1. Herda L., Polo E., Kelly P.M., Rocks L, Hudecz D., Dawson K.A. Eur J Nanomed. 2014 (6): 127–139.
2. Hudecz D., Rocks L., Fitzpatrick L., Herda L., Dawson K.A. Eur J Nanomed. 2014 (6): 185–193.
3. Herda L., Polo E., Lo Giudice M.C. Hristov D., Dawson K.A. Optimising immunogold based mapping for use with biologically relevant particles. Unpublished.
4. Lo Giudice M.C., Herda L., Polo E., Dawson K. A. In situ characterization of the nanoparticle biointerface. Submitted.

CONFERENCE PRESENTATIONS

- March 2016 The 11th Conference on Biological Barriers, Saarbrucken (Germany): poster presentation, conference session chairing, oral presentation in a satellite meeting.
- Dec 2014 The Nanotracking Cluster Meeting, Gold Coast/Brisbane (Australia): oral presentation
- Nov 2014 Microscopy Symposium, Glasgow (UK): poster presentation
- Sept 2014 International Meeting on Signal Transduction at the BBB, Dublin (Ireland): poster presentation

Leticia Higa

PhD
Programa de Nanomedicinas
Universidad Nacional de Quilmes
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Leticia Higa obtained her degree in Biotechnology and PhD in Basic & Applied Sciences National University of Quilmes (UNQ). After that, she continued her postdoctoral degree at Nanomedicine Research Program (NRP) laboratory at UNQ. Nowadays, she is an Assistant Research and a Chemistry professor in the Science and Technology Department at the same university. Her research is focused on the development of nanoparticles (nanoparticle lipid solid and nanostructured lipid carriers, liposomes, archaeosomes) to deliver anti-inflammatory or antigens by oral and topical via under supervision of Prof. Eder Romero and Maria Jose Morilla. Her scientific contribution has been published in International Journal of Pharmaceutics, Colloids and Surfaces B: Biointerfaces, International Journal of Nanomedicine Nanomedicine: Nanotechnology, Biology and Medicine, BMC Biotechnology, Human Vaccines and Immunotherapeutics, Journal of Biomaterials and Tissue Engineering. Besides, she is a Chemistry Assistant Professor at UNQ.

SCIENTIFIC PAPERS (PEER REVIEWED)

- Ultradeformable Archaeosomes for Needle Free Nanovaccination with Leishmania braziliensis Antigens. Leticia H. Higa, Laura Arnal, Mónica Vermeulen, Ana Paula Perez, Priscila Schilrreff, Cecilia Mundiña-Weilenmann, Osvaldo Yantorno, María Elena Vela, María José Morilla, Eder Lilia Romero. PLOS ONE. February 2016
- Enhanced photodynamic leishmanicidal activity of a hydrophobic zinc phtalocyanine within archaeolipids-containing liposomes". AP Perez, A Casasco, P Schilrreff, MV Defain Tesoriero, L Dumpelmann, J Altube, L Higa, MJ Morilla, P Petray, EL Romero. International Journal of Nanomedicine, 2014.
- Archaeosomes display immunoadjuvant potential for a vaccine against Chagas disease. LH Higa; MJ Morilla; R Corral; E Romero; P Petray. Human Vaccines and Immunotherapeutics 2013.
- The intervention of Nanotechnology against epithelial fungal diseases. LH Higa, P Schilrreff, AP Perez, MJ Morilla and EL Romero. Journal of Biomaterials and Tissue Engineering 2013
- Ultradeformable archaeosomes as new topical adjuvants". Higa L, Schilrreff, Perez AP, Iriarte M, Roncaglia D, Morilla MJ and Romero EL. Nanomedicine, Nanotechnology, Biology and Medicine, 2012.

BOOK CHAPTER

Nanotechnology and Drug Delivery, Volume Two: Nano-Engineering Strategies and Nanomedicines against Severe Diseases. Jose L. Arias. Capítulo del libro: Nanomedicines against Infectious Diseases; Leticia H. Higa, Ana Paula Perez, Priscila Schilrreff, Maria Jose Morilla and Eder Lilia Romero. 2016

GRANTS AND AWARDS

- Grant for Researchers: Secretary of Research, UNQ 2016
- Doctoral: National Scientific and Technical Research Council (CONICET) 2007–2012
- Posdoctoral: CONICET 2013–2014

PROFESSIONAL ASSOCIATION

- Asociación Argentina de Nanomedicina: vocal suplente de la Comisión Directiva 7/2010–present

Cordula Hirsch

Empa – Swiss Federal Institute for
Materials Science and Technology
Department Materials-Biology
Interactions, St. Gallen,Switzerland

Dr. Cordula Hirsch was born in 1978 in Germany. She studied Biology at the University of Konstanz (Germany) starting from 1997. In the field of nervous system regeneration she graduated from there in 2002 with a Diploma in Neurobiology. In the beginning of 2003 she started her PhD Thesis at the University of Freiburg (Germany) at the Institute of Molecular Medicine and Cell Research. The main focus was to elucidate the influence of the canonical Wnt-signaling pathway on proliferation and differentiation of neural progenitor cells from mouse forebrain. Cordula successfully defended her PhD in 2007. After a short Postdoc period still in Freiburg on a Systems Biology of the Liver project she started 2008 as a Postdoc at Empa in St. Gallen in the field of Nanomaterial-Cell Interactions where she's still working as a scientist.

Delyan R. Hristov

PhD
Research Experience

Jan 2016–present: PostDoc Position, Center for BioNano Interactions, University College Dublin, School of Chemistry and Chemical Biology, Dublin, Leinster, Ireland
Sep 2011–Sep 2015: PhD Student, University College Dublin, School of Chemistry and Chemical Biology, Dublin, Leinster, Ireland
Apr 2009–Dec 2010: Technical Assistant, Bulgarian Academy of Sciences, Department of Optical Materials, Sofia, Sofia-Capital, Bulgaria
Jun 2008–Aug 2011: Laboratory Assistant, Sofia University "St. Kliment Ohridski", Department of Inorganic Chemistry, Sofia, Bulgaria

SKILLS & ACTIVITIES

Skills: Nanomaterials, Surface Modification, Polymers, Surface Functionalization, Nanoparticle Synthesis, Silica Nanoparticles, Material Characterization, Nanobiotechnology, Medical Nanotechnology

Selected Journal Publications:

- Delyan R. Hristov, Louise Rocks, Philip M. Kelly, Steffi S. Thomas, Andrzej S. Pitek, Paolo Verderio, Eugene Mahon, Kenneth A. Dawson, Scientific Reports 12/2015; 5:17040. DOI:10.1038/srep17040
- Delyan R Hristov, Eugene Mahon, Kenneth A Dawson, Chemical Communications 10/2015; 51(98). DOI:10.1039/c5cc06598d
- Anna Salvati, Andrzej S Pitek, Marco P Monopoli, Kanlaya Prapainop, Francesca Baldelli Bombelli, Delyan R Hristov, Philip M Kelly, Christoffer Aberg, Eugene Mahon, Kenneth A Dawson, Nature Nanotechnology 01/2013; 8(2). DOI:10.1038/nnano.2012.237
- Eugene Mahon, Delyan R Hristov, Kenneth A Dawson, Chemical Communications 07/2012; 48(64):7970-2. DOI:10.1039/c2cc34023b

Diána Hudecz

Marie Curie Early Stage Researcher
Centre for BioNano Interactions,
University College Dublin, Belfield,
Dublin 4, Ireland
Mobile: +353 89 440129
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I am a Marie Curie Early Stage Researcher and PhD fellow at the Centre for BioNano Interactions, University College Dublin, Ireland. The aim of my research is to understand the basic mechanisms by which nanoparticles are taken up and subsequently pass through the blood-brain barrier (BBB) using *in vitro* BBB models, live cell imaging and other complementary techniques.

Since I started my project, I have had the opportunity to present my results on the Marie Curie Consortium meetings and various international conferences. I had an oral presentation on the 11th Conference and Workshop on Biological Barriers in Saarbrücken, Germany (March, 2016), a poster presentation on the 4th Cold Spring Harbor conference on Blood Brain Barrier in Long Island, USA (Dec, 2014), and a poster presentation on the 17th International Symposium on Signal Transduction at the Blood-Brain and Blood-Retina Barriers in Dublin, Ireland (Sept, 2014).

I hold two master's degrees, one in Chemical Engineering from the Technical University of Denmark, Denmark (2011) and one in Pharmaceutical Sciences from the University of Copenhagen, Denmark (2013). During my chemical engineering degree, I received a sponsorship from H. Lundbeck A/S, where I spent 2 months in a summer internship and 7-month master's project (Lumås, Denmark) between 2010 and 2011. I was always interested in pharmaceutical research, but I got closer to biology and nanomedicines in Prof. S. Moein Moghimi's group during my second master's project, where I was working with polyethylenimine coated polystyrene nanoparticle for nucleic acid delivery.

Additionally, I also hold a bachelor degree in Chemical Engineering from the Budapest University of Technology and Economics, Hungary (2009). My bachelor studies contained a two-year long internship at Gedeon Richter Plc. (Budapest, Hungary), where I modelled human drug absorption through the gastrointestinal tract and the BBB in the early phase of drug discovery using the Parallel Artificial Membrane Permeability Assay method. The results of this study were presented as oral presentations in national conferences, such as in XXXI. KEN Conference, Szeged, Hungary (Oct, 2008). I won a first prize on the university round of the Conference of Scientific Students' Associations in 2008, therefore I was entitled to present my work in the highly prestigious national round, where I won the first prize in in Section of Biochemistry and Biotechnology (April, 2009).

PUBLICATIONS:

1. Hudecz D, Rocks L, Fitzpatrick LW, Herda LM, Dawson, KA. Reproducibility in biological models of the blood-brain barrier. EJNM. 2014, 6(3):185–93.
2. Herda LM, Polo E, Kelly P, Rocks L, Hudecz D, Dawson KA. Designing the future of nanomedicine: current barriers to targeted brain therapeutics. EJNM. 2014, 6(3):127– 39.
3. Parhamifar L, Andersen H*, Wu L*, Hall A*, Hudecz D*, Moghimi SM. Polycation-mediated integrated cell death processes. Adv Genet. 2014, 88:353-98. (*Contributed equally)
4. Abstract: Hudecz D, Permeability assay in the early phase of drug discovery. Published in: KRISZTINA LÁSZLÓ: Conference of MSc students - Abstracts of the best contributions October 2008, Per. Pol. Chem. Eng., 2009, 53(3):19-27.

Cristian Iacovita

Oltului, 36/8, Cluj-Napoca, Romania
Tel: 004 0743 421 147
E-mail: cristian.iacovita@umfcluj.ro
crissiac@yahoo.com

PROFESSIONAL EXPERIENCE:

Since February 2013: Assistant Professor in the group of Prof. C. M. Lucaciu at Department of Pharmaceutical Physics and Biophysics, „Iuliu-Hatieganu" Univeristy of Medicine and Pharmacy, Cluj-Napoca, Romania.

February 2012–February 2013: Postodoctoral researcher in the group of Prof. C. M. Lucaciu at Department of Pharmaceutical Physics and Biophysics, „Iuliu-Hatieganu" Univeristy of Medicine and Pharmacy, Cluj-Napoca, Romania.
February 2011–January 2012: Postodoctoral researcher in the group of Prof. T. A. Jung, Department of Physics, University of Basel, Switzerland.
October 2009–January 2011: Postodoctoral researcher in the group of Prof. T. A. Jung, Department of Physics, University of Basel, Switzerland.
November 2005–August 2009: PhD student in the group of Prof. Jean-Pierre Bucher, IPCMS, University of Strasbourg, France.
September 2004–June 2005: Master student (Socrates-Erasmus fellowship) in the group of Prof. D. R. T. Zhan, Institute of Physics, University of Technology, Chemnitz, Germany.

CURRENT RESEARCH TOPICS:

1. the design of magnetic nanoparticles with improved heating capabilities for hyperthermia applications.
2. the development of biocompatible noble metal nanoparticles with potential applications in nano-medicine and the investigation of their physical-chemical properties
3. the study of the interaction of biocompatible nanoparticles with different types of cancer cells.
4. the study of the interaction between different pharmaceutical compounds and the surface of noble metal nanoparticles by means of Surface Enhanced Raman Spectroscopy.

Christina Janko

Dr. rer. nat. Christina Janko studied Biology at the Friedrich-Alexander-University of Erlangen-Nuremberg from 2002 to 2007. From 2007 to 2012 she was a PhD student at the Institute of Clinical Immunology and Rheumatology within the Department of Internal Medicine 3 of the University Hospital Erlangen. In her PhD thesis in 2012 she focused on CRP-mediated effects in the clearance of dying and dead cells. Since 2013 she is working as postdoctoral researcher in the Section of Experimental Oncology and Nanomedicine (SEON) at the Department of Otorhinolaryngology, Head and Neck Surgery, at the University Hospital Erlangen in the group of Prof. Dr. med. Christoph Alexiou. Here she is responsible for the toxicological analyses of nanoparticles for medical applications.

Sae Rin Jean

4th year PhD student
Biological Chemistry, Kelley Lab,
University of Toronto
Tel: (647)7796891
E-mail: saerin.jean@gmail.com
MSB 5326 (1 King's College Circle,
M5S 1A8)

EDUCATION

Sept 2012 – Present: University of Toronto,Toronto, ON; PhD student Biological Chemistry; PI: Dr. Shana O. Kelley; Rationally designed and synthesized various anticancer drugpeptide conjugates to control the biological activity in cellulo and *in vivo* while maintaining the ability to evade multidrug resistance and secondary toxicity

Adept in operating and maintaining various scientific equipment such as HPLC, NMR, peptide synthesizer, fluorescence and confocal microscope, Western blot apparatus, gel imaging systems, flow cytometer, (q)PCR, spectrophotometer, tissue culture, rotary evaporator, speed vacuum concentrator, and lyophilizer

cumulative GPA: 3.9

Sept 2007 – Apr 2012: University of Waterloo, Waterloo, ON; Bachelor of Science, Honours Biochemistry Coop; Graduated on Dean's Honours List with cumulative average of final 2 years: 90% (GPA: 4.0)

WORK EXPERIENCE

May – Aug 2010/Jan – Sept 2011: Kinectrics Inc., Toronto, ON Analytical Chemistry/Radiochemistry

Developed novel procedures for metal separation and analyzed various samples using spectrophotometer, turbidimeter, conductivity meter, distillation apparatus, muffler oven, pH meter, dissolved oxygen meter, alpha and gamma spectrometry and alkalinity titrators

PUBLICATIONS

- Jean, S.R. *, Zhang, L.*, Sargent, E.H., and Kelley, S.O. (2016) Self-assembled quantum dot DNA hydrogel for drug and siRNA delivery. (manuscript in preparation)
- Wisnovsky, S., Jean, S.R., and Kelley, S.O. (2016) Proteins involved in mitochondrial repair and replication revealed with organelle-specific chemical probes. Nature Chemical Biology (under review)
- Buondonno, I., Gazzano, E., Jean, S.R., Kopecka, J., Salaroglio, I.C., Costanzo, C., Serra, M., Ghigo, D., Kelley, S.O., and Riganti, C. (2016) Mitochondriatargeting doxorubicin: a new therapeutic strategy against doxorubicinresistant osteosarcoma. Clinical Cancer Research (under review)
- Huang, S.Y., Rosa, I.D., Khiati, S., Tulumello, D.V., Michaels, S.A., Jean, S.R., Agama, K., Murai, J., Gao, R., Jenkins, L.M., Kelley, S.O., and Pommier, Y. (2016) TyrosylDNA Phosphodiesterase 2 (TDP2) and its short isoform in mitochondria. Proceedings of the National Academy of Sciences (under review)
- Jean, S.R.*, Tulumello, D. V.*, Riganti, C., Liyanage, S. U., Schimmer, A. D., and Kelley, S. O. (2015) Mitochondrial targeting of doxorubicin eliminates nuclear effects associated with cardiotoxicity. ACS Chemical Biology 10(9), 20072015.

*joint first authorship

Leena Karimi

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Mobile: (0045)-53 69 26 56
E-mail: Karimi@bmb.sdu.dk

EDUCATION TRAINING

2009-2012: University of Southern Denmark, Odense, Denmark, B.Sc. Biomedicine; Bachelor's Thesis awarded 12(A), entitled "The effect of intravenous injection of anti-TNF therapy on ischemic brain damage''.

2012-2015: University of Southern Denmark, Odense, Denmark; Cand. Scient Biomedicine; Master's Thesis awarded 12 (A), entitled "Evaluation of fatty acid metabolism as target for breast cancer therapeutics''. ITEK-project awarded 10 (B) entitled "High-throughput protein quantification of PTEN in breast cancer stem cells''. Individual study activity project (10 ECTS) awarded 12(A), entitled '' New protein biomarkers in invasive tumor cells in gliomas".

2015–Pres.: Ph.D. student at University of Southern Denmark, Odense, Denmark

WORK EXPERIENCES

2015–2015: Research Assistant, SPSE, University of Southern Denmark, Odense, Denmark

2011–2015: Academic Mentor, Elite & Support, University of Southern Denmark, Odense, Denmark

2012–2014: Instructor at Southern University of Denmark, Odense, Denmark

SUMMARY OF QUALIFICATIONS

Experience in a wide variety of experimental techniques, listed below:

•Cell culturing •Invasion assay •Migration assay •Mammosphere formation assay •Cytotoxicity assay •siRNA gene silencing• Quantification of enzymatic activity •Purification of RNA, DNA and plasmids• Extraction of RNA and nuclear proteins •cDNA synthesis •Gel Electrophoresis •PCR and Real-time qPCR •CHIP-PCR •Western Blotting •FACS-analysis •Immuno-cytochemical staining • fluorescence staining • Mouse brain tissue processing •Infarct volumetric analysis •Toluidine Blue Staining •Production of monoclonal antibodies, immunisation, fusion, selection and cloning •ELISA •Quantitative radio-immunoassay (RIA) •Immuno-electrophoresis and immune-diffusion techniques •Animal experiments •Mouse behavioural studies •Confocal time lapse microscopy

PUBLICATIONS

Bettina-Hjelm Clausen, Mathilda Degn, Nellie-Anne Martin, Yvonne Couch, Leena Karimi, Maria Ormhøj, Maria-Louise Bergholdt Mortensen, Hanne-Birgit Gredal, Chris Gardiner, Ian IL Sargent, David E Szymkowski, Géraldine H Petit, Tomas Deierborg, Bente Finsen, Daniel Clive Anthony and Kate Lykke Lambertsen (2014). "Systemically administered anti-TNF therapy ameliorates functional outcomes after focal cerebral ischemia". Journal of Neuroinflammation 11(1): 1-17.

Anna Khimchenko

Anna Khimchenko is a PhD student in Biomedical Engineering at the Biomaterials Science Center, University of Basel, Switzerland.
Anna Khimchenko did her B.Sc. in Medical Acoustic and Bioacoustic Instruments and Devices at National Technical University of Ukraine "KPI" and received her M.Sc. in Medical Acoustic and Bioacoustic Instruments and Devices at National Technical University of Ukraine "KPI" in 2012 and M.Sc. in Biomedical Engineering at University of Bern in 2013. During her studies she worked as research assistant at Wolfson Brain Imaging Centre, University of Cambridge in 2010 and from 2012 until 2013 as a scientific assistant at the Bern University of Applied Sciences. She is currently working towards her PhD degree on the micro- and nanoanatomy of human brain tissues. As a member of the SNSF-funded project 147172, her research interests include hard X-ray tomography, grating interferometry, phase contrast imaging and soft tissue visualization. Recently, Anna Khimchenko won the H. Don Wolpert from the Bioinspiration, Biomimetics, and Bioreplication SPIE conference sponsored by the Optical Society of Southern California, USA.

Klara Kiene

I am 25 years old and last year I finished my studies in pharmacy with a Master's degree and a Swiss diploma as a pharmacist. Afterwards I have started my PhD in the field of Pharmaceutical Technology at the University of Basel in the group of Prof. Dr. J. Huwyler.
2010 I completed the secondary school in Germany with an A-level "very good". Following, I started to study Pharmaceutical Sciences at the University of Basel, where I also finished my Bachelor of Pharmaceutical Sciences and my Master of Pharmacy in 2015.
During my studies, I had the opportunity to deepen my knowledge about Pharmaceutical Technology by doing my Master Thesis in the group of Prof. Dr. J. Huwyler under the supervision of Dr. F. Porta on the topic "Chitosan – From Shrimps to Nanoparticles: Formation of a Self-Assembling Hydrogel and Synthesis of Nanoparticles via Ionic Crosslinking and Nanoprecipitation". During these six months of my master thesis project, I worked with the biopolymer chitosan and created chitosan nanoparticles and chitosan hydrogels. In detail, I characterized the nanoparticles via Dynamic Light Scattering and Transmission Electron Microscopy, performing homogenous and nicely separated chitosan nanoparticles. Moreover, I modified chitosan resulting in thiolated chitosan and maleimide chitosan, forming a self-assembling hydrogel via Michael-addition. I characterized the obtained hydrogel by release testing of bovine serum albumin, showing that even large molecules, such as proteins, are released mediated by diffusion and not held back by small pores within the gel. The pores were also analyzed by Scanning Electron Microscopy in order to image the microporous structure of the chitosan hydrogel.
After four years of studying I concluded my Master in Pharmacy with an additional practical year in a public pharmacy as well as in a hospital pharmacy. As I was also very much interested in the pharmaceutical industry I did two internships at F. Hoffmann-La Roche, in Basel in the Quality Assurance Drug Product Biologics in 2012 and 2013.
Since I began my PhD in November 2015 I have been working with poly(dimethylsiloxane)-poly(2-methyloxazoline) (PDMS-PMOXA) polymersomes which were targeted with asialofetuin (AF). We synthesized polymeric vesicles using AF as targeting moiety for the HepG2 cell line due to significant expression of asialoglycoprotein receptors on their cellular membrane. We could show receptor mediated uptake of such formulation and investigated intracellular trafficking studying the permanence of the vesicles in early and late endosomes. Moreover, we worked with zebrafish as an early in-vivo tool to investigate the real time blood circulation of the AF-modified PDMS-PMOXA polymersomes.
Superiors, colleagues and friends describe me as a very good motivator and team player, highly energetic, always co-operative, helpful, positive, open-minded, well-balanced and well able to deliver in times of high pressure.
Coauthor Porta Fabiola

Małgorzata Konopka

Małgorzata Konopka, is a MSc Student at the Department of General Biophysics at the University of Lodz. She received her BSc degree in Biotechnology from the University of Lodz in 2014. Her research focused on the cytoxicity (*in vitro*) of different groups of dendrimers and their applications in medicine, especially as autofluorescent agents. Now she is involved in studies in fluorescence of PAMAM dendrimers.
Małgorzata Konopka is also a participant of the project "Intrinsically fluorescent dendrimers - spectrofluorimetric and cell biology studies"" supported by the National Science Centre.

Gergely Tibor Kozma

Nanomedicine Research and
Education Center, Semmelweis University,
Budapest, Hungary; SeroScience Ltd.,
Budapest, Hungary

Gergely Tibor Kozma, MSc, PhD, immunologist, senior research fellow at the Nanomedicine Research and Education Center at Semmelweis University, Budapest, Hungary and at SeroScience Ltd.. He received his MSc degree in bioengineering at Technical University Budapest, Faculty of Chemical Technology and Biotechnology; thereafter he obtained PhD in immunology and molecular biology at Semmelweis University. He was working at Semmelweis University and at several companies as a researcher studying mainly the immunological mechanisms of allergy, and the nano-drug induced hypersensitivity mediated by the complement system. He spent one and half a year in Rome as a researcher sponsored by the Marie Curie Research Training Network to investigate the antigen presenting processes of dendritic cells. Besides research he was also involved at assay developments including e.g. protein engineering in E. coli, ELISA and monoclonal antibody development, and detailed phenotyping of immune cells by flow cytometry. His current field of research is the immunological study of nano-drug induced hypersensitivity reaction including mainly the complement activation related processes and immunogenicity to develop predictive tests for patients. He has co-authored 18 original papers, with more than 400 citations.

Melanie Kucki

Empa – Swiss Federal Institute for Materials Science and Technology
Department Materials meet life
Laboratory for Particles-Biology Interactions, St. Gallen, Switzerland

Dr. Melanie Kucki obtained her diploma in biology from the University of Kassel, Germany. She completed her PhD studies at the University of Kassel in the field of biological photonic crystals/diatom research in 2009. Melanie then joined the BMBF-project NanoKon at the INM –Leibniz Institute for New Materials, Saarbruecken, Germany. Her research focus was on the investigation of the impact of nanoscale contrast agents on human intestinal cells in vitro, as well as on the determination of endotoxin contaminations in nanoparticle dispersions. In 2013, she joined Empa - Swiss Federal Institute for Materials Science and Technology as research associate in frame of the EU-Flagship Graphene, WP2 Health and Environment. Her current research focus is on the investigation of the interaction of graphene-related materials with human barrier models, such as the GI tract and the placental barrier in vitro.

Sharon Lee Wei Ling

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EDUCATION

Aug 15–Present: National University of Singapore, NUS
- Recipient of Singapore-MIT Alliance for Research and Technology (SMART) Graduate Fellowship
- Doctor of Philosophy, Yong Yoo Lin School of Medicine (Year 1)

Aug 11–Jul 15: National University of Singapore, NUS
- Bachelor of Engineering (Biomedical Engineering), Honours
- Date of graduation: July 2015, CAP: 4.3/5.0

Jan 14–May 14: University of California, Davis , UCD (Student Exchange Programme, SEP), Davis, California, United States
- Bachelor of Engineering (Biomedical Engineering)

RESEARCH EXPERIENCE

Aug 15–Present: Singapore-MIT Alliance for Research and Technology, PhD Student
- Under the BioSyM Interdisciplinary Research Group (IRG), spearheads ongoing collaboration to elucidate the role of myeloid cells in T cell immunotherapy through microfluidic platform
- Experienced in techniques in microfluidics, multi-colour confocal microscopy, flow cytometry, cell isolation and culture

Aug 14–May 14: Singapore-MIT Alliance for Research and Technology, Final Year Project Student
- Under the BioSyM IRG, engaged in project that investigated the effect of size on nanoparticle margination with and without a protein corona, for applications in novel drug delivery methods
- Employed techniques in microfluidics, nanoparticle hydrodynamics and fluorescence microscopy

May 14–Jul 14: The Johns Hopkins University, Baltimore, Summer Research Intern, Baltimore, Maryland, United States
- Responsively collaborated with a global team of scientists to optimize a clinical imaging method, producing 200 files of high-quality brain scans in 10 weeks

Jan 14–May 14: The University of California, Davis, Student Research Assistant, Davis, California, United States
- Independently planned and executed project that investigated the effect of flow-induced stress on the inflammatory response of endothelial cells, for better understanding of atherosclerosis development
- Employed techniques in microfluidics, fluorescence microscopy and cell culture

May 13–Aug 13: Institute of Materials Research and Engineering, A*STAR, Research Intern
- Co-author of paper that was published in the Journal of Applied Polymer Science
- Determined the critical micellization concentration and micelle formation thermodynamics of novel amphiphilic polymers, for development of novel polymers in several applications
- Produced interesting results for challenging project on carbon dot encapsulated silica particles

ACHIEVEMENTS & AWARDS

- Lee Foundation Travel Fellowship for 250th ACS National Meeting and Exposition International Conference
- Merit Award for "Novel Investigation Into the Margination of Nanoparticles with a Protein Corona" in the Faculty of Engineering 29th Innovation & Research Award, May 2015
- Merit Award for "Development of Novel Glaucoma Drainage Devices" in the Faculty of Engineering 29th Innovation & Research Award, May 2015
- Merit Award for Students' Design of Glaucoma Drainage Device at the Biomedical Engineering Society, (BMES) (Singapore), 8th Scientific Meeting, May 2014
- 'Prestigious Director' Award for Glaucoma Drainage Device in NUS Biomedical Engineering (BME) Design

Neill Liptrott

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PROFILE

Dr Liptrott has a background in pharmacology, immunology and molecular cell biology. His research is focused on investigating biocompatibility and immunological safety of conventional and nanotechnology-enabled medicines. Dr Liptrott's research to date has helped underpin the successful translation of solid drug nanoparticle formulations through GMP manufacture towards healthy volunteer bioequivalence studies. Dr Liptrott also worked as a guest researcher at the National Institutes of Health (NIH) National Cancer Institute's (NCI) Nanotechnology Characterisation Laboratory (NCL) based in Frederick, Maryland, USA.

In 2015 Dr Liptrott was awarded a tenure-track fellowship within the department of Molecular and Clinical Pharmacology and heads the nanotechnology biocompatibility research programme. Additionally Dr Liptrott is a member of the Executive Board and core expert team (CET) of the recently established European Nanomedicine Characterisation Laboratory (EU-NCL). He leads the University of Liverpool work packages on nanoparticle biocompatibility and structure-activity relationships. Dr Liptrott continues to receive numerous national and international invitations to speak as well as being a peer reviewer for a number of scientific journals including those with a focus on nanomedicine.

RESEARCH

2015–present: Tenure track fellow – Department of Molecular and Clinical Pharmacology, University of Liverpool, UK. Biocompatibility, Nanotoxicology and Immunopharmacology

2012–2015: Senior Postdoctoral Research Fellow – Department of Molecular and Clinical Pharmacology, University of Liverpool. Towards nanomedicine interventions in HIV/AIDS.

2011–2012: Postdoctoral Research Associate - Department of Mo-

lecular and Clinical Pharmacology, University of Liverpool. Determining the interaction between nanoformulated drug delivery systems and the human immune system.
2009–2011: Postdoctoral Research Associate - National Biomedical Research Centre for Microbial Disease, Royal Liverpool and Broadgreen University Hospital Trust, Liverpool. Investigating the mechanisms governing the intracellular pharmacology of HIV antiretrovirals in primary immune cells.
2007-2009: Research Associate - National Biomedical Research Centre for Microbial Disease, Royal Liverpool and Broadgreen University Hospital Trust, Liverpool, UK.

EDUCATION

2004–2007: Ph.D. – Department of Molecular and Clinical Pharmacology, University of Liverpool. Pharmacological and Immunological Factors that Influence Antiretroviral Drug Therapy.
2003-2004: M.Sc. Human Immunity, University of Liverpool, UK.
1999–2003: B.Sc. (Hons) Molecular Biology, University of Liverpool, UK.

Mengjiao Liu

Mengjiao Liu received her MSc degree in Sichuan Agricultural University, PR China, in 2015. After obtaining a scholarship from Chinese government, she joined Prof. Lammers's group at the department of Nanomedicine and Theranostics, Which is part of the institute for Experimental Molecular Imaging (ExMI) at RWTH Aachen University, to start her PhD career in September. Her research focus on developing multifunctional microbubbles to enhance drug delivery across the blood-brain barrier (BBB) and to improve the treatment of brain tumors.

Maria Cristina Lo Giudice

Centre for BioNano Interactions,
University College Dublin, Ireland
maria-cristina.lo-giudice@cbni.ucd.ie

I graduated in Chemistry in 2009 at the University of Catania (Italy), with a thesis on the confinement of supported lipid bilayers on topographically and chemically patterned surfaces, which I performed in part during the course of an internship in the department of applied physics of the Chalmers University, Göteborg (Sweden). In 2011, I took a Master's Degree in Material Chemistry at the University of Catania (Italy) with a thesis on the inhibition of Proteasome by cationic porphyrins, which resulted in a peer-reviewed publication (Santoro A.M., Lo Giudice M.C., D'Urso A., Lauceri R., Purrello R., Milardi D., Cationic porphyrins are reversible proteasome inhibitors, J Am Chem Soc. 2012;134(25):10451-7). Subsequently, I joined the biomaterial group of prof. J.A. Jansen of the UMC St. Radboud, Nijmegen (The Netherlands), where I worked on the fabrication and functionalization electrospun scaffolds for bone tissue engineering (Castro A., Lo Giudice M.C., Vermonden T., Leeuwenburgh S.C.G., Jansen J.A., van den Beucken J.J.J.P., Yang F., A Top-down approach for the preparation of highly porous, biodegradable PLLA micro-cylinders, submitted).
In September 2013, I spent three months as a visiting student at the Centre for BioNano Interactions, University College Dublin, Ireland, where, in January 2014, I started a PhD program under the supervision of prof. Kenneth A. Dawson. My research project is focused on the development of new methodologies for the sub-molecular characterization of the bio-nano interface, and on elucidating the correlation between nanoparticle biomolecular corona and nanoparticle-cell interactions. My research so far has been the subject of three manuscripts, two recently submitted (Lo Giudice M.C., Herda L.M., Polo E., Dawson K. A. In situ characterization of the nanoparticle bio-interface, submitted, and Lo Giudice M.C., Meder F., Polo E., Thomas S.S., Alnahdi K., Lara S., Dawson K.A. Bifunctional nanoparticle-protein conjugates and advanced charac-terization of their exposed recognition motifs) and one in preparation (Herda L.M., Polo E., Lo Giudice M.C. Hristov D., Dawson K.A. Optimising immunogold based mapping for use with biologically relevant particles, unpublished).

Hender Lopez

Dr Hender Lopez; obtained his PhD in Electronic Engineering from the Universidad Autonoma de Barcelona, Spain in 2010. From 2011 to 2013, he worked at the Mathematical Science Department at Loughborough University were his research focus was on Mathematical Modelling of Complex Liquids. From 2013 to 2015 he was part of the University College Dublin team of the EU project MembraneNanoPart developing computational models to simulate the interactions of Nanoparticles with Proteins and Lipids. From the end of 2015, he joined the Centre for BioNano Interactions (CBNI) at University College Dublin were he has continued to develop computational and mathematical models to study nanobiointeraction.

Constantin Lucaciu

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I was born in 11.11.1957 in Cluj-Napoca, Romania. I graduated the Faculty of Physics, Babes Bolyai University, Cluj-Napoca in 1982, and obtain the Ph.D in Biophyics in 1998, under the supervision of prof. dr. Vasile V. Morariu with the thesis entitled "Pathological changes of biological membranes permeability to divalent cations" I worked as a research scientist at the National Institute for Research and Development for Isotopic and Molecular Technology, Cluj-Napoca and since 1991 as assistant professor, lecturer, associate professor and professor at the "Iuliu Hatieganu" University Of Medicine and Pharmacy Cluj-Napoca, Romania.Actually I am Professor, Head of the Department of Pharmaceutical Physics and Biophysics at the "Iuliu Hatieganu" University Of Medicine and Pharmacy Cluj-Napoca, Romania.
My main research interest are:

- effects of strong electromagnetic fields on biological cells: electrofusion, electroporation, electrorotation, dielectrophoresis;
- conductance properties of some natural and synthetic oligopeptides;
- transport of ions through biological membranes, changes in pathologies;
- applications of Electron Paramagnetic Resonance in biological systems: spin labelling, spin trapping, assessment of antioxidant activity of different substances;
- synthesis,characterisation and functionalisation of metalic nanoparticles (Au, Ag), magnetic nanoparticles, lipid nanoparticles (liposomes);
- Raman Spectroscopy, Surface Enhanced Raman, DFT with applications in life sciences.

Pernille Lund Hansen

POSTDOC (2014–)

The Molecular Oncology Group, Institute for Molecular Medicine, Faculty of Health Sciences, University of Southern Denmark.

EDUCATION

PhD (2009–2013) Set-Up and Testing of Components for Therapeutic SiRNA Delivery
The Molecular Oncology Group, Institute for Molecular Medicine, Faculty of Health Sciences, University of Southern Denmark.
Master in biomedicine (2002–2009)
Health and Natural Science faculties, the University of Southern Denmark.
Master project: Osteogenic differentiation in 3D-osteospheres: Novel approach for studying *in vitro* bone formation. Performed at the Molecular Endocrinology Laboratory at Institute for Molecular Medicine, Faculty of Health Sciences, University of Southern Denmark
Bachelor Project: Osteogenic differentiation of human bone marrow derived mesenchymal stem cells immortalized by telomerase and grown as 3D multicellular spheroids. Performed at the Molecular Endocrinology Laboratory at Institute for Molecular Medicine, Faculty of Health Sciences, University of Southern Denmark.

PUBLICATIONS

- **Tunable CD44-Specific Cellular Retargeting with Hyaluronic Acid Nanoshells** Morten F Ebbesen, Morten TJ Olesen, Mikkel C Gjelstrup, Malgorzata M Pakula, Esben KU Larsen, Irene M Hansen, Pernille L Hansen, Jan Mollenhauer, Birgitte M Malle, Kenneth A Howard; Pharmaceutical Research, 2014 Nov.
- **The pattern recognition molecule Deleted in Malignant Brain Tumors 1 (DMBT1) and synthetic mimics inhibit liposomal nucleic acid delivery** Pernille Lund Hansen, Stephanie Blaich, Caroline End, Steffen Schmidt Jesper B. Moeller, Uffe Holmskov, and Jan Mollenhauer; Chemical Communications, 2011, 47, 188–190
- **Parameters in three-dimensional osteospheroids of telomerized human mesenchymal (stromal) stem cells grown on osteoconductive scaffolds that predict *in vivo* bone-forming potential** Pernille Lund Rasmussen, Jorge S. Burns, Kenneth H. Larsen, Henrik D. Schrøder, Moustapha Kassem; Tissue Engineering, Part A. 2010 Jul;16(7):2331-42..

ORAL PRESENTATIONS

- Presentation of research project at Oncology Department, Odense University Hospital, Odense, Denmark, May 23rd 2013. "Development of cancer stem cell targeting nanodrugs"
- 1st NanoCAN meeting Odense, Denmark April 14th 2011 "Construction of synthetic cancer stem cells" –
- 4th Joint meeting, Danish Stem Cell Center (DASC) Sønderborg, Denmark January 22– 24, 2006 "3D-culture of mesenchymal stem cells modelling osteogenesis"

AWARDS

2008–2009 Novo Scholarship Programme in Biotechnology and Pharmaceutical Sciences. DKK 72.000

Amit Ranjan Maity

My current interest is finding the limiting factors of subcellularly targeted Drug Delivery Systems (DDSs) that will deliver specific drugs to the nuclei of the target cells and thus, will enhance efficacy and reduce toxicity of these drugs. To achieve the goal presently I'm using Quantum Dots (QDs) as a model nano-DDSs decorated with specific targeting residues.

SELECTED PUBLICATIONS:

- Maity, A. R.; Stepensky, D. Efficient subcellular targeting to the cell nucleus of quantum dots densely decorated with nuclear localization sequence peptide. ACS Appl. Mater. Interfaces (revision submitted), 2016.
- Maity, A. R.; Stepensky, D. Limited efficiency of drug delivery to specific intracellular organelles using subcellularly "targeted" drug delivery systems. Mol. Pharm. (Article ASAP, in press). 2015.
- Maity, A. R.; Stepensky, D. Delivery of drugs to intracellular organelles using drug delivery systems: Analysis of research trends and targeting efficiencies. Int. J. Pharm., 2015, 496, 268-274.
- Maity, A. R.; Chakraborty, A.; Mondal, A.; Jana, N. R. Carbohydrate coated, folate functionalized colloidal graphene as a nanocarrier for both hydrophobic and hydrophilic drugs. Nanoscale, 2014, 6, 2752-2758.
- Sarkar, S.; Maity, A. R.; Karan, N. S.; Pradhan, N. Fluorescence energy transfer from doped to undoped quantum dots. J. Phys. Chem. C, 2013, 117 (42), 21988-21994.

CONFERENCE PRESENTATIONS:

- Functionalized Nanoparticles as Cellular Imaging Probe by Amit Ranjan Maity and Nikhil Ranjan Jana (Poster Presentation) in International Conference on Nano Science and Technology (ICONSAT), 2012, Hyderabad, India.
- Chitosan–Cholesterol-Based Cellular Delivery of Anionic Nanoparticles by Amit Ranjan Maity and Nikhil Ranjan Jana (Poster Presentation) in International Conference on Fundamental & Applications of Nanoscience and Technology (ICFANT), 2011, Kolkata, India

Pooria Mansoori

M.D
Doctorate of Medicine (M D), Tehran University of Medical Sciences, Tehran, Iran (1997-2003)
E-mail: pooriyamansoori@yahoo.com

PUBLICATIONS:

1. Taheri A, Atyabi F, Salman Nouri F, Ahadi F, Derakhshan MA, Amini M, Ghahremani MH, Ostad SN, Mansoori P, Dinarvand R. Nanoparticles of conjugated methotrexate-human serum albumin: Preparation and cytotoxicity evaluations. J Nanomater. 2011;2011: Article ID 768201.
2. Taheri A , Dinarvand R, Atyabi F, Ahadi F, Salman Nouri F, Ghahremani MH, Ostad SN, Taheri Borougeni A, Mansoori P. Enhanced anti-tumoral activity of methotrexate-human serum albumin conjugated nanoparticles by targeting with luteinizing hormone-releasing hormone (LHRH) peptide. Int. J. Mol. Sci. 2011;12:4591-4608.
3. Taheri A, Dinarvand R, Atyabi F, Salman Nouri F, Ahadi F, Ghahremani MH, Ostad SN, Taheri Borougeni A, Mansoori P. Targeted delivery of methotrexate to tumor cells using biotin functionalized methotrexate-human serum albumin conjugated nanoparticles. J. Biomed. Nanotechnol. 2011;6:743-753.
4. Ghorbani A, Soltani Shirazi A, Sametzadeh M, Mansoori P, Taheri A. Relation of resistive and pulsatility indices with graft function

after renal transplant. Exp Clin Transplant. 2012;10 (6):568-572.

ABSTRACTS:

1.Taheri A, Dinarvand R, Atyabi F, Taheri BorougeniA, Mansoori P, Trastuzumab decorated methotrexate-human serum albumin conjugated nanoparticles for targeted delivery to HER2 positive tumor cells, The 5th Iranian Controlled Release Conference (ICRC 2011), 4-6 October, 2011, Mashhad, Iran.

2. Taheri A, Dinarvand R, Atyabi F, Salman Nouri F, Taheri BorougeniA, Mansoori P, Targeted delivery of methotrexate to tumor cells using biotin functionalized methotrexate-human serum albumin conjugated nanoparticles, The 5th Iranian Controlled Release Conference (ICRC 2011), 4-6 October, 2011, Mashhad, Iran.

3. Taheri A, Dinarvand R, Atyabi F, Ahadi F, Taheri BorougeniA, Mansoori P, Enhanced anti tumoral activity of methotrexate-human serum albumin conjugated nanoparticles by targeting with LHRH peptide, The 5th Iranian Controlled Release Conference (ICRC 2011), 4-6 October, 2011, Mashhad, Iran.

4. Taheri A, Dinarvand R, Mansoori P, Khorramizadeh M, The *in vivo* antitumor activity of LHRH targeted methotrexate-human serum albumin nanoparticles in 4T1 tumor-bearing Balb/c mice. 13th Iranian pharmaceutical Sciences Conference, 3-6 Sep 2012, Isfahan,Iran.

5. Taheri A, Mohammadi M, Mansoori P. The use of cellulose nanocrystals for potential application in drug delivery to skin. TWAS-ROCASA Young Scientists Conference on "Nanoscience & Nanomaterials", 18-20 Feb 2015, Bangalore, India.

RESEARCH PROJECTS:

- Preparation of trastuzumab targeted methotrexate-human serum albumin conjugated nanoparticles.
- Preparation of biotin targeted methotrexate-human serum albumin conjugated nanoparticles.
- Preparation of LHRH targeted methotrexate-human serum albumin conjugated nanoparticles.
- Preparation of gel wound dressing using chitosan.
- Preparation and evaluation of doxorubicin-pectin-LHRH conjugates as a targeted drug delivery system to cancer.
- Preparation and characterization of wound dressing using L-arginine-bacterial nanocellulose for use in wound healing
- Preparation and characterization of wound dressing using L-arginine-chitosan nanofibers for use in wound healing
- Preparation and characterization of wound dressing using L-arginine-lignin nanofibers for use in wound healing

Natalia Martin

French National Research Agency (ANR), France

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Dr Natalia Martin is responsible for transnational collaborations in the Biology & Health department at the French National Research Agency (ANR), which is involved in 15 ERA-NETs, 3 JPIs and 7 multilateral collaborations. After 12 years of experience in research in the biomedical field and a training in business management, she managed a 3-year European CSA project (Rare Disease Platform) before joining the ANR in September 2010. She manages a 7-people team dedicated to representing the ANR in several transnational collaborations, she is workpackage leader in several ERA-NETs and she is coordinator of the EuroNanoMed II ERA-NET. In this context, she has participated in the elaboration of the Nanomedicine Strategic Research and Innovation Agenda (2016-2030) in collaboration with ETPN.

Coauthor Ignacio Baanante

Sandra Lara Martinez

PhD

Centre for BioNano Interactions, UCD, Belfield, Dublin 4, Ireland

Currently I am studying a PhD at the Centre for BioNano Interactions (CBNI), University College Dublin (UCD), Ireland. I started on January 2014, and my research is focusing on the interactions of nanoparticles in complex biological milieu with specific cellular receptors, in order to identify those receptors involved in the specific recognition of the nanoparticles-biomolecular complexes. In order to achieve that, a model of functional receptor library hosted is used in HEK-293T cells. The main techniques used in this project are Flow Cytometry to assess the transfection efficiency for specific receptors and the nanoparticle uptake, in parallel with western blots, PCR, and imaging.

During my PhD I have been trained in several techniques and softwares, such as Motion Tracking, at MPI-CBG, Dresden, Germany (April 2015) and participated in a number of conferences (November 2014 NanoBio & Med, Barcelona, Spain).

In 2013 I was a visiting researcher at the CBNI for 6- Months of experimental work oncell cultures, including knock-out techniques.

Publications: Lara, S. et al. Biological Recognition of Biomolecular Corona. Unpublished. (2016)

IN MY PREVIOUS EDUCATION:

I studied from 2011 to 2012 a Master in Science by Research in Regenerative Biomedicine, Department of Anatomy & Pathology, at University of Granada (UGR), Spain. MSc Thesis: "A Review of Induced Pluripotent Stem Cells and Applicability in Regenerative Biomedicine".

During the master I completed a 5-month internship of Experimental Work on Cell Cultures at "Instituto de Biopatología y Medicina Regenerativa" (IBIMER) Centro de Investigación Biomédica (CIBM), Granada (Spain). Work included cell cultures, PCR and practice in several techniques in biomedical laboratories

From 2006 to 2011 I studied a 5-year Degree in Biology, at University of Girona (UDG), Catalonia, Spain. Speciality: Cell & Molecular Biology.

Marc Masa

Marc Masa, biologist, is working at Leitat, Technological Centre since 2008 as founder member of the new Biomed unit at the Technological centre focused in preclinical oncology. He has participated in several domestic and international projects, either as a partner or as PI, dealing with biological cancer therapy discovery/development and diagnosis (including cancer but not limited to). In 2009, he co-funded the biotech company Lykera Biomed, a privately held biotech company based in Barcelona. Lykera is developing biologics for the treatment of cancer by targeting not only the tumour cells but also the stromal cells and tumour angiogenesis.

From 2014, he was appointed as biosensor group leader. The group is made up of several KETs experts (nanotechnology, micro-nanoelectronics) and scientist from environment, microbiology and biomedicine field focused on biosensor development.

From 1999–2007, Marc Masa worked at Merck Serono. The research activities performed in this period were focused on target discovery/validation on novel cancer therapeutic targets. Additionally, he leaded a drug discovery kinase inhibitor programme coordinating groups from Spain and Germany for the identification of potential leads. Beside the research activities, he was involved in resource allocation for project execution and time control dedicat-

ed projects at Merck's Bioresearch Laboratory in Barcelona.
He has published several academic papers and he's co-inventor of 10 patent applications.

RECENT PUBLICATIONS:

- Cellular uptake and cytotoxic effect of EGFR targeted and plitidepsin loaded co-polymeric polymersomes on colorectal cancer cell lines Goñi-de-Cerioa F, Thevenot J, Oliveira H, Masa M, Suárez-Merinoa B, Lecommandoux S, Heredia P.Journal of Biomedical Nanotechnology. 10/2015;
- Surface decorated poly(ester-ether-urethane)s nanoparticles: a versatile approach towards clinical translation. Piras AM, Sandreschi S, Malliappan SP, Dash M, Bartoli C, Dinucci D, Guarna F, Ammannati E, Masa M, Múčková M, Schmidtová L, Chiellini E, Chiellini F. Int J Pharm. 2014 Nov 20;475(1-2):523-35.
- In vivo anticancer evaluation of the hyperthermic efficacy of anti-human epidermal growth factor receptor-targeted PEG-based nanocarrier containing magnetic nanoparticles. Baldi G, Ravagli C, Mazzantini F, Loudos G, Adan J, Masa M, Psimadas D, Fragogeorgi EA, Locatelli E, Innocenti C, Sangregorio C, Comes Franchini M. Int J Nanomedicine. 2014 Jun 24;9:3037-56.
- S100P antibody-mediated therapy as a new promising strategy for the treatment of pancreatic cancer. Dakhel S, Padilla L, Adan J, Masa M, Martinez JM, Roque L, Coll T, Hervas R, Calvis C, Messeguer R, Mitjans F, Hernández JL. Oncogenesis. 2014 Mar 17;3:e92
- Therapeutic targeting of tumor growth and angiogenesis with a novel anti-S100A4 monoclonal antibody. Hernández JL, Padilla L, Dakhel S, Coll T, Hervas R, Adan J, Masa M, Mitjans F, Martinez JM, Coma S, Rodríguez L, Noé V, Ciudad CJ, Blasco F,Messeguer R. PLoS One. 2013 Sep 4;8(9):e72480.

Sofiya Matviykiv

Sofiya Matviykiv is a Ph.D. student at the Biomaterials Science Center of the University of Basel and is funded via an Excellence Program of the Swiss Government.
Sofiya Matviykiv did her B.Sc. in Biotechnology and received her M.Sc. in Pharmaceutical Biotechnology at Lviv Polytechnic National University (Lviv, Ukraine) in 2014. During her Master studies (2013-2014), Sofiya spent one year as an exchange student at University of Trento (Trento, Italy), attending Master courses in Cellular and Molecular Biotechnology. She conducted her Master thesis internship at the Biotech Research Center (Trento, Italy). Her research was focused on the preparation and characterization of silk fibroin film for tissue regeneration. In June-July 2015 she participated in the fellowship program at the University of Würzburg (Würzburg, Germany), working on the fabrication and characterization of the composite bone cement for bone regeneration.
Currently, Sofiya is working towards her Ph.D. degree in Nanosciences, within the "NO-Stress" project, previously funded by the Swiss National Science Foundation via the program NRP 62 "Smart Materials". Her work is focused on the fabrication and characterization of the artificial mechano-sensitive liposomes for targeted drug delivery against atherosclerosis. Her scientific interests include dynamic light scattering technique, enzyme-linked immunosorbent assay, atomic force microscopy technique, applications of drug delivery systems for the cardiovascular diseases and tissue engineering.

Rinat Meir

Rinat Meir received her B. Sc. and M.Sc. degrees in Chemistry with honors from the Hebrew University in Jerusalem. Her Masters research in Computational Chemistry, under the supervision of Prof. Sason Shaik led to two scientific papers as first author and one paper as a co-author. She is currently pursuing her Ph.D. in Bioengineering in the laboratory of Prof. Rachela Popovtzer at the Faculty of Engineering at Bar-Ilan University (BIU).
Rinat's research focuses on the development of a novel nanoparticle-based imaging technique which she named CT3 - Computed Tomography for Cell Tracking in Cell Therapy. Cell therapy is the transplantation of living cells for the treatment of diseases and injuries. Such therapy offers a promising solution for the treatment of various pathologies that conventional medicine cannot cure effectively, thus encouraging future medical breakthroughs. For instance, cancer-fighting T cells may be injected in the course of cancer immunotherapy, and stem cells may treat neurodegenerative diseases, heart disease, muscular dystrophy and diabetes. A major obstacle in the advancement and implementation of cell therapy is the challenge of no-invasively tracking transplanted cells in the body. In vivo cell tracking could elucidate essential knowledge regarding mechanisms underlying the success or failure of therapy. An optimal solution for the challenge of cell tracking does not yet exist hence the need for an accurate imaging technique. Rinat's research suggests developing a novel methodology for longitudinal and quantitative *in vivo* cell tracking, based on the combination of CT as an imaging modality and gold nanoparticles as labeling agents. Uniting the superior visualization abilities of classical CT with state-of-the-art nanotechnology is the key for high-resolution cell tracking. In the future, this technology has the potential to be applied clinically and to serve as an early warning system for patients after cell transplantation. So far this research subject led to the publication of three papers.
Rinat was recently awarded an excellence scholarship by the Israeli Ministry of Science, Technology & Space. This year, Rinat received the Rector's Prize at BIU. In addition, she was awarded with BIU Nano-center scholarship and BIU "president scholarship" for excellent Ph.D students. During her years at the Hebrew University Rinat appeared on the Dean's list and received several prizes for outstanding students.

Tamás Mészáros

Tamás Mészáros, MSc, research fellow at Nanomedicine Research and Education Center, Semmelweis University and SeroScience Ltd., Budapest, Hungary. He received his MSc degree as an Immunologist from Eötvös Lóránd University in 2008, Budapest, Hungary. He is currently pursuing his PhD degree at Semmelweis University. His research interest is complement system, liposomes and nanomedicine. His special skills include *in vitro* assays and techniques.

Gergely Milosevits

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After graduating from Semmelweis University in Budapest, dr. Gergely Milosevits has been working as a medical doctor at the University's II. Department of Pediatrics and also as a research fellow in the laboratory of Professor János Szebeni at the Nanomedicine Research and Education Center in Budapest, Hungary. He teaches both Hungarian and international medical students in practical classes of Pediatrics. He is especially interested in flow cytometry, liposomes, exosomes and CARPA.

Dennis Müller

Dennis Müller studied chemistry at the RWTH Aachen, Germany, and was graduated from the RWTH in 2011. He than moved to the University of Fribourg were he started his PhD under the supervision of Prof. Andreas Zumbühl working on the synthesis and characterization of artificial phospholipids.

Coauthor Zumbuehl Andreas

Ángeles Muñoz Fernández

Dr. Mª Ángeles Muñoz Fernández is a specialist in Immunology, Head of Section at the Hospital General Universitario Gregorio Marañón, and Scientific Director of HIV HGM Biobank. She is a doctor of Medicine and Surgery, as well as a doctor in Molecular Biology. Since 1994, main laboratory research studies have been primarily related to the infection by the human immunodeficiency virus (HIV). Among different research lines include the HIV pediatric infection, the searching for vaccines against HIV both in adults and children, and novel preventive strategies based on microbicides and nanotechnology. The HIV pediatric research has allowed the development of guidelines for the clinical practice at a national and international level and is member of the PENTA Network. The second research line is actively working with the group of vaccines from Spanish AIDS Research (RIS) Network including nanosystems. Since 2003, Mª Ángeles Muñoz Fernández started an innovative approach based on nanotechnology and more specifically on nanoparticles and dendrimers. These nanosystems can have negative charges (polyanionic) and positive charges (polycationic). Polyanionic dendrimers are used in the development of topical microbicides against HIV and other sexually transmitted diseases such as herpes simplex virus type 2 (HSV-2) or hepatitis C virus (HCV). After testing the proof-of-concept in BALB/c mice and humanized BLT mice and showing that the microbicide can be used against HIV and HSV-2, we are developing a phase I clinical trial. Polycationic dendrimers are used as carriers for siRNA delivery, as vectors in gene therapy, as carriers of drugs, and as therapeutic and preventive nanovaccines against HIV.

Dr. Mª Ángeles Muñoz Fernández has led more than 50 national and international projects as principal Investigator and/or coordinator, including outstanding studies at European level in the field of the nanotechnology and development of vaccines. She has 15 patents as an inventor based on the development of dendrimers as new tools in nanomedicine, as well as she has more than 375 research articles in first decil or quartil (since 2011) and an H factor of 33. She has directed 42 Doctoral Thesis, 11 with Mention International and 9 with Ph.D. extraordinary award. Moreover, she has written 34 book chapters, 7 clinical practice guidelines, and 4 reference books in the biomedical field. She also has 37 awards and distinctions from public and private entities, aimed at both business management and research excellence. It should be noted the Award Join at the Civil Order of Health with the category of Single Cruz, awarded by the Ministry of Health.

Her management work is completed with the development, as a founding member, of 2 start ups (GENOMADRID SL, Dendrico SL) and 2 spin-offs (Ambiox Biotech SL and SimCosmetics Biotech). She is also accredited as an evaluator of research projects at national

Xabi Murgia

Xabi Murgia graduated in biochemistry at the University of the Basque Country and achieved a Master ' s degree in Neuroscience in 2010. From 2009 to 2013 he worked at Cruces University Hospital, in Bilbao, where he investigated the delivery of surfactant and perfluorocarbon aerosols as a treatment for experimental Respiratory Distress Syndrome. Since January 2014, he is part of the Department of Drug Delivery at the Helmholtz Institute for Pharmaceutical Research of the Saarland (HIPS) as a Marie Curie fellow within the framework of the PATHCHOOSER initial training network (www.pathchooser.eu). Currently he focuses his investigations on the cellular and non-cellular barriers of the lung and, in particular, in the interaction between nanoparticles and the bronchial barrier, including the pulmonary mucus.

Sara Nogueira

In 2008, I initiate my bachelor of science in Biology, in University of Tras-os Montes e Alto Douro, Vila Real, Portugal. During my bachelor studies, I did an internship in University of Gdanks, Poland under ERASMUS PROGRAMME and curricular internship in collaboration with University of Porto, where I developed a scientific work entitled "Preliminary studies of a biosensor for detection of the growth rate of Escherichia coli". In 2012, I pursued my master degree in Biophysics and Bionanosytems in University of Minho, Braga, Portugal. Under my master degree, I did several advanced courses, including 'Nanoparticles and the Immune System: Risks and Therapeutic Opportunities', Cancer Therapies: From Basic Research to Clinic', 'Applied Physics in Biodevices based on MEMS'. My thesis dissertation entitled "Development of new DODAX:MO:DC-Chol nanoparticles containing BRAF-siRNA for colorectal cancer therapy" was developed between the collaboration of Biology and Physics department of University of Minho. Furthermore, I presented my thesis in oral communication at 6th Iberian Meeting on Colloids and Interfaces, 8th to 10th of July of 2015, Guimarães, Portugal, with the title 'Monoolein-based nanocarriers for therapeutic siRNA delivery in colorectal carcinoma treatment'. In March of 2015, I initiate an internship in Max Planck Institute for Colloids and Interfaces, Group of Prof. Gerald Brezesinski to investigate physiochemical characterization of novel transfection lipids. Since November of 2015, I initiate my PhD studies in BioNTech mRNA pharmaceuticals, Mainz, Germany, under supervision of Prof. Peter Langguth and Heinrich Haas.

Coauthor Ziller Antje

Tarun Ojha

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Utrecht University
Utrecht, The Netherlands
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I did my bachelor's in Microbiology from Fergusson College (Pune, India) followed by master's in Nanotechnology and Nanoscience from Amity University (Noida, India). For my master dissertation, I joined Prof. Dr. Justin Hanes's research group at Center for Nanomedicines, Johns Hopkins University (Baltimore, USA). My master thesis was focused on developing chemo drug loaded mucus penetrating biodegradable nanoparticles for treating lung and cervix cancer.
After my master's, I worked in Prof. Dr. Twan Lammers's research group (Nanomedicines and Theranostics) at the institute for Experimental Molecular Imaging (ExMI), RWTH University Hospital Aachen (Aachen, Germany). During my twelve months of work tenure at ExMI, I worked on vascular normalization strategy for enhancing drug delivery in solid tumors and gained experience on several imaging modalities like Computed Tomography (CT), Fluorescence Molecular Tomography (FMT) and Ultrasound. I recently joined the research group of Prof. Dr. Gert Storm at department of Pharmacy, Utrecht University (Utrecht, Netherlands) as a PhD student. Currently I am working on developing materials and methodology to locally open blood brain barrier.

Erik Örfi

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STUDIES:

2015–: Nanomedicine Research and Education Center (Semmelweis University, Budapest) PhD Student; Thesis title: „Pathophysiology of nanomedicines, especially cardiovascular an renal effects"

2009–2015: Semmelweis University Faculty of Pharmacy, Budapest (MSc, Pharm. D.)
2003–2009: ELTE Trefort Ágoston Secondary School, Budapest

SKILLS:

„Experimental animals – animal experiments" („B" level course)

PROFESSIONAL EXPERIENCES:

- 3 months industrial practical studies at Egis Pharmaceuticals (Servier), Dept. of Structure Analyis, Budapest, Hungary (NMR, Raman spectroscopy, IR, X-ray crystallography) (2011)
- Semmelweis University Pharmacy (organising pharmaceutical management, drug supply of clinical emergency, doing laboratory work especially prepare the missing drugs to supply clinics, Transporting of special pharmaceutical products, Quality control of Infusions, making customized infusions for special therapies, experiences in institutional pharmacy in practice) (2012, 2015)
- Materia Medica Pharmacy, Budapest, Hungary (Pharmaceutical managemenet) (2014-)
- Nanomedicine Research and Education Center, Budapest, Hungary (Studies of complement activation related pseudoallergy (reaction) in liposomal nanodrugs *in vivo* and *in vitro* (2013)

Marion Paolini

PhD
Marion is PhD Candidate and Research Associate in the Biology department at NANOBIOTIX. She graduated from Ecole Polytechnique and Paris Descartes University (2014) in BioMedical Engineering, Molecular and Cellular Biotherapies. She contributed in research projects in the field of DNA repair at the Lawrence Berkeley National Laboratory (Berkeley, CA), and in the study of cellular motors at Columbia University (NYC, NY) and at the University of Chicago (Chicago, IL). The topic of her PhD is "New compositions for the administration of therapeutic agents. Nanomedicine as a means to optimise the benefit-risk ratio of treatments." She also graduated from HEC Paris and SciencesPo Paris in Corporate and Public Management, with experiences in Business Development and Strategic Planning at IPSEN and NANOBIOTIX.

Vertika Pathak

I did my bachelor's in basic sciences (Zoology, Botany, and Chemistry) from D.S.B College (Nainital, India) in 2010. I secured second highest rank in my university and was awarded with 'Vice chancellor's Silver medal' from Kumaun University and 'All India UGC Post Graduate merit scholarship scheme for university rank holders at undergraduate level 2010-2012' from Government of India. After completing my bachelors, I did dual degree master course in Nanotechnology and Nanoscience from Amity University (Noida, India). For my master dissertation, I joined Prof. Dr. Justin Hanes's research group at Center for Nanomedicines, Johns Hopkins University (Baltimore, USA) in 2013. My master thesis was focused on developing chemo drug load mucus penetrating biodegradable nanoparticles for treating lung and cervix cancer. After my master's, I joined Prof. Dr. Twan Lammers's research group (Nanomedicines and Theranostics) at the institute for Experimental Molecular Imaging (ExMI), RWTH University Hospital Aachen (Aachen, Germany) in November 2015. During my 6 months of work tenure at ExMI, I worked on multiple projects and gained experience on techniques like Immunohistochemistry, several imaging modalities like Computed Tomography (CT), Fluorescence Molecular Tomography (FMT) and Ultrasound. Recently I have been awarded prestigious DAAD stipend to work on materials and methods to improve drug and oxygen delivery to tumors, in particular to brain tumors. The primary focus of my work is on alleviating tumor-associated hypoxia, via strategies such as nanomedicine-mediated vascular normalization, in order to improve the efficacy of combined modality anticancer therapy.

Viorica Patrulea

University of Geneva, Switzerland

Viorica graduated from West University of Timişoara, Romania, Department of Chemistry, in 2009. She received the M.Sc. degree in Chemistry from the same university in 2011.
In 2011, she started her Ph.D. studies at the University of Timişoara, Department of Chemistry, on Chitosan and its application in industries, with focus on heavy metals and azo-dyes adsorption onto chitosan beads. Being selected for a fel-

lowship within the framework of the Scientific Exchange Program NMS-CH (Sciex), she has been continuing her PhD at the University of Geneva, Department of Pharmaceutical Sciences, Biopharmacy, since 2013. In 2015, she obtained her doctorate degree in Biopharmacy form University of Geneva and started to work as a postdoctoral research fellow at the same University.
Her current research is mainly focused on Chitosan application in the biomedical field, including development of new biopolymers derived from chitosan. Grafted with different peptides, these biopolymers could serve as scaffolds for accelerated dermal wound healing.

Nathalie M. Pinkerton

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EDUCATION

• **Princeton University, Princeton, NJ**
Doctor of Philosophy in Chemical and Biological Engineering, **March 2014**
Master of Arts in Chemical Engineering, **June 2010**
• **Massachusetts Institute of Technology, Cambridge, MA**
Bachelor of Science in Chemical Engineering, **June 2008**;

RESEARCH EXPERIENCE

• **L'Institut des Technologies Avancées en Sciences du Vivant - CNRS-USR3505, Toulouse, France**
Developing a tumor targeting nanocarrier with NIR-light triggered drug release capabilities and evaluating its efficacy in 3D microtumor models. Investigating the interactions between nanocarriers and cancerous cells in 3D tumor microenvironments using live 3D imaging. Developing tunable, dual T1 MRI and fluorescent imaging probes for whole animal imaging. Advised by Dr. Stefan Chassaing. **March 2014 to present**
• **Princeton University - Department of Chemical and Biological Engineering, Princeton, NJ**
Developed a versatile drug delivery system that specifically targets the lungs. The platform is an IV injectable, microgel particle loaded with active drug containing nanoparticles. Developed dual T2 MRI and fluorescent imaging probes for *in vivo* liver metastasis detection. Advised by Dr. Robert K. Prud'homme. **Jan 2009 to March 2014**
• **Novartis Research Center, Basel, Switzerland**
Formulated nanosuspensions of hydrophobic active pharmaceutical ingredients for oral and parenteral delivery. Investigated process parameters relating to formulation stability and developed a thermodynamic model to predict the stability of nanosuspensions. Developed a novel hydrophobic ion pairing formulation approach to enable the encapsulation of weakly hydrophobic, ionizable molecules into nanoparticles. Advised by Dr. Bernd Riebesehl, Dr. Jörg Brozio and Dr. Andreas Fisch. **Summer 2011**

PUBLICATIONS AND PATENTS

• "Ionic Flash NanoPrecipitation: a one-step, controlled self-assembly process for the formation of inorganic-organic hybrid nanoparticles" N.M. Pinkerton, L. Behar, B. Amouroux, K. Hadri, C. Mingotaud, D.R. Talham, S. Chassaing, J.D. Marty, Angew. Chem. Int. Ed. Engl., in preparation
• "Red-emitting EtTP-5-based organic nanoprobes for two-photon imaging in 3D multicellular biological models" N.M. Pinkerton*, C. Frongia, V. Lobjois, B.K. Wilson, M.J. Bruzek, R.K. Prud'homme, J. Anthony, F. Bolze, S. Chassaing, RSC Advances, under review
• "Single-step assembly of multimodal imaging nanocarriers: MRI and long-wavelength fluorescence imaging" N.M. Pinkerton, M.E. Gindy, V.L. Calero-DdelC, R.F. Pagels, D. Adler, D. Gao, S. Li, M. Zevon, N. Yao, C. Pacheco, M.J. Therien, C. Rinaldi, P.J. Sinko, R.K. Prud'homme, Adv. Healthc. Mat., 2015, 4, 1376
• "Gelation chemistries for the encapsulation of nanoparticles in composite gel microparticles for lung imaging and drug delivery" N.M. Pinkerton, S.W. Zhang, R.L. Youngblood, D. Gao, S. Li, J. Anthony, H.A. Stone, P.J. Sinko, R.K. Prud'homme, Biomacromolecules, 2014, 15, 252
• "Formation of stable nanocarriers by in situ ion pairing during block-copolymer-directed rapid precipitation" N.M. Pinkerton, A. Grandeury, A. Fisch, J. Brozio, B.U. Riebesehl, R.K. Prud'homme, Mol. Pharmaceutics, 2013, 10, 319

GRANTS AND AWARDS

• Recipient of an RITC and InNaBiosanté foundation grant with Stefan Chassaing, 2014
• Poster, 2nd place CLINAM Poster Prize, 2013
• Poster, Honorable Mention at NJACS Polymers in Drug Delivery Symposium, 2010
• Princeton University High Meadows Foundation, Sustainability Grant Winner, 2009
• National Science Foundation Graduate Research Fellowship (NSF GRFP), 2009

Simona Pînzaru

Function: Associate professor
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EDUCATION:

1998: Doctor in Physics of Babes-Bolyai University,Cluj-Napoca, Romania;.

EXPERTISE:

Optical nanosensing, nano-bio interface; SERS spectroscopy, lasers, optoelectronics, nanotechnology for medicine, experimental physics, applied laser Raman spectroscopy techniques in biomedical, pharmaceutical and environmental field.
Expert evaluator of national and international projects;

EMPLOYMENT:

2014–2016: Senior Researcher at the University of Dubrovnik, Croatia, NEWFELPRO Project manager "JADRANSERS" (2014-2016) NEWFELPRO Grant Nr. 5,– Marie Curie FP7-PEOPLE-2011-COFUND program Ministry of Science, Education. and Sports Croatia,
2003–present: Associate professor, Biomolecular Physics Dept., Babes-Bolyai University, Cluj-Napoca, Romania; Coordinator of research projects: PN_II_ID_2284/ 2008-2011; Grant Director
• Member in other projects grants teams **–2005, 2004, 2003, 2002**
1999–2000: World Bank Grant Director for Young Researchers: BM-T Grant Director
2000–2002: Grant Director CNCSIS –T
2003, 2004, 2005: Visiting scientist, University of Wùrzburg, Germany
1998–2003: Lecturer, Optics and Spectroscopy Department, Babes-Bolyai University
1995–1998: Assistant professor, Babes-Bolyai University

AWARDS

"Excellentia" Award 2015, (CSUBB); The Prize of Excellence for scientific research, Babes-Bolyai University, 2011;
Nomination: Dhamelincourt Prize, ECSBM 2007, Paris

PUBLICATIONS

76 papers in ISI ranked journals (Hirsch Index 15); more than 200 contributions in conferences proceedings, 46 oral presentations in conferences, 15 invited lectures;
5 Books, book chapters; Complete list of publications: Researcher ID: A-4543-2011.

Ester Polo

Centre for BioNano Interactions,
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I obtained a degree in Biochemistry in 2006 and the M.Sc. in Molecular and Cellular Biology in 2008 from the University of Zaragoza. After that, I obtained my PhD from Zaragoza University (Spain) in 2013, working in the Institute of Nanoscience of Aragon. My doctoral thesis involved the development of several nanoparticle-based biosensors for cancer marker detection. During the PhD, I worked extensively on nanoparticle synthesis and functionalization with molecules of biological relevance. The main objective of the research was the development of nano-immunoconjugates used to improve the bioperformance of well-known optical biosensors and to develop new ones based on the ability of plasmonics nanoparticles to convert light into heat. This work results in several peer reviewed articles (Osante, I. et al. Journal of Nanoparticle Research 16 (2), 2014; Polo, E. et al Chemical Communications, 49 (35), 3676-3678, 2013; Kosaka, K et al. Analyst, 138 (3), 863-872, 2013; Puertas, S. et al. ACS Nano, 5(6), 452d1-4528, 2011)

Then, after working as a postdoctoral researcher at the Institute of Nanoscience of Aragon, under the project Development of a universal toolkit for remote release of drugs using magnetic hyperthermia, I joined the group of Prof. Kenneth Dawson as a postdoctoral research fellow at the Centre for BioNano Interactions in Dublin. My research so far, supporting by the Science foundation Ireland, involves nanomaterial synthesis for studying the bionano-interface and the development of new tools for elucidating the biological identity of nanoparticles dispersed in biological fluids and the resultant mechanisms. During this period the following munis-cripts have been published: Kelly, P.M. et al., Nature Nanotechnology 2015 (10): 472–479; Herda L.M., et al European Journal of Nanomedicine, 6 (3), 127-139, 2014.

Karina Pombo-García

PhD Candidate
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EDUCATION

01/2012–present: Ph.D. candidate at TÜ-Dresden and Helmholtz Zentrum Dresden Rossendorf, Germany. “Ultrasmall nanoparticles as multimodal agents for cancer imaging” Thesis submitted
12/2012 - 07/2013: PhD research stay at Monash University, Melbourne, Australia. Lab of Prof. Spiccia Department of Medicinal Chemistry, School of Pharmacy and Pharmaceutical Sciences.
10/2014 - 12/2014: PhD research stay at Bio21, Department of proteomics, University of Melbourne, Australia.
07/2014 - 08/2014: PhD research stay at University of Marburg, Germany. Department of Biophotonics, School of Physics. Lab of Prof. Wolfgang Parak
02/2010 - 08/2011: Master (M.Sc.), School of Pharmacy, USC, Spain. “Nanovaccines: nanocapsules for the association of the rHBsAg”. Suma. Lab of Prof. Alons
10/2005 - 02/2010: M.Pharm. Pharmacy Degree, School of Pharmacy, USC, Spain.
09/2008 - 09/2009: Erasmus scholarship Università degli Studi di Urbino Carlo Bo, Italy.

AWARDS AND FELLOWSHIPS

2015: -Young scientist speaker award at European Parliament, Brussels, Belgium.
-RISE DAAD Scholarship to host/supervise a Canadian student, German Government.
2014: -RISE DAAD Scholarship to host/supervise American student, German Government.
-1st prize at Famelab science slam Kassel & Finalist at the National Famelab, Germany.
2013: A.T. Kearny Fellowship as finalist at the Falling Walls Lab, Berlin, Germany.
2011: Master research fellowship, Spanish Ministry of Science, Spain & Bill Gates Foundation.

PUBLICATIONS

- Karina Pombo-García, et al. (Cover). Zwitterionic-coated “Stealth” nanoparticles for biomedical applications: Recent advances in countering biomolecular corona formation and uptake by the mononuclear phagocyte system. Small, 2014, 13, 2516-29.
- Karina Pombo-García, et al. Design, Synthesis, Characterisation and *in vitro* studies of hydrophilic colloidal-stable 64Cu. (II) labeled Ultra-small iron oxide nanoparticles in a range of human cell lines. RSC Adv., 2013, 3, 22443-54.
- K.Viehweger, (.), Karina Pombo-García, et al. EGF receptor-targeting peptide conjugate incorporating a near-IR fluorescent dye and a novel 1,4,7-triazacyclononane-based 64Cu(II) chelator assembled click chemistry. Bioconjugate Chemistry, 2014, 25, 1011-22.
- K. Zarschler (.), Karina Pombo-García et al. Ultrasmall Nanoparticles: a forward look to nanomedicine, NNBM, 2016. DOI: http://dx.doi.org/10.1016/j.nano.2016.02.019.
- Karina Pombo-García et al. Zwitterionic polymer-coated USPIONs with low protein interaction and high *in vivo* stability. Submitted.

Alfonso Maria Ponsiglione

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Alfonso Maria Ponsiglione received his Diploma in Classical Studies in 2007 with 100/100 and achieved his Master’s Degree in Biomedical Engineering in 2013 with 110/110 cum laude at the University of Naples “Federico II”, defending a dissertation entitled “A multiparametric approach for Foetal Heart Rate Variability signals assessment: Symbolic Dynamics and Frequency Domain Analysis”.

Between 2014 and 2015, he worked as a software developer at GESAN S.r.l. (Caserta, Italy) and as a healthcare ICT consultant at Engineering Ingegneria Informatica S.p.A. (Naples, Italy).

After working in the area of medical informatics, he worked as a trainee in the field of biomaterials and nanotechnology at Istituto Italiano di Tecnologia - Center for Advanced Biomaterials for Healthcare (Naples, Italy). Since November 2015, he is a Ph.D. student at University of Naples “Federico II” - Department of Chemical Engineering, Materials and Industrial Production (D.I.C.Ma.P.I.) and has been continuously working at Istituto Italiano di Tecnologia - Center for Advanced Biomaterials for Healthcare (Naples, Italy).

His research experiences are in different fields such as signal processing, data analysis, software programming and development, systems modelling and simulations, characterization of polymer nanostructures through small angle x-ray scattering, scanning electron microscopy, isothermal titration calorimetry and nuclear magnetic resonance. His research is mainly focused on characterization and modelling of nanostructured contrast agents for Magnetic Resonance Imaging.

Coauthor Torino Enza

Fabiola Porta

Dr. Fabiola Porta has studied Medicinal Chemistry and Pharmaceutical Technology in Milan at Universita' degli studi, where she obtained her Master degree in Pharmacy in 2008. She then moved to the Leiden Institute of Chemistry where she graduated, in 2012, in chemistry with a special focus in nanoparticles synthesis and characterization. After the completion of her PhD she started to work as PostDoc researcher at FHNW in the synthesis and formulation of nanoparticles for enzyme immobilization. In 2013, she joined the group of Prof. Huwyler at University of Basel as PostDoc. In this group she is developing novel polymer based nanoparticles as drug delivery systems. She is particularly interested in the design of innovative smart responsive nanovesicles. Moreover, she is using alternative animal models as Danio rerio to understand the behaviour of the nanoparticles in a physiological environment.

PUBLICATIONS:

- Ke Peng, Chao Cui, Itsuro Tomatsu, Fabiola Porta, Annemarie H. Meijer, Herman P. Spaink and Alexander Kros, Cyclodextrin/dextran based drug carriers for a controlled release of hydrophobic drugs in zebrafish embryos, Soft Matter, 2010, 6, 3778-3783
- Fabiola Porta, Gerda E. M. Lamers, Jeffrey I. Zink and Alexander Kros, Peptide modified mesoporous silica nanocontainers, PCCP, 2011, 13, 9982-9985
- Fabiola Porta, Faiza Sharif, Annemarie H. Meijer, Alexander Kros and Michael Richardson, Mesoporous silica nanoparticles as a compound delivery in zebrafish embryos, Int. J. of Nanomed., 2012, 7, 1875-1890
- Fabiola Porta, Gerda E. M. Lamers, Claude Backendorf, Marcel Schaaf, Jeffrey I. Zink and Alexander Kros, Folic acid modified silica nanoparticles as drug delivery systems for anticancer drugs, Adv. Health. Mat., 2013, 2, 281-286
- Fabiola Porta and Alexander Kros, Colloidosomes as single implantable beads for the *in vivo* delivery of hydrophobic drugs, Particles & Particles Systems Characterization, 2013, 30, 606-613
- P. Nadrah, Fabiola Porta, O. Planinšek, A. Kros, M. Gaberšček, Poly(propylene imine) dendrimer caps on mesoporous silica nanoparticles for redox-responsive release: smaller is better, PCCP, 2013, 15, 10740-10748
- D. Witzigmann, S. Sieber, Fabiola Porta, P. Grossen, A. Bieri, N. Strelnikova, T. Pfohl, C. Prescianotto-Baschong and J. Huwyler, Formation of lipid and polymer based gold nanohybrids using a nanoreactor approach, RCS Adv., 2015, 5, 74320

Marina Pöttler

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Head and Neck Surgery
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Marina Pöttler is a biologist with the main focus in nano-medicine and cancer research. After she studied biology at the Paris Lodron University in Salzburg (Austria), where she finished her master in zoology/ cell biology and physiology with excellent degree, she stated her PhD studies at the Medical University Vienna (Austria), in the field of oncology with main research area of molecular signal transduction and malignant diseases. Herby, she focused on the development of tumor markers in solid tumor as well as in tumor angiogenesis. As a PostDoc she investigated tumor-immunological questions at the Moore Cancer Center at the University of San Diego (CA, USA). Working as a PostDoc at the Section of Experimental Oncology and Nanomedicine, (SEON, University Hospital Erlangen) she strived on toxicological evaluations of superparamagentic iron oxide nanoparticles for the use in cancer therapy and diagnosis as well as tissue engineering using nanotechnology aimed at formation of 3D cell structures via magnetic cell guidance.

Suma Prabhu

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DOB: 14th October, 1988

EDUCATION

- Master of Science (M. Sc.) - Biochemistry, JSS College, Autonomous, Affiliated to the University of Mysore, Karnataka, India, 77.95%, June, 2011
- Bachelor of Science (B. Sc.) - Biochemistry, St Philomena's College, Affiliated to the University of Mysore, 83.39%, July, 2009

PHD DISSERTATION

Targeted Delivery of Ligand Loaded Iron Oxide naoparticles for Human Glioma in Mouse Xenograft model: The work involves engineering of iron oxide based targeted drug delivery for human Gliobalstoma Multiforme using mouse xenograft model. Where the engineered nano formulate involves bio-conjugation of ligands to the polymer, and *in vivo* and *in vitro* evaluation of the optimized formulation in comparison to that of the pure drug along with detailed toxicity profiling.

CONFERENCES

- Suma Prabhu1, Srinivas Mutalik2, Satyamoorthy K1, N. Udupa4, B. S. Satish Rao1*. Toxicity Profile of Superparamagnetic Iron Oxide Nanoparticles. 6th Bangalore India Nano Conference (Poster Presented December 2013)
- Bhabani Shankar Mohanty, Suma Prabhu, Jayant Sastri Goda, B. S. Satish Rao. Micro Computerized Tomography: An Imaging Modality for Visualization of Intracerebral Glioma in Mouse. International conference on promotion of animal research, welfare and harmonization of laboratory animal science. Laboratory Animal Scientists' Association (India) and ACTTREC, Navi Mumbai. (Poster Presentation in October 2015)
- Suma Prabhu, Srinivas Mutalik, N. Udupa, Satyamoorthy K., B. S. Satish Rao. Targeted Multifunctional Polymeric Magnetite Nanoparticles against Glioma in Mouse Orthograft Model. International conference on Nanomaterials and Nanotechnology, KSR-Nano 15, Tamil Nadu, India. (Oral Presentation in December 2015)
- Suma Prabhu, Jayant Sastri Goda, Bhabani Shankar Mohanty, Srinivas Mutalik, Sharada Rai, Nayanabhrama Udupa, Pradip Chaudhari, Rahul Thorat, Bola Sadashiva Satish Rao. Radio labeled Polymeric Magnetite Nanoparticles Targeted against Human Glioma in Mouse Orthotopic Xenograft Model

International Conference on Radiation Research: Impact on Human Health and Environment (Poster Presentation, February 2016)

PUBLICATIONS

- Suma Prabhu, Srinivas Mutalik, Sharada Rai, Nayanabhrama Udupa, Bola Sadashiva Satish Rao*. PEGylation of Superparamagnetic Iron Oxide Nanoparticle for Drug Delivery Applications with Decreased Toxicity: An *in vivo* Study. J. Nanoparticle Research. 2015. DOI: 10.1007/s11051-015-3216-x
- Trupti Kaleb, Kiran Bendalea, Suma Prabhu, K. K. Singh, Pradip Chaudhari*. Albumin Based Iohexol Nanoparticles for Computed Tomography: An In vivo Study. J. of Biomedical Nanotechnology (Communicated)
- Suma Prabhu, Jayant Sastri Goda, Bhabani Shankar Mohanty, Srinivas Mutalik, Sharada Rai, Nayanabhrama Udupa, Pradip

Chaudhari, Rahul Thorat, Bola Sadashiva Satish Rao*. Multifunctional Polymeric Magnetite Nanoparticles Targeted against Human Glioma in Mouse Orthotopic Xenograft Model (Manuscript under preparation)

- Divya N, Nagamani JE*, Suma Prabhu. Antioxidant and Antihemolytic activity of Bombax Ceiba Pentandra Spike and Fruit extracts. International Journal of Pharmacy and Pharmaceutical Sciences. Vol 4, Suppl 5, 2012.

AWARDS & ACHIEVEMENTS

- Award for securing first in M. Sc, JSS College, University of Mysore, Sept, 2011
- Merit Scholarship, for higher studies - M. Sc, Indira Gandhi Scholarship for Single Girl Child for Merit Scheme, University Grants Commission, Delhi, India, Oct, 2009
- First Place, Eco-friendly Utilisation of Natural Resources, State Level Science Exhibition, Karnataka Science & Technology Academy, Bangalore, India, Held at Belgaum, Mar, 2009
- Second Place, Eco-friendly Utilisation of Natural Resources, Zonal Level Science Exhibition, Karnataka Science & Technology Academy, Bangalore, India, Held at Mysore, Feb, 2009

WORK EXPERIENCE

- Worked as a quality control and R&D executive at Urja Foods Pvt. Ltd
- Worked as Scientist I at Strand Life Sciences Pvt. Ltd., Bangalore

EXTRACURRICULAR

I have won many prizes in Elocution and singing competition. I' am good at Drawing and painting. Passionate about singing, reading, listening to music, traveling, and also passionate to learn underwater/deep sea diving.

Simon Sebastian Räsch

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Saarland University, Campus Bldg. E8.1,
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EDUCATIONAL BACKGROUND

2012–to date: PhD candidate; Biopharmaceutics and Pharmaceutical Technology, Saarland University / Helmholtz Institute for Pharmaceutical Research Saarland (HIPS)
2012: Certification as Pharmacist
2012: Diplom-Pharmazeut (equivalent to MPharm)
„Establishment, validation and evaluation of an *in vitro* permeation method for cystine delivery across bovine corneas"
2011: Postgraduate research at the Department of Ophthalmology, University of Auckland, New Zealand
2005–2011: Studies of pharmacy, Saarland University, Saarbrücken

RESEARCH INTERESTS

Polymeric nanoparticles, Nanoparticle Corona, Nano-Bio Interactions at the Cellular and Non-Cellular Barriers of the Lungs, in particular with Pulmonary Surfactant, Analytics

AWARDS AND GRANTS

Dec. 2015: Paper of the Month Award, Helmholtz Centre for Infection Research, Raesch, S. S., et al. Proteomic and Lipidomic Analysis of Nanoparticle Corona Upon Contact with Lung Surfactant Reveals Differences in Protein, but Not Lipid Composition. ACS Nano 2015, 9, 11872-11885
Nov. 2014: Research Grant "QualityNano"

Lilla Ravasz

PhD
My name is Lilla Ravasz. I graduated at Eötvös Lorand University, Institute of Biology, Faculty of Molecular-, Immun- and Microbiology as a biologist. Here, in 2014 I started my PhD studies within the Physiology and Neurobiology Program at the Laboratory of Proteomics and Systems Neurobiology. My research is about the role of fast spiking interneurons in the mechanisms of physiological mood swings and psychiatric diseases. I investigate it from the view of physiology and genomics by patch clamp and one cell sequencing approaches with the mentoring of Dr Adrienna Katalin Kékesi, Dr Dobolyi Árpád and Dr Juhász Gábor.

Paul Retif

Paul Retif obtained his Ph.D. in 2016 at the Université de Lorraine (Nancy, France) under the supervision of Prof. Bastogne and Prof. Barberi-Heyob. He is currently a Medical Physicist, Head of the Medical Physics Department at the C.H.R. Metz-Thionville (Metz, France) since 2015. His main research interests are the interactions between X-rays and metallic nanoparticles and numerical prediction of the *in vitro* and *in vivo* ranking of those nanoparticles.

Maria Russo

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In 2007, Maria Russo received High School Diploma in Scientific Science. In 2013 she achieved her Master's Degree in Biomedical Engineering from the University of Naples "Federico II", discussing a thesis entitled "Production of crosslinked Hyaluronic Acid Nanoparticles (cHANPs) and MRI Contrast Agents encapsulation by Microfluidic Nanoprecipitation System". During her thesis experience, she had the opportunity to work with advanced instrumentations to conduct her experimental research based on the design and the development of new systems for the encapsulation of biologically active molecules by microfluidic devices. Maria Russo implemented her knowledge on the microfluidics and on the nanotechnologies, taking up an internship at the Centre for Advanced Biomaterials for Health Care of the Italian Institute of Technology (IIT@CRIB) until June 2014.
To date, Maria Russo is Ph.D. student at University of Naples "Federico II", Department of Chemical Engineering, Materials and Industrial Production. At the same time, she is Research Fellow at IIT@CRIB and her specific research fields include: diagnostic, drug delivery, nanomedicine, regenerative medicine, lab-on-a-chip, microfluidics and biomedical micro-devices.

EXPERTISE

During her work experience she improved her Computing, Communication, Organizational and Managerial skills. Furthermore, due to the multi-ethnic environment, she implemented her English language skills. Present field of R&D: bottom-up approach methods for continuous flow production of nanoparticles; production and characterization of different formulations including liposomes, microspheres, polymeric micelles, micro- and nano-particles for drug

delivery and for diagnostic applications by microfluidic approach. She has participated in various national and international conferences in the field of Nanotechnologies.
Coauthor Torino Enza

Jonas Schnittert

In 2009, Jonas Schnittert started his study in Biomedical Engineering at the University of Twente. In 2013 he obtained his bachelor's degree and continued with his masters in Biomedical Engineering. During his master's thesis he investigated novel miRNA delivery strategies in the section Targeted Therapeutics of the Department of Biomaterials Science and Technology, University of Twente. After completing his master thesis in 2015, he started his PhD with focus on novel targets in tumor stroma and gene delivery system in the section Targeted Therapeutics of the Department Biomaterials Science and Technology, University of Twente.

Dima Shamrakov

Dima received his M.Sc. (1982) and Ph.D. (1987) in Chemistry from the Gorky State University, Russia. In 1991 his family moved to Israel, where, in 1994, he completed his post-doctoral training at the Hebrew University of Jerusalem, specializing in the field of composite glasses for solid state dye lasers, luminescent solar concentrators, sensors, integrated and nonlinear optics applications.
Since then, for the last 22 years, Dima works in the pharmaceutical industry, where he gained diverse experience in analytical and process development, QC, along with a vast exposure to production and formulation, CMC and regulation, personnel training and technology transfer. He held analytical R&D managerial positions in a number of major Israeli pharma companies and taught analytical chemistry to pharmaceutical engineering students. Currently Dima heads analytical chemistry of liposomal drug delivery systems at Lipocure and Ayana Pharma, located in Jerusalem.
Dima has 25 publications, including 2 US patents.

Ewelina Sobierajska

Ewelina Sobierajska is a Master Student at the Department of General Biophysics, University of Lodz. Her research interest is focused on studying dendrimers.

Etienne Stalder

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EDUCATION

Doctor Rerum Naturalium, Chemistry, University of Fribourg, Fribourg, 2012–2016 (ongoing); Post formulation modification of vesicles, Prof. Andreas Zumbuehl
Master of Science, Chemistry, University of Geneva, Geneva, 2010–2012; Post formulation modification of vesicles, Dr. Andreas Zumbuehl
Bachelor of Science, Chemistry, University of Geneva, Geneva, 2007–2010

PUBLICATIONS

- "Phosphate Test 2.0", Etienne Stalder, Andreas Zumbuehl, CHIMIA International Journal for Chemistry, 67, (2013), 819-82
- "The synthesis of an amine-bearing polymerizable phospholipid", Pierre-Léonard Zaffalon, Etienne Stalder, Illya A. Fedotenko, France Favarger, Andreas Zumbuehl, Tetrahedron Letters, 52, (2011), 4215–4217.

RESEARCH EXPERIENCE

Master internship, Department of Organic Chemistry, University of Geneva, Geneva, 2010 • Synthesis of amine-bearing polymerizable phospholipid (Dr. Andreas Zumbuehl) • Phospholipid synthesis and characterization
Master internship, Department of Organic Chemistry, University of Geneva, Geneva, 2011 • Synthesis of palladium precatalyst containing labile ligand (prof. Peter Kuendig) • Enantioselective synthesis, inert condition organic synthesis
Master internship, Department of Inorganic Chemistry, University of Geneva, Geneva, 2011 • Synthesis of a segmental ligand for helicoidal self-assembly heteroatomic complexes (prof. Claude Piguet)) • Organic synthesis
Master thesis, Department of Organic Chemistry, University of Geneva, Geneva, 2010 • Post formulation modification of vesicles (Dr. Andreas Zumbuehl) • Liposome formulation and characterization

TEACHING EXPERIENCE

Teaching Assistant, General chemistry laboratory course for 1st year pharmaceutical and medicine students, Department of Chemistry, University of Fribourg, Fribourg, 2013–2014 • Supervising laboratory work, grading student performances.
Teaching Assistant, General Chemistry laboratory course for 1st pharmaceutical, biology and geology students, Department of Inorganic Chemistry, University of Geneva, Geneva, 2010–2011 • Supervising laboratory work, grading student performances.
Teaching Assistant, Chemistry laboratory course for 3rd and 4th year high-school student, College Calvin, Geneva, 2012• Supervising laboratory work

Azade Taheri

PhD
Assistant Professor
Department of Pharmaceutics, Faculty of Pharmacy and Novel Drug Delivery System Research Center, Isfahan University of Medical sciences, Isfahan, Iran
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azadetaheri@yahoo.com

EDUCATION:

- Doctorate of Pharmacy (Pharm D), Tehran University of Medical Sciences, Tehran, Iran (2001-2007) (A:17.70)
- Doctorate of Pharmaceutics (PhD), Tehran University of Medical Sciences, Tehran, Iran (2007-2011) (A:18.20)

HONOR AND AWARDS:

- Awarded as the best Ph.D thesis in the 13th Avecina Festival, Tehran University of Medical Sciences (2012)
- Top student between pharmaceutics Ph.D students in Tehran University of Medical Sciences, Tehran, Iran (2007-2011).
- One of the 3 top between Pharm.D students in Tehran University of Medical Sciences, Tehran, Iran (2001-2007).
- Awarded as the Grade One for best poster presenter in the 12th Iranian pharmacy students seminar, 2006, Sari, Iran.
- Awarded as the Grade One for best poster presenter in the 5th

Iranian Controlled Release Conference (ICRC 2011), 4-6 October, 2011, Mashhad, Iran.

ACADEMIC POSITION:

- From January 2012-to June 2014: Assistant Professor of Department of Pharmaceutics at Faculty of pharmacy, Zanjan University of Medical sciences, Zanjan, Iran.
- From July 2014 up to Now: Assistant Professor of Department of Pharmaceutics at Faculty of pharmacy, Isfahan University of Medical sciences, Isfahan, Iran

THESIS & DISSERTATION:

- Preparation and characterization of in situ gel forming systems using PEO-PPO-PEO polymers (Pharm.D. Thesis).
- Preparation and characterization of targeting drug delivery systems using human serum albumin (HSA) nanostructures surface modified with monoclonal antibody (Ph.D. Dissertation).

PUBLICATIONS:

- Khanbanha N, Atyabi F, Taheri A, Talaie F, Mahbod M, Dinarvand R. Healing efficacy of an EGF impregnated triple gel based wound dressing: *in vitro* and *in vivo* studies. Biomed Res Int 2014; 8:493732.
- Taheri A, Bastami Z. Nanomedicine for Diagnosis and Treatment of Cancer in Global Market. J Mazandaran Univ Med Sci. 2014; 24 (115):203-218 (In Persian).
- Bastami Z, Taheri A, Soltanpour S. Formulation, Optimization and characterization of Gemfibrozil Nanocrystals prepared by wet milling technique. Asian J Pharm. 2015;9(1):19-22.
- Taheri A, Mohammadi M. The use of cellulose nanocrystals for potential application in topical delivery of hydroquinone. Chem Biol Drug Des. 2015;86(1):102-106.
- Taymouri S, Taheri A. Use of nanotechnology in diagnosis and treatment of hepatic fibrosis; A review. Curr Drug Deliv. 2015

ABSTRACTS:

- Bastami Z, Taheri A, Soltanpour S. Formulation, Optimization and characterization of Gemfibrozil Nanosuspension prepared by wet milling technique. The 1st Middle East & The 6th Iranian Controlled Release Conference, 25-27 February 2014, Tehran, Iran.
- Taheri A, Mohammadi M, Mansoori P. The use of cellulose nanocrystals for potential application in drug delivery to skin. TWAS-ROCASA Young Scientists Conference on "Nanoscience & Nanomaterials", 18-20 Feb 2015, Bangalore, India.

Shima Tavakol

Assistant Professor, Drug nanocarriers Research Core, Razi Drug Research Center, Iran University of Medical Sciences, Tehran, Iran
E-mail: Tavakol.sh@iums.ac.ir

ACADEMIC BACKGROUND

Ph.D of Medical Nanotechnology, Tehran University of Medical Sciences, School of Advanced Technologies in Medicine, Tehran, Iran (2014).
Master of Medical Nanotechnology, Tehran University of Medical Sciences, School of Advanced Technologies in Medicine, Tehran, Iran (Feb 2010).
Bachelor of Medical Laboratory sciences, Isfahan University of Medical Sciences, Isfahan, Iran (Feb 2006)

AWARDS AND HONORS

- Recognized and encouraged as the best Ph.D graduate of Nanotechnology in Iran by Iranian Nanotechnology society.2014.
- Recognized and encouraged as the best Ph.D graduate of School of Advanced Technologies in Medicine by Tehran University of Medical Sciences. 2014.
- Ranked First, among Ph.D students in the Board exam. 2012
- Ranked 3rd, in the Ph.D Entrance Examination held by Ministry of Health and Medical Education. 2010
- Winner of the oral presentation prize in the 4th nanotechnology student's conference; Tehran. 2008

SOME ARTICLES PUBLISHED TO REFEREED JOURNALS:

- Chimeric self-assembling nanofiber containing bone marrow homing peptide's motif induces motor neuron recovery in animal model of chronic spinal cord injury; an in-vitro and in-vivo investigations. Shima Tavakol, Reza Saber, Elham Hoveizi, Hadi Aligholi, Jafar Ai, Seyed Mahdi Rezayat. Molecular Neurobiology. (2015) doi:10.1007/s12035-015-9266-3.
- Acidic pH derived from cancer cells may induce failed reprogramming of normal differentiated cells adjacent tumor cells and turn them into cancer cells. Shima Tavakol. Medical Hypothesis (2014) 13;83(6):668-672.
- In vitro comparative survey of cell adhesion and proliferation of human inducedpluripotent stem cells on surfaces of polymeric electrospun nanofibrous and solution-cast film scaffolds. S Ebrahimi-barough, Shima Tavakol, M Nabiuni. Journal of Biomedical Materials Research Part A. (2015) DOI: 10.1002/jbm.a.35420
- Differential effect of Activin A and WNT3a on definitive endoderm differentiation onelectrospunnanofibrous PCL scaffold. Elham hoveizi1, Jafar Ai, Somayeh Ebrahimi-barough and Shima Tavakol. Cell Biology International. (2015) DOI: 10.1002/cbin.10430.
- Neuroprotective effect of transplanted neural precursors embedded on PLA/CS scaffold in an animal model of multiple sclerosis. Elham hoveizi, Shima Tavakol, Somayeh Ebrahimi-barough, Molecular Neurobiology. (2014) 51 (3), 1334-1342. DOI: 10.1007/s12035-014-8812-8.

PATENT

- Hydrogel based peptide nanofiber containing long motif of laminin for application in medical studies; International category A61, Patent no 82433.
- Biodegradable and biocompatible nano composite t-plate implant and a method of synthesizing the same. Jafar Ai, Mahmood Azami, N Bahrami, Shima Tavakol. United States Patent Application 14/301,306:20140356410.

BOOK (COMPILATION)

- Nanomedicine. 2 chapters, Jahad Daneshgahi, Tehran, Iran.
- Introduction of Physiology (Persian) Publisher; Taaliye Andishe, Tehran, Iran.
- Embryology summery (Persian) 2014 Publisher; Taaliye Andishe, Tehran.

Okan Tezcan

I received my Bachelor's Degree in Biology at Ankara University (Ankara, Turkey) and followed my Master's Degree in Biology at Middle East Technical University (Ankara, Turkey). My thesis was conducted in molecular biology and cancer research laboratory. The subject entitled "metastatic behavior of doxorubicin resistant MCF-7 breast cancer cells after vimentin silencing". After my master graduation, I started my PhD studies at RWTH Aachen University (Aachen, Germany), Department of Experimental Molecular Imaging (ExMI) with a prestigious DAAD scholarship to work on the relationship between multidrug resistance and metastasis in Nanomedicine and Theranostics research group, under the supervision of Prof. Twan Lammers. More specifically, I generate multidrug resistant cells from drug sensitive parental lines and develop nanomedicine formulations to overcome multidrug resistance in metastases, and employs imaging techniques to longitudinally monitor therapeutic efficacy.

Nguyen TK Thanh

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Prof. Nguyen TK Thanh FRSC is Professor of Nanomaterials and has over 15 years of research experience in materials chemistry, synthesis and biofunctionalisation of nanoparticles. Her work is focussed on the chemical synthesis of various Au, Ag and magnetic nanoparticles. She has published over 70 papers and book chapters. Her papers received over 550 citations in 2015 alone. Research highlights include the development of biocompatible iron oxide magnetic nanoparticles using co-precipation method using sodium carbonate in a microwave reactor, polyol synthesis, functionalisation of superparamagnetic iron oxide nanoparticles as potential MRI contrast agents and a novel strategy for delivering functionalised superparamagnetic iron oxide NPs to the outer surface of pancreatic islet grafts for tracking them by magnetic resonance imaging (MRI). She has been an invited speaker over 120 institutes and scientific meetings and served on over 20 scientific committees for many major international conferences on Nanoparticles such as MRS, EMRS, MRS Singapore, ACS, RSC-SCI UK Colloids 2011, 2014 and 2017. She has served on organisation and scientific committees for many major international conferences on NPs research, e.g. " Multifunctional nanostructures for diagnosis and therapy of diseases" Symposium at 2016 E-MRS Spring Meeting, Lille, France http://www.european-mrs.com/2016-spring-symposium-r-european-materials-research-society. She was the Guest Editor of Philosophical Transactions of the Royal Society A on "Nanoparticles" theme issue published in September 2010. She edited a seminal book: "Magnetic nanoparticles: From fabrication to clinical applications" published by CRC Press/Taylor and Francis, 2012. She is also the Editor of Royal Society of Chemistry (RSC) Nanoscale themed issue "Functional nanoparticles for biomedical applications" published in 2013. She was the scientific chair and editor of RSC Faraday Discussion 175 "Physical Chemistry of Functionalised Biomedical Nanoparticle". She is advisor of Mag(net)icFun, an EU Training Network which focuses on the application of functionalized magnetic nanoparticles in Chemistry and Biomedicine. She also serves on the Management Committee for EU COST Action TD1402 Multifunctional Nanoparticles for Magnetic Hyperthermia and Indirect Radiation Therapy (RADIOMAG).

RECENT PAPERS:

1. R. Hachani, M. Lowdell, M. Birchall, A. Hervault, D. Merts, S. Begin-Colin, N.T.K. Thanh*. (2016) Polyol synthesis, functionalisation, and biocompatibility studies of superparamagnetic iron oxide nanoparticles for potential MRI contrast agents. Nanoscale. DOI: 10.1039/C5NR90206A.
2. R. M. Pallares, X. Su, S. H. Lim, N. T. K Thanh* (2016) Fine-Tuning Gold Nanorods Dimensions and Plasmonic Properties Using the Hofmeister Salt Effects. Journal of Material Chemistry C. 4: 53-61. Front Cover
3. C. Blanco-Andujar, P. Southern, D. Ortega, S.A. Nesbitt, Q.A., Pankhurst and Thanh, N. T. K*. (2016) Real -time tracking of delayed-onset cellular apoptosis induce d by intracellular magnetic hyperthermia. Nanomedicine. 11: 121-136.
4. R. M. Pallares, S. L. Kong, H. R. Tan, Thanh, N.T.K, Y. Lu and X. Su (2015) A plasmonic nanosensor with inverse sensitivity for circulating cell-free DNA quantification. Chemical Communications. 51, 14524 - 14527
5. L. T. Lu, N. T. Dung, L. D. Tung, C. T. Thanh, O. K Quy, N. V. Chuc and N. T. K. Thanh* (2015) Synthesis of magnetic cobalt ferrite nanoparticles with controlled morphology, monodispersity and composition: the influence of solvent, surfactant, reductant and synthetic condition. Nanoscale. 7: 19596-19610. Front Cover noscale.. 7: 1768-1775

Jean-Sébastien Thomann

PhD
Dr Thomann is pharmaco-chemist, specialized in the synthesis of nano-carrier for vectorization. He has a strong knowledge on organic and colloidal synthesis. In 2008, he defended his PhD in pharmaco-chemistry at Strasbourg University (France) in the field of synthetic nanovaccine design. After a post-doc in the field of targeted nanoemulsion at CEA LETI (Grenoble, France), he joined the Luxembourg institute of science and technology (LIST) in 2012 where he developed a new Drug Delivery System (patent filed) based on supported lipid bilayer on mesoporous silica. This system is currently under preclinical investigation for cancer therapy. He is focused on applied research and has filed 6 patents and published 15 papers in chemical and biomedical engineering.
Coauthor Gaelle Corne

Enza Torino

Enza Torino gradueted in Chemical Engineering at the University of Salerno (Italy) in 2006. Life time goal of her research interests has always been obtaining of nanostructures and the exploitation of their fascinating properties. Since her bachelor degree, she worked on carbon nanotubes to increase polymer strength and later on, during my master degree, she used the thermodynamics to improve the characteristic of the nanoparticles in the pharmaceutical field (size, shape and charge). She gained a PhD in Chemical Engineering on the development of novel technologies for nanoparticle production, addressed to the study, characterization and development of new processes and materials, at University of Salerno (ITALY) – Supervisor Prof Ernesto Reverchon- Thesis Title: Nanoparticles Production by SUPERCRITICAL-CO2- Her last ten years of research have always been devoted to the nanotechnologies in the medical field. Starting from her background in chemical engineering, she was involved in a project for the pharmaceutical industry in Switzerland to design a process to increase the bioavailability of several drugs and later she spent part of my PhD to study how nanoparticles can be modified using surfactants to increase their delivery properties in a biological environment. Indeed, during her PhD she also worked as visiting scientist at University of Texas at Austin – Texas (USA), studying "Research on Colloidal systems: emulsion and microemulsion formation and stability for pharmaceutical and energy applications – supervisors Prof. Keith P. Johnston – and was also involved in a Collaboration project on EOR (Enhanced Oil Recovery) supported by Dow Chemicals and Petroleum and Chemical Engineering Department at UT at Austin (TX). After her PhD, she worked as Guest scientist at the "School in Advanced Optical Technologies" (SAOT) established at the University of Erlangen-Nuremberg - Department of Chemical and Bioengineering within the framework of the Excellence Initiative of the German Federal and State governments, where she studied the mechanism of precipitation involved on drug nanoparticles production by Supercritical Antisolvent technique using on situ laser diagnostic technique and the Control and manipulation of pharmaceutical emulsions to produce nanospheres or nanocapsules by Microfluidic technique. Currently, she is working as Post Doc Researcher at Italian Institute of Technology – Center for Advanced Biomaterials for Health Care- coordinated by Prof. Paolo Antonio Netti – at Theranostic Engineered Nanoshuttle (TeNs) Platform, where she design new processes to obtain novel polymer based engineered nanoshuttles for *in vivo* application in diagnostic and therapy.

Rudolf Urbanics

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MD, PhD, Head of the *in vivo* laboratory of Nanomedicine Research and Education Center of Semmelweis University, and SeroScience Ltd., an immunotoxicity CRO, since 2008 in Budapest, Hungary.
He obtained MD diploma and the PhD degree at Semmelweis Medical School, Budapest, Hungary. He had teaching and research activity at the parent university (2nd Institute of Physiology) and held in between various research/collaboration positions in Germany and in the USA. He was the Deputy R&D Director and Head of CNS Pharmacology Department at Biorex R&D Co., worked at IVAX/ Drug Research Institute Budapest, as Scientific Adviser, Leading researcher in Safety and CNS Pharmacology and later in IVAX/Drug Research Institute, Subsidiary of TEVA as Head of In Vivo Pharmacology Group.
He is working with *in vivo* models of nano drug - nano carrier induced, complement activation related pseudoallergic reactions (CARPA), clarifying their immuno-toxicological and safety hazards.

Ildikó Vashegyi

MSc, PhD
Position: postdoctoral research fellow
Company: SeroScience Ltd.
E-mail: ivashegyi@seroscience.com

Dr. Ildikó Vashegyi obtained her MSc degree in Biology in 2007 from the Faculty of Sciences, Eötvös Loránd University (ELTE), Hungary. She received her PhD degree in 2012 from the Molecular- and Nanotechnologies PhD School, Pannon University, Hungary.
As a research associate at the Hungarian Academy of Sciences, her major research interests were molecular genetics and cellular signal transduction. She was principal investigator of a Hungarian Scientific Research Fund (OTKA) project, and she also worked as a research fellow in EU FP7 studies. For her scientific activity, she won János Bolyai Research Fellowship and Outstanding Young Researcher Prize of the Hungarian Academy of Sciences.
Since January 2016, Dr. Ildikó Vashegyi is a postdoctoral research fellow at SeroScience Ltd. In the team of Dr. Rudolf Urbanics, she works with *in vivo* models of complement activation related pseudo-allergic reactions induced by nanomaterials (nanodrugs and nanocarriers) clarifying their immuno-toxicological and safety hazards. Her recent research activity focuses on the connections between complement activation and coagulation in porcine CARPA model.

Christine Vauthier

PhD.
Institut Galien Paris Sud, CNRS,
Univ Paris-Sud, Faculty of Pharmacy,
Université Paris Saclay
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France
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Christine VAUTHIER, received her Ph. D. in polymer chemistry in 1986 from the University Louis Pasteur at Strasbourg, France. She joined the University of Paris South, Faculty of Pharmacy in 1987 as a research assistant. Presently, she is Director of Research at the CNRS (Centre National de la Recherche Scientifique) at the Institut Galien Paris Sud, UMR CNRS 8612, Université Paris Sud. During her carrier, she was visiting scientist at the Center for Chemical Controlled Delivery, University of Utah, USA (1995-1996) and at the Federal University of Pernambuco, Recife, Brazil (2008). Her research activities are focussed on the understanding of the role of physicochemical characteristics of nanomedicines on their interactions with biological systems to develop rational basis for the design of functional polymer based nanomedicines improving drug delivery by mucosal and intravenous administration. She is performing a multidisciplinary research in characterising polymer nanoparticles on a physicochemical stand point and studying their interactions with proteins, the immune system, cells and evaluating their *in vivo* fate. She is authors and co-author of more than 100 research papers and over 20 review papers and book chapters on nanoparticle preparation and characterization methods and on the application of nanoparticles as drug delivery systems. She has presented over 100 communications and many invited conferences. She serves as an editor for Pharmaceutical Research.

POSITIONS

2002–current: Research Director at the C.N.R.S. (http://www.cnrs.fr) Institut Galien Paris Sud, UMR CNRS 8612, Université Paris-Sud, Université Paris Saclay, Chatenay-Malabry, France (http://www.umr-cnrs8612.u-psud.fr/)
1988–2002: Assitant Researcher at CNRS, Physico-chimie, Pharmacotechnie and Biopharmacie, URA CNRS 1218, Université Paris-Sud, Chatenay-Malabry, France
July 1995–July 1996: Visiting Scientits, Center for Chemical Controlled Delivery, Jindrich Kopecek Group, University of Utah, USA

EDUCATION

1992: Habilitation à Diriger les Recherches, University of Paris XI, CHATENAY-MALABRY.
1986: PhD in Polymer Science, University of Strasbourg, Supervisor: Françoise CANDAU, Research topic: Inverse microemulsion polymerization of acrylamide
1983: Master Degree in Cellular and Molecular Biology, University of Strasbourg, Research topic Identification of oligonucleotides able to block E. coli protein synthesis.

SCIENTIFIC INTEREST

Christine Vauthier' research is developed at the interface between the physicochemistry of polymer colloids and conception of nanomedicines for mucosal and intravenous administration of drugs including for instance antisense oligonucleotides and siRNA. Starting from the characterization of the polymer nanoparticles, her main interest is to better understand interactions of nanomedicines with biological systems in relation with the *in vivo* fate hence their biodistribution and safety. Christine Vauthier is also interested in developing methods for the characterization of the biological identity of nanomedicines.

SCIENTIFIC PRODUCTION

H Index: 41 (C. Vauthier (married name) or C. Holtzscherer (patronym)); 110 International Publications; 16 Review articles; 23 Book chapters; 25 Invited conferences in international meetings;Supervision of 19 Ph.D

EDITORIAL ACTIVITIES

Editor for Pharmaceutical Research (http://link.springer.com/journal/11095)
Editorial Board of Journal of Colloid Science and Biotechnology (http://www.aspbs.com/jcsb.html)

Donatella Vecchione

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BIOSKETCH

In 2007, Donatella Vecchione received High School Diploma in Scientific Science. In 2013, she achieved her Master's Degree in Biomedical Engineering from the University of Naples "Federico II", discussing a thesis entitled "Design and development of a removable medical device to support the areas of Pelvic Organ Prolapse". During her thesis experience, she had the possibility to be involved in the different steps of production of a medical device and in drafting of a patent. After her thesis experience, she had the opportunity to work with advanced instrumentations to conduct her experimental research based on the design, the development and the characterization of new nanovectors for the encapsulation of active compounds by complex coacervation for multimodal imaging at Istituto Italiano di Tecnologia IIT@CRIB. Donatella Vecchione implemented her knowledge on the biomaterials and on the nanotechnologies, taking up an internship at the Centre for Advanced Biomaterials for Health Care of the Italian Institute of Technology (IIT@CRIB) until June 2014.To date, Donatella Vecchione is Ph.D. Student at University of Naples "Federico II", Department of Chemical Engineering, Materials and Industrial Production (DICMAPI). At the same time, she is Research Fellow at Istituto Italiano di Tecnologia IIT@CRIB and her specific research fields include: diagnostic, multimodal imaging, drug delivery, nanomedicine, theranostic and *in vitro* and *in vivo* study.

EXPERTISE

During her work experience she improved her Computing, Communication, Organizational and Managerial skills. Due to the multiethnic environment, she improved her language skills. Present field of R&D: different methods to produce micro and nanoparticle using high pressure homogenizer; characterization of different formulations for theranostic and multimodal diagnostic applications as integrated PET/MRI and optical imaging.

Coauthor Torino Enza

Kseniya Vlasova

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EDUCATION

September 2008–July 2012: B.Sc. in Chemistry, Moscow State University.

August 2012–June 2013: M.Sc. in Chemical Enzymology, Department of Chemistry, Moscow State University.

July 2013–present: PhD program in Chemical Enzymology, Department of Chemistry, Moscow State University.

EXPERIENCE

September 2008–November 2010: Kurnakov Institut of General and Inorganic Chemistry, Laboratory of Chemical synergi: synthesis of nanoparticles of zirconium oxide doped by Eu, by using methods of "soft chemistry". Superviser :Dr. A.E. Baranchikov

November 2010–present: MSU, Chemistry Department, Department of Chemical Enzymology, Laboratory of chemical design of nanoboimaterials: study of catalical activity of recombinant proteins and using of them for drug delivery. Research in the field of nanomedcine and nanozymes. Superviser: Prof. Dr. N.L. Klyachko, Dr. M. Sokolsky- Papkov, Prof. Dr. A.V. Kabanov, Dr. M. Abakumov

October 2011–present: RSMU, Department of Medical nanobiotechnologies: synthesis of nanoparticles(liposoms, micelles and "nanogels") for address drug delivery *in vivo*. Superviser: Prof. Dr. V.P. Chechonin, Dr. N.V. Nukolova

January–February, 2012 June–July, 2012: University of Nebraska Medical Center(Omaha, the USA), Department of Pharmaceutical Sciences and Center for Drug Delivery and Nanomedicine: preparation and characterization of magnetic nanoparticles (DLS, TGA, ICP-MS). Superviser: Dr. M. Sokolsky-Papkov, Prof. Dr. A.V. Kabanov

AWARDS AND PRIZES

May, 2012: Winner in poster presentation in 3rd Russian-Hellenic Symposium "Biomaterials and bionanomaterials: recent problems and safety issues"

March, 2012: Winner in the Berezin competition of scholarships

PUBLICATIONS/ABSTRACTS

- O.V.Ivashkov, N.V. Nukolova, K.Y. Vlasova, V.P. Baklyshev "Active transport of Biochemical substances by cationic lyposoms invitro, 7th The International Pirogov scientific medical conference of students and young scientists 2012.
- K.Y. Vlasova, M. Sokolsky, N.L. Klychko, A.V. Kabanov "Synthesis of magnetic nanoparticles of iron oxide, coated with superoxide dismutase 1 and increasing the activity of enzyme by magnetic field acting", 3rd Russian-Hellenic Symposium "Biomaterials and bionanomaterials: recent problems and safety issues",2012.
- K.Y. Vlasova, N.L.Klychko, A.V. Kabanov "Activation of superoxide dismutase 1 immobilized on the surface of iron oxide magnetic nanoparticles by application of AC magnetic field", conference "Nanomedcine: from molucles to diagnostic and therapy", 2012.
- K.Y. Vlasova, M.A.Abakumov, M. Sokolsky, N.L. Klychko, A.V. Kabanov "Stabilization of superoxide dismutase 1 on the surface of MNPs", 8th The International Pirogov scientific medical conference of students and young scientists 2013.
- Vlasova K.Y., Sokolsky M., Vishwasrao H., Golovin Y. I., Klychko N.L,Kabanov A.V. " Magnetic field regulated nanoparticles coated with SOD1" 7th The International Mendeleev scientific chemical conference of students and young scientists 2013.

Klaus-Michael Weltring

Dr. Klaus-Michael Weltring is a molecular biologist by training with a PhD and a Habilitation degree from the University of Münster. Since 2001 he is the managing director of bioanalytik-muenster responsible for the development of the Münster region into a leading nanobioanalytic location at the European level. He has set-up a local network of researchers from different disciplines and SMEs and organizes the marketing of the region at international events and fairs. Between 2003 and 2008 he was the deputy-coordinator of the Nano2Life Network of Excellence and leader of the "ELSA" Board in this network. He co-managed the Nanomedicine Round Table and the EuroNanoBio projects and participated in the NANOMED2020 project (FP7 CSA projects). Since 2009 he is a member of the Executive Board of the ETP Nanomedicine leading the ELSA Advisory Group of this platform. Since March 2015 he is the chair of the German platform NanoBioMedicine. At the local level he is the Chief Scientific Officer of the Nano-Bioanalytik-Zentrum Münster (NBZ) and manages the Nano-Characterization-Lab Muenster (www.NCL-Muenster.de) interfacing 11 local companies, which develops new and certified methods for characterization of Nanomaterials in consumer products and biological systems. Currently he is partner in the EU-projects ENATRANS and EU-NCL.

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Peter Wick heads the Research Laboratory for Particles-Biology Interactions at Empa the Swiss Federal Laboratories for Materials Science and Technolo-gy. He received his PhD degree in cell and molecular biology at the Univer-sity of Fribourg, Switzerland. In 2002, he moved to Empa and began his re-search in nanosafety. His research interest focuses on studying the interactions of nanomaterials with human tissues including barrier tissue *in vitro* in order to obtain detailed mechanistic understanding about their uptake, transport and biological effects. He is board member of the Swiss Action Plan for Synthetic Nanomaterials and editorial board member of the Journal Nanotoxicology and associated Editor of the new launched journal NanoImpact. Currently he has published more the 100 articles including peer-reviewed papers and book chapters.

Antje Ziller

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I was born in on the 17th of May 1989 in Bad Salzungen (Thuringia) and graduated with my A-levels in 1997. In October 2007 I started to study pharmacy at Friedrich Schiller-University in Jena, which I finished with the second state examination in September 2011. My admission as a pharmacist I got in June 2013 after passing the third state examination.
During and after my studies I did several internships, starting with a month-long internship in a pharmacy “Stadionapotheke” in March 2008 as well as in the dispensary of the local hospital in August 2008. In September 2009 I worked in a lab at the N.M. Emanuel Institute of Biochemical Physics of the Russian Acadamy of Science in Moscow. Topic of the work was: “Antioxidative effect of essential oils”.
From November 2011 to April 2012 I worked in the laboratory of Prof. Alfred Fahr at the Department of Pharmaceutics at University of Jena. The work was published as my diploma thesis in July 2013 with the title: “The effect of PEGylation of Invasomes on the percutaneous penetration into human skin”.
As part of my practical year to get admission as pharmacist, I did a six-month internship from May 2012 to October 2012 at Merck KGaA in Darmstadt, where I worked in the sterile filling of the development department. Immediately after this, I worked six month in the pharmacy “Südapotheke” in Frankfurt.
Since September 2013 I am doing my PhD-thesis in the working group of Prof. Peter Langguth at the Department of Biopharmaceutics and Pharmaceutical Technology at Johannes Gutenberg-University of Mainz. Topic of my work is the development and characterization of liposomal RNA/DNA-formulations.
Beside this, I am doing emergency service in the pharmacy “Kurapotheke” in Wiesbaden. Furthermore, in 2014 I started a three-year training to become expert pharmacist for pharmaceutical technology.

Andreas Zumbuehl

Andreas Zumbuehl graduated from ETH Zurich in 1999 and also received his PhD from ETH in 2004 working under the guidance of Professor Erick M. Carreira. He then spent his postdoctoral years with Professor Robert Langer at the Massachusetts Institute of Technology in Cambridge (USA) and Professor Joachim Seelig at the Biozentrum Basel. In 2008 he became Maître Assistant at the University of Geneva where he started his independent research on the synthesis and applications of artificial phospholipids. In 2012 he moved to the University of Fribourg as a Swiss National Science Foundation Professor.

CLINAM

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ABSTRACTS SPEAKERS

SELF-ASSEMBLING POLYPEPTIDE NANO-PARTICLES (SAPNS): THEIR USE IN BIOMEDICAL APPLICATIONS

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Because of the need to limit side effects, nanoparticles are increasingly being employed for drug delivery and targeting, antigen display and other biomedical applications. We used computer modeling to design a novel type of biocompatible and biodegradable nanoparticle composed of polypeptides as building blocks, so-called self-assembling polypeptide nanoparticles (SAPNs). We verified the computer models via solid-phase peptide synthesis and biophysical analyses. In near-physiological buffer, these SAPNs self-assemble from single recombinant polypeptides. They exhibit regular polyhedral (e.g., icosahedral) symmetry with an outer diameter of about 18 nm and a 6-nm-diameter central cavity. The polypeptide is composed of two distinct coiled-coil oligomerization domains with different oligomerization states, i.e. forming 3- and 5-stranded alpha-helical coiled coils, joined by a short linker segment. Such SAPNs are ideally suited for medical applications such as drug targeting and drug delivery systems or imaging devices, or they may be used for repetitive antigen display in synthetic vaccine design (see below). Our data suggest that the SAPNs are non-specifically taken up by the reticuloendothelial system. Low uptake in the pancreas indicates that there is little or no specific targeting of the native radioactive SAPNs. In particular, the uptake in the spleen, which is a primary organ of the immune system, highlights the potential of the SAPNs as vaccine carriers. Also, the decrease in liver and spleen radioactivity with time implies that the SAPNs are broken down and cleared. This is an important finding, as it shows that the nanoparticles can be safely used as a vaccine platform without the risk of prolonged side effects.
As an example, using our SAPNs as a platform to display a tandem repeat of the B cell immuno-dominant epitope $(DPPPPNPN)_2D$ of the rodent malaria parasite Plasmodium berghei has conferred a long-lived, protective immune response in mice without the need for a heterologous adjuvant. Similarly, our SAPNs also induced protective antibody and a $CD8^+$ T-cell response to the protozoan parasite Plasmodium falciparum (one of the parasite species causing malaria in humans) circumsporozoite protein (CSP). Currently, this novel vaccine platform is tested in humans. By the same approach an anti-smoking vaccine has been produced and is ready for clinical tests. Last but not least, we are working on an anti-flu vaccine using our SAPNs as a repetitive antigen display platform.

LIGHT-EMITTING ∏-PROBES FOR DIAGNOSTIC APPLICATIONS

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π–probes based on conjugated polymer nanoarchitectures are interesting biomimetic materials in view of application to chemical and biological sensor devices. These conjugated π–probes are unique in altering photoluminescence and resonance Raman scattering and/or in changing electronic property, caused by perturbation of probes' electronic state and energy transfer upon specific binding events. Based on these optical and electronic characteristics, we can utilize these probes as label-free detection agents for chemical and biological targets. In this presentation, we demonstrate strategy of interfacial design of nanoarchitectured probe materials achieving the label-free and rapid detection capability. A strikingly rapid detection of biological targets within 2~30 min. was also enabled by designing 3-dimensional materials involving columnar and porous interfaces showing higher surface area that enhanced accessibility and mass transfer rate of the target molecules. We will discuss current challenges using the light-emitting π–probes in developing diagnostic systems for biomarkers of cancer and infectious disease.

THERMOSENSITIVITY OF TEMPERATURE-SENSITIVE LIPOSOMES IS AFFECTED BY THEIR IN VIVO BIOMOLECULAR PROTEIN CORONA

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Thermal triggered drug release from temperature-sensitive liposomes (TSL) holds great promise for cancer therapy. Different types of TSL have been designed recently for heat triggered drug release inside tumor blood vessels or after accumulation into the tumor interstitium. However, justification of drug release profiles was mainly based on *in vitro* release data. While these methods could be good enough to give early indication about the thermal sensitivity of TSL, they are still far from being optimum. This is because these methods do not take into consideration the actual biomolecular protein adsorption (protein corona) formed onto TSL *in vivo* and the influence this could have on drug release. Therefore, in this study we compared thermal triggered drug release profile of two different types of TSL; namely the lysolipid-containing TSL (LTSL) and traditional TSL (TTSL) after *in vivo* recovery. Ex-vivo release profile at 42°C was then tested either in the presence of full plasma (to represent intravascular drug release) or after removal of unbound plasma proteins (i.e. protein corona coated TSL to represent interstitial release). Our data showed that the influence of the environment on drug release profile was very much dependent on the type of TSL. LTSL release profile was consistently characterized by ultrafast drug release independent on the conditions tested. On the contrary, TTSL release profile changed significantly. Doxorubicin release from *in vivo* recovered TTSL liposomes was slow and incomplete in the presence of unbound plasma proteins, whereas very rapid drug release was detected from *in vivo* recovered TTSL liposomes in the absence of unbound proteins. Using mass spectrometry and quantification of protein adsorption, we confirmed that this discrepancy is due to the changes in biomolecular protein adsorption onto TTSL when heated in the presence of unbound proteins leading to reducing in drug release. In summary this study showed that designing TSL for thermal triggered release cannot be predicted based on chemical composition and *in vitro* release studies only, but the environment of drug release should be taken into account.

KINETICS OF FUNCTIONALISED CARBON NANOTUBE DISTRIBUTION IN MOUSE BRAIN AFTER SYSTEMIC INJECTION: SPATIAL TO ULTRA-STRUCTURAL ANALYSES

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Earlier studies proved the success of using chemically functionalised multi-walled carbon nanotubes (f-MWNTs) as nanocarriers to the brain. Little insight into the kinetics of brain distribution of f-MWNTs *in vivo* has been reported. This study employed a wide range of qualitative and quantitative techniques with the aim of shedding the light on f-MWNT's brain distribution following intravenous injection. γ-Scintigraphy quantified the uptake of studied

radiolabelled f-MWNT in the whole brain parenchyma and capillaries while 3D-single photon emission computed tomography/computed tomography imaging and autoradiography illustrated spatial distribution within various brain regions. Raman and multiphoton luminescence together with transmission electron microscopy confirmed the presence of intact f-MWNT in mouse brain, in a label-free manner. The results evidenced the presence of f-MWNT in mice brain parenchyma, in addition to brain endothelium. Such information on rate and extent of regional and cellular brain distribution are needed before further implementation into neurological therapeutics can be made.

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MAGNETIC PARTICLE IMAGING (MPI) – AN INNOVATIVE IMAGING TECHNIQUE USING SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES (SPION)

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INTRODUCTION:

Superparamagmetic Iron Oxide Particles (SPIONs) offer a great potential for many different biomedical applications, like *in vitro* and *in vivo* diagnostics (imaging) and drug delivery in different disease patterns like infections, arteriosclerosis and cancer. Due to their big surface SPIONS can bind and deliver drugs in high amounts. In addition to that, targeting of the diseased area can be achieved by either secondary surface modifications capable of recognizing molecular target structures, e.g. on cancer cells, or by magnetic fields, because of their magnetic properties. But SPIONs can also be used for imaging in Magnetic Resonance Imaging (MRI) (Fig. 1) and are the tracers for the new imaging technology of magnetic particle imaging (MPI). Taking all these properties together, SPIONs can be seen as a unique platform for theranostic applications. Here, the great advantage of Magnetic Particle Imaging is that SPIONs can be imaged and quantified. Therefore, with MPI it could be possible to estimate the drug load in the diseased area after the application by measuring the content of the SPIONs used for delivering the drug.

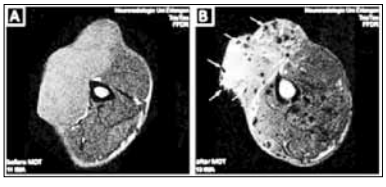

Figure 1: MRI imaging of SPIONs in a VX2-rabbit tumor. MRI-imaging before (A) and after (B) the accumulation of SPIONs in a tumor by Magnetic Drug Targeting. The signal extinction due to SPIONs is marked by green arrows.

The aim of the Section of Experimental Oncology and Nanomedicine (SEON) is to utilize SPIONs for the treatment of cancer and arteriosclerosis by MDT. Therefore, SEON over a period of several years developed SPIONs optimized for the purpose of magnetic drug delivery. These particles are very stable in human and animal blood, can carry a more than sufficient drug load[1], be accumulated in a target area by magnetic fields and first results show, that these particles even can be heated after magnetic accumulation in tumors. These SPIONs were also suitable for MRI-imaging and the aim of this preliminary study was to investigate the potential of these multicore nanoparticles for Magnetic Particle Imaging.

MATERIALS AND METHODS:

SEONLA-BSA-nanoparticles were synthesized according to[1]. In short, the nanoparticles were synthesized by coprecipitation of iron salts and stabilized by a dual coating with lauric acid and bovine serum albumin. Subsequently, a dilution series was measured with Magnetic Particle Spectroscopy (MPS) at Universitätsklinikum Hamburg-Eppendorf and after that a sample of (2 mm * 2mm * 1mm) was measured with MPI.

RESULTS:

It could be shown that the MPS-spectrum of the SEONLA-BSA-multicore nanoparticles in comparison to the MPS-signal of Resovist® was weaker at higher frequencies but comparable at frequencies below 100kHz (Fig. 2). Nevertheless, it was possible to visualize the particles with Magnetic Particle Imaging in a 20 µl sample with the dimensions of (2 mm * 2mm * 1mm) (Fig. 2)

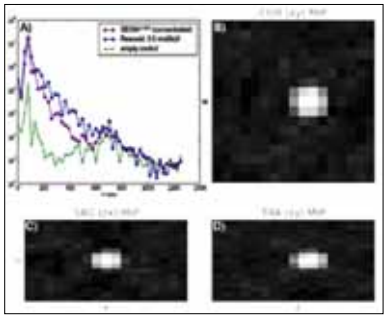

Figure 2: A) Comparison of the MPS signal of SEONLA-^{BSA}-nanoparticles and Resovist® at different frequencies. B) – D) MPI-signal of a point sample of SEONLA-^{BSA}-nanoparticles

CONCLUSION:

The experiments showed that the MPS signal of SEONLA-BSA-nanoparticles is comparable to that of Resovist® at frequencies below 100 kHz, although sample were taken for the tests, that had an approximate iron concentration of 50% compared to the concentration of Resovist®. The first MPI-measurements also showed that with this concentration a MPI-signal can be generated.
Further experiments now have to elucidate, if this signal is enough to measure the distribution of these nanoparticles in a tumor after their accumulation by MDT[2].

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NANOMEDICINE: BALANCING RISK OF TRIAL PARTICIPATION AND RELEVANCE OF THE NEW TREATMENT FOR PATIENTS – THE CLINICAL RESEARCHER`S VOICE

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Results from clinical trials help to answer questions and provide guidance for practicing health care professionals. All of these trials have their own benefits and risks, depending on the type of trial and their particular focus. The spectrum of these characteristics range from the advantageous perspective, that the attendance may help the participant in presence and others to get a better treatment for their disease in the future and allow researchers to learn more about how diseases can be prevented, identified or managed. Furthermore there is the potential that the treatment being studied is more effective than the standard treatment resulting to be among the first to benefit. Possible risks in joining clinical trials may include unfavorable or severe side effects, no benefit comparing to standard treatments and time consuming, additional visits at the study institution. The most challenging part for the clinical researcher performing a trial is to define respective patients. Therefore detailed knowledge about the severity of the patient`s disease is obligatory for everyone involved in the potential new (e.g. nanomedicine) driven therapy and intensive and transparent discussions between physicians/researcher and the patient is essential. Detailed information about the pros and cons of the new treatment modality has to be given and compared to standard treatment options. Benefitial may be the inclusion of patient`s confidants in this decision process, if possible. Taken together, clinical trials are of outstanding importance to promote medical achievement potential for improvement of the patient`s care. This should be performed in a climate of confidence and transparency and sponsored by independent funding sources.

STRUCTURAL FINDINGS, AT NANOSCOPIC SCALE, IN RECTAL MUCOSA OF PATIENTS WITH HIV/AIDS AND ANORECTAL PHATOLOGIES.

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INTRODUCTION

Gastrointestinal pathologies/disorders (GI) are common during the course of HIV infection. 35% of patients have GI symptoms as clinical presentation of HIV. These are usually very varied and include weight loss, dysphagia, anorexia, gastric disorders and diarrhea, the latter being one of the most common symptoms occurring in 30% to 50% of patients in North America and Europe. Affectation that produces HIV along the digestive tract may compromise the mouth, esophagus, stomach, small intestine, colon and terminal portions as anus and rectum[1,2,3,4,5]. Among the most frequent anorectal diseases the following have been reported: condyloma acuminata (human papilloma virus [HPV]), fistulas, perianal abscesses, hemorrhoids, fissures, herpes perianal, nonspecific proctitis, rectal masses (polyps, diverticula, Kaposi's sarcoma). Opportunistic and neoplastic anal and rectal infections have also been described in these patients, with a variety of symptoms that can range from anorectal pain, tenesmus and discharge from the rectum[6,7,8].
The intestinal mucosa (IM) plays an important role in the pathogenesis as well as a persistent HIV area despite the fact that Highly Active Antiretroviral Therapy (HAART) is effective[9,10]. The rectal mucosa (RM) is the main gateway into groups of men who have sex with men (MSM). The virus can penetrate by direct trauma of the RM, after which it has access to the microcirculation, however, even without existing trauma, HIV can penetrate the RM by viral absorption through specialized epithelial cells -called M- present in the epithelium associated follicles[10], by transcytosis across epithelial cells or by direct sampling of dendritic cells from the lamina propia. HIV temporarily may open tight junctions between epithelial cells generating a gradient that drives viral migration of dendritic cells through R5 receptor[11]. In addition the IM contains a high percentage of cells that are the main target of HIV and represents the ideal site of viral replication and depletion of CD4 + cells[12,13]. Previous research has shown that viral replication remains in lymphoid tissue for at least two years despite the fact that HAART has achieved complete suppression of viral load in peripheral blood. This also demonstrates incomplete suppression of viral replication as well as the increased activation of the immune system and persistent intestinal inflammation at the level of the IM9,10,14. It is relevant to notice an important finding which is the existence of efflux pumps that are expressed in the intestinal epithelium called P glycoprotein-1 (P-gP), which eject different substances outside the cells and carrying various substrates across the cell membrane including antiretroviral drugs as protease inhibitors (PI) and non-nucleoside inhibitors of reverse transcriptase (NNIRT). Increased expression of intestinal P-gp may reduce the absorption of drugs that are substrates of P-gp and may result in reduced bioavailability and subtherapeutic plasma levels[15].
Electron microscopy studies have detected the presence of the virus and changes in the lymphoid composition in IM with depletion of T lymphocytes[11,16]. GI disorders caused by HIV are due in part to the presence of opportunistic pathogens or hidden enteric infections, or due to indirect effects of HIV on the intestinal epithelium or due to indirect secondary effects of immunological abnormalities in the absence of enteric pathogens unidentifiable (eg : lysing infected cells expressing viral proteins on their surface); as well as injury or damage of the intestinal mucosa by direct cytopathic effect[17,18,19].
Studies in patients with HIV co-infection with HPV showed a high prevalence of HPV anorectal infection and Squamous Intraepithelial Lesion (SIL) in MSM ; the incidence of SIL of high degree (precancerous lesions) was higher in patients receiving HAART. It is believed that this is due to increased expression of HPV secondary to the interaction between the TAT gene of HIV-1 and the p97 protein which prevents the repression of gene E2 of HPV. SIL progression to invasive cancer requires several years , due to prolonging survival with HAART, paradoxically involved in the increased risk of anal cancer[21,22,23].

OBJECTIVE

To determine the ultrastructural pathological findings, at nanoscopic scale, of rectal mucosa of HIV/AIDS patients with anorectal disease.

MATERIALS AND METHODS

A prospective, descriptive study was performed of a selected sample of cases in which 5 patients between 18 and 51 years were included with anorectal disease (4 patients with HIV co-infection with HPV and 1 HIV-negative patient with HPV infection (control Patient). They were referred to the consultation of the ColoProctology Unit of the University Hospital of Caracas that were scheduled in surgical plans for performing rectoscopy and biopsy (Bx), on an outpatient basis, the first two weeks of March 2016 . After signing an informed consent and after prepositioning of rectal enema (Fleet enema ®) by the patient they underwent individually Bx of RM. Prior to the procedure the patient was placed in knee-chest position for anal inspection and then cleaning the area with soap solution of betadine. Then sample collection of RM was performed through rectal anoscope, with a special Bx clip, after placement of a local anesthetic (Lidocaine 2%). All patients were instructed to get antibiotic prophylaxis before the study (oral Metronidazole and ciprofloxacin). RM samples were properly identified in each pa-

tient, they were placed in a sterile plastic bottle with 1 mL of 2% gluraraldehído for subsequent histological processing. Later samples were immediately transported for routine processing of fine cut for Transmission Electron Microscopy (TEM). They were fixed with Karnovsky solution with Millonig phosphate buffer (pH 7.4 and 320 mOsm) and post-fixed with OsO4 under the same conditions of pH and osmolarity. After dehydration was applied in increasing concentrations of ethanol and inclusion in epoxy resin. The fine cuts or thin sections (60-90 nm) were obtained with a Porter-Blum MT2-B ultramicrotome. Grids were examined in a Transmission Electron Microscope JEM-1011 (80 kV).

ETHICS STATEMENT

The use of rectal mucosa of HIV/AIDS patients was approved by the Ethical Committee of the University Hospital of Caracas. A written consent was obtained from patients.

RESULTS:

In process…

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NANOMEDICINES TO ENABLE INNOVATIVE CANCER MEDICINES – AURORA KINASE INHIBITOR NANOPARTICLES IMPROVE THERAPEUTIC INDEX

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One of the major challenges to innovative medicines development remains a lack of therapeutic index[1]. Nanomedicines can enable new drug products by changing a candidate drug's distribution and increasing the drug concentration at tumour sites relative to healthy tissue. This biodistribution change in combination with careful selection of drug release rate from a nanoparticle has the potential to improve both efficacy and safety, thus enabling promising treatments otherwise limited by narrow therapeutic index.

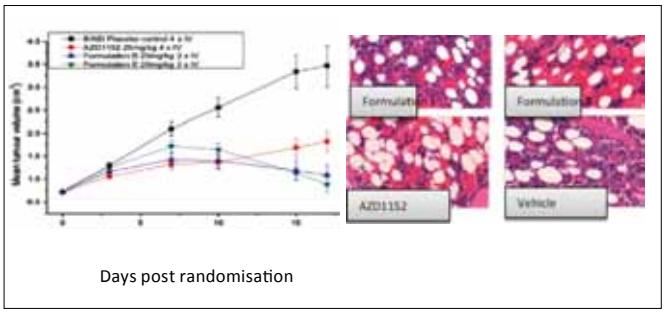
Aurora B kinase plays a pivotal role in cell cycle progression and inhibiting Aurora B kinase results in mitotic catastrophe and cellular apoptosis, however Aurora kinase inhibitors have shown disappointing single-agent activity to date at tolerable doses in the clinic, particularly in solid tumours[2] and challenges in combination therapy due to overlapping, enhanced, or unexpected toxicities. AZD2811 is a potent and specific small-molecule Aurora B kinase inhibitor. Its water-soluble dihydrogen phosphate prodrug, AZD1152, has been tested in clinical trials in various tumours, however despite showing significant improvement in complete response rate in acute myeloid leukaemia compared to standard of care in a randomised phase 2 trial[3], the requirement for continuous intravenous infusion in this setting and the toxicity profile in other indications limited broader application in the clinic.

This presentation will describe the development and characterisation of AZD2811 encapsulated into ACCURIN® polymeric nanoparticles, using an ion-pairing agent, to enable clinic development[4, 5]. ACCURINS® are composed of block copolymers of poly-D,L-lactide (PLA) and poly(ethylene glycol) (PEG). A range of AZD2811 ACCURIN® nanoparticles containing pharmaceutically acceptable organic acids as ion pairing agents were prepared. These displayed continuous drug release for more than 1 week *in vitro* and showed prolonged circulation times in rat pharmacokinetic studies and corresponding extended pharmacodynamic reduction of tumour phosphorylated histone H3 levels *in vivo* for up to 96 hours after a single administration. The efficacy and bone marrow tolerability of two AZD2811 ACCURIN® nanoparticles with different release rates were evaluated in nude rats bearing SW620 xenograft tumours. Both these ACCURIN® nanoparticles (formulations B and E) administered at half the dose intensity of AZD1152 inhibited tumour growth by 92 and 101%, respectively, and were more effective than

prodrug AZD1152, which inhibited tumour growth by 58% (Figure 1a). Hematoxylin and eosin (H&E) staining of bone marrow showed that AZD1152 and formulations B (but not formulation E) reversibly reduced bone marrow cellularity at day 5, and that formulation E had minimal effect on bone marrow (Figure 1b).

Figure 1: The effects of AZD1152 and ACCURINS® B & E on SW620 tumour growth and bone marrow.

a b



SW620 tumours were excised from rats treated with AZD1152 or ACCURIN® encapsulated AZD2811, and were analysed by imaging MS to characterize the intra-tumoural distribution of AZD2811. In animals treated with AZD1152 prodrug, AZD2811was detected in the tumour at 2 and 6 hours after dosing, but was undetectable at 24 hours however, in animals treated with nanoparticles AZD2811 was detected across the entire tumour cross-section for up to 6 days after the last nanoparticle administration.

The Target Product Profile defined during formulation development included sufficiently high drug loading to enable practical clinical dosing and a similar slow release profile to enable extended exposure and minimal effect on bone marrow. A number of hydrophobic ion pairing agents were investigated to increase drug loading and encapsulation efficiency so improving cost of goods. A pamoic acid lead formulation has been selected for clinical evaluation (ClinicalTrials.gov Identifier NCT 02579226).

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SELF-NANOEMULSIFYING DRUG DELIVERY SYSTEMS OF ARTEMETHER AND LUMEFANTRINE

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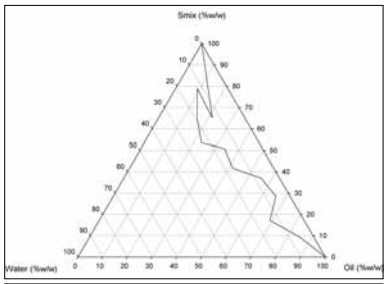

Fig. 1. Pseudoternary phase diagram

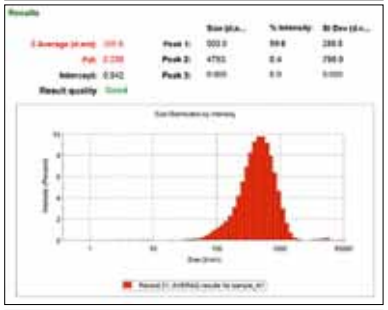

Fig. 2. Malvern particle size analysis result

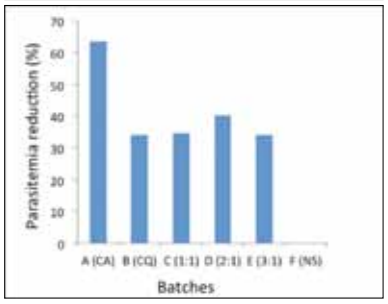

Fig. 3. Antimalarial activity (SNEDDS = C, D and E, 4/24 mg/kg; CA = Coartem®, 4/24 mg/kg; CQ = chloroquine, 10.5 mg/kg; NS = Normal saline)

This study was designed to develop and evaluate novel self-nanoemulsifying drug delivery systems (SNEDDS) containing artemether (ART)/lumefantrine (LFT) (ratio 1:6). The antimalarial activity of the SNEDDS containing the antimalarial drugs was compared with chloroquine and Coartem® using Peter's 4-day suppressive protocol in Plasmodium berghei infected mice. Solubility studies of ART and LFT guided the choice of the following excipients– oleic acid and Capmul® MCM (oils) and Tween® 80 and Cremophor® EL (surfactant/cosurfactant: S_{mix} - 1:1, 2:1, 3:1), which were used to develop pseudoternary phase diagrams (e.g. Fig. 1) using water titration method. The SNEDDS were characterized by measurement of pH, viscosity, emulsification time, robustness to dilution and drug content analysis in addition to thermodynamic studies. pH, viscosity and thermodynamically-stable and robust nanoemulsions formed within 2 minutes with drug contents greater than 78.9%, z-average particle sizes less than 550 nm (Fig. 2) and polydispersity indices ranging from 0.267 to 0.451. Antimalarial activity (Fig. 3) indicated that activity of the SNEDDS containing 2:1 Tween® 80/Cremophor® EL was higher than that produced by chloroquine but lower than that produced by Coartem®. This study points to the fact that SNEEDS is a promising delivery platform for these antimalarial drugs.

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HOW CAN STAKEHOLDERS ACCESS EUNCL?

SIMON BACONNIER

INTRODUCTION

The use of nanotechnologies in healthcare promises to tackle major medical challenges. However, the manufacturing process of nanomedicines is potentially complex and inconsistencies must be carefully assessed before clinical applications can be considered. Furthermore, safety concerns related to the use of nanomaterials must be addressed as early as possible during product development.

The European Nanomedicine Characterization laboratory (EU-NCL) will address these issues by providing the critical infrastructure and characterization services required to analyze physical and chemical attributes, *in vitro* biological properties, and *in vivo* characteristics of nanomedicines under development.

The EU-NCL services will be accessible to all organizations developing candidate nanomedicines, whatever the development maturity. Product developers will benefit from a detailed and confidential characterization data set that supports their decision making for further product development As the First Call for access campaign has been recently completed, we will present you the access procedures, what should be a successful application, the lessons learned and the evolution to be implemented in the future Call campaigns.

CHARACTERIZATION PLAN ACCESS PROCEDURES

The EU-NCL characterization process will be conducted by the Core Expert Team (CET), a group of nanoparticles and nanomedicine experts within top-level European institutions using high-end methods. The Core Expert Team (CET) will be in charge of validating the adequacy of the sponsor proposal, material and development strategy and the EU-CNL capacities and to define the most relevant characterization plan to be performed to provide the most reliable and relevant data-set for future development.

The access selection is organized in a two steps process:

A first submission step based on a light proposal intended to give EU-NCL external and internal reviewers an overview of the strategy, without requiring investigators to prepare costly, time-consuming proposals. This format will allow to briefly describe the background, the strategy or the concept of action of the innovative nanomedicine, a synthesis of the already accessible characterization data, a description of the innovation, the clinical impact and a scale-up compatibility description, *in vitro* and *in vivo* testing data.

In case of success at the first step the sponsor will be invited to submit a second step proposition. The level of details requested for the second step application is much higher and will be evaluated against more stringent criterion.

To fulfill its advisory role, the EU-NCL will feedback to the sponsors successful or not. The objective is to motivate and support the sponsor to submit again in case of failure.

The selection process should not exceed 75 days after which the material transfer should start in accordance with the characterization plan and agreed sample typology and quantity.

THE SUCCESSFUL APPLICATION PROFILE

There is no best sponsor profile, actually, any Med-NP provider, including Industry, academia or government agencies, can apply to the EU-NCL services. But there are best applications.

Actually, some critical information should be detailed in the submission process among which the Med-NPs initial characteristics including inherent toxicity, or previous physico-chemical characterization data.

In addition, the Med-NP manufacturing environment should be fully mastered by the sponsor. Actually, two independent product batches will be requested. The detailed production process description and the related scaling up strategy should be described

Finally, the application should include a demonstrated efficacy in a biological system as well as the strategy to transfer the technology to the clinical environment and an anticipated impact on clinical application.

Globally, the maturity of the product as well as the production management and the anticipation of the translation to the patients will be accurately assessed.

THE LESSONS LEARNED

After only one year of existence, EU-NCL, the integrated nanomedicine characterization laboratory gathering 6 European institutions' expertise and capacities, has launched its first call for access.

As opposed to the NCI-NCL, our US cousin expert nanocharacterization infrastructure, EU-NCL is distributed among Europe. This unique shared infrastructure offers large variety of characterization techniques and equipment within a very heterogeneous legal and administrative environment.

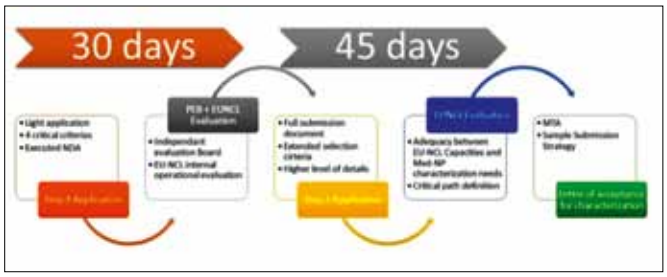
One of the first lessons learned has been to work under a consensual framework, implying that a harmonized legal and administrative process had to be developed for decision-making easiness.

Another lesson learned, was to integrate the extensive diversity in the type of material to be analyzed. Actually, considering that the Med-NPS to be characterized can be organic, inorganic, metallic, drug associated or loaded, with therapeutic or diagnostic objectives, targeting specific target, charged or not, the EU-NCL partners will have to be able to adapt and optimize the characterization plan strategy and capacities.

Finally, one of the most important lessons learned from our close collaboration with the NCI-NCL is the strong need from sponsors for quality data produced through standardized methodologies in accordance with established standards and controls. The very stringent analysis framework is the only way to provide top quality data to the future providers of patients' innovative treatments and diagnostic tools.

THE EVOLUTION TO BE IMPLEMENTED IN THE FUTURE CALL CAMPAIGNS.

Considering the strong awareness EU-NCL is raising, we will have to push forward the access strategy. Its is planed to make it more flexible and have open calls with bi-annual review sessions (April and October). The objective is to allow early stage product a better visibility to access EU-CNL characterization capacities and facilitate the interactions with potential sponsors within a continuous stream.



A POINT-OF-CARE DIAGNOSTIC PLATFORM FOR INFECTIOUS DISEASES (DISCOGNOSIS)

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Tropical infectious diseases are a major global health threat. According to the World Malaria Report of the World Health Organization (WHO) in 2013, there were still 207 million cases of malaria and 627,000 deaths reported globally. Among these, a percentage reaching 80% were reported in Africa. Yet, in tropical regions there are often diseases exhibiting the same symptoms as in malaria, i.e., acute fever, but originate from pathogens of different nature such as viruses and bacteria. It is of utmost importance to identify these pathogens in order to proceed to the proper treatment, however the pure clinical manifestation is not sufficient to achieve this. Moreover, even technologies that are considered as "gold standard" often fail to differentiate between parasites, viruses, or bacteria. Indicatively, microscopy is suitable mainly for malaria diagnosis; rapid diagnostic tests (RDTs) although cost-efficient, exhibit insufficient sensitivity and specificity. Bacterial culture needs up to 2-3 days for the results, which may result in severe complications or, even worse, death of the patient. Nucleic acid amplification technologies (e.g., PCR) are sensitive, on one hand, but require qualified personnel and well-equipped laboratories, which may be rare in resource-limited settings.

The DiscoGnosis project aims to fill in the existing gaps, as aforementioned, by developing a centrifugal microfluidic based diagnostic tool that integrates nucleic-acid amplification and multiple targets for rapid, reliable, and highly specific detection of the pathogenic agents at the point of care. Some of the key technological innovations are:

Centrifugal microfluidics: The core of the platform is the LabDisk, a plastic disposable cartridge that implements centrifugal and capillary forces in order to handle liquids (LabDisk, Fig.1). The LabDisk is handled by a corresponding LabDisk Player, which provides mechanical, thermal, and optical units for the disc processing, the heating, and the detection, respectively.

Modular platform for fully automated sample-to-answer analysis: The LabDisk consists of "unit operations", which are microfluidic modules performing dedicated functions (e.g., nucleic acid extraction and purification, aliquoting, reagent storage in dedicated pouches). These can be interfaced at will, in order to carry out a series of complex functions (that would otherwise be done on the bench) in a fully automated way.

Multiplexing: As mentioned, the overlap of clinical symptoms is a major difficulty in reliable diagnosis. Furthermore, missing to identify a pathogen may lead to serious mistakes in treatment. Finally, a big challenge emerges when a "standard" malaria test proves negative, but the patient still has fever. To tackle these, we have integrated a large panel of pathogens in the same cartridge, namely: malaria (pan-malaria, P. falciparum, P. vivax, P. ovale, P. malariae); Salmonella Typhi and Paratyphi; Streptococcus pneumoniae; Dengue virus (serotypes 1-4); and Chikungunya virus. In this way, co-infections can be easily identified in one shot, avoiding sequential tests.

Isothermal amplification: The loop-mediated isothermal amplification (LAMP) is used in this project. Being isothermal in nature, it overcomes the drawbacks of the traditional PCR, thereby offering fast results (assay times between 10 and 60 min, depending on the assay) with lower power consumption, which would allow a potential battery-based use.

Production technology: The fabrication technology is microthermoforming of polymer foils. It is an adaptation of "traditional" blister package technology, adapted to microscale features. It is scalable and allows batch manufacturing of the cartridges. Back-end processing includes the filling of the structured foil with the biochemical components (dried magnetic beads, primers), lyophilized amplification reagents, buffers in pouches, and eventually sealing, cutting and packaging.

The development steps have taken into account local particularities such as high temperature and humidity (stability tests have been performed) but considered also the general applicability of the system in developed countries. The system has been designed in such way as to rapidly respond to possible epidemics or urgent needs, due to its adaptable nature.

Fig.1. Foil-based disc-shaped microfluidic cartridge (LabDisk) and the corresponding LabDisk Player, integrating user-defined frequency and temperature protocols as well as an optical detection unit (fluorescence).

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NETWORKS, EMERGENCE, HIV, AND CANCER

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Motto: "The essence is simple, only the details are complicated.

The hierarchy of our knowledge is grounded in hearsay and accidental observations. Scientific data are usually acquired under controlled conditions, but we must be able to detect patterns and understand relations to transform raw data first to information then knowledge. Understanding the principles enables us to simplify complexity and predict behavior of systems.

Complex Systems may simply be modeled as nodes, connections, and flow between the nodes. Dynamics of such a system are determined by the actual topology and by the nature of flow (information exchange, supply, etc.) Connectivities may follow various patterns, which result in properties that all large complex networks share. Examples are genetic networks, human relations, transportation systems (road, air), the internet, etc. Identifying common properties of these networks helps us to understand their characteristic and shared features. For example, in a disease network, diseases are connected if they have a common genetic or functional origin. Understanding the functionally of relevant interactions in the human disease network leads to a better understanding of the pathophysiology of human diseases. Another example is the social network of human-to-human interactions, which plays a central role in the spread of communicable diseases. The notion of "emergence" is becoming increasingly important in a variety of disciplines, especially in cross-disciplinary investigations. Complex adaptive systems, punctuated equilibrium, and symmetry breaking are all examples of emergent phenomena. Simple interactions at one systemic level may produce new, and often surprising outcome at the next higher level when emergence sets in. Then, the result goes beyond the particulars of the individual subsystems, and the whole becomes more than simply the sum of its parts.

The human body is a unique example of different sub-systemic networks connected by emergence. Understanding this feature has numerous important consequences, which will be illustrated in this presentation looking at the challenges of a systemic disease, cancer. To successfully conquer cancer, we have to consider its behavior as part of a dynamic complex system and acknowledge the existence of systemic properties with all consequences in our approach.

NANO-MEDICINE: SMALL DOSE-BIG PROBLEM?

IAN BANKS

If only the ethical issues surrounding nano-medicine were as small as the vehicles. Informed consent is almost an oxymoron when it comes to cutting edge medical technologies. The gap between people knowledge' and 'expert knowledge' is continuing to widen despite all the advances of communication. Even health care professionals (HCPs) are struggling to maintain their insight and understanding, so not surprisingly, people/patients simply cannot keep up with the galloping strides of medical advance.

Involving 'the patient voice' has become a mantra 'nothing about us without us'. An early input from patient advocates organisations (PAs) makes sense if you don't want to waste an awful lot of money and time not to mention endangering patient safety. Yet there is less than 6% genuine involvement of this resource for the initial stages of research and clinical trials.

Is it 'ethical' to deliver treatments which have been developed without such considerations? More to the point is it 'ethical' to expect people/patients to accept such treatments without sufficient knowledge and insight which could be provided by such collaboration? The Patient Voice' is not just a 'small tick box when it comes to ethical practice, it is a major essential prerequisite.

SELECTIVITY OF DRUG RELEASE RATE ON NANO-LIPOSOME BASED NANO-DRUGS: ONE OF THE KEYS TO DETERMINE NANO-DRUGS THERAPEUTIC INDEX

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It is clear that nano-drugs such as Doxil® are superior to the same APIs used in the conventional way. However it is also clear that nano-drugs and nano-medicine did not meet the high expectations as a revolution in anticancer therapy. The current nano-drugs' prolonged pharmacokinetics superiority of the nano-drug over the API administered conventionally together with the nano-size allow for nano-drug to take advantage on the tumor unique EPR effect. These resulting in passive targeting to tumors, which does not occur for the API administered in the conventional way. (EPR) effect was firstly demonstrated in cancer patients in first Doxil clinical trial performed by us in Jerusalem (1991-1993, Gabizon and Barenholz.) EPR effect has a large impact on tumor physics. It can be used for therapy as the tumors' "Achilles' heel". However today it has become clear that many aspects relating between therapeutic efficacy and the EPR effect remain controversial despite the extensive relevant research done so far.The numerous studies with various nano-drugs during the last 25 years have clearly demonstrated that even though this passive targeting reduces, some-times even dramatically, toxicity and side effects, this is not sufficient to provide a quantum leap in the improvement of the therapeutic efficacy. While delivering of API as nano-drugs improves their bio-distribution, which results in passive targeting to tumors due to the EPR effect, the tumor high interstitial pressure (IFT), prevent the diffusion of APIs and of the nano-drugs within the tumors, while the chaotic extracellular matrix and the suppressed convection due to low or almost no blood and lymphatic flows further reduces the intra-tumor distribution of the nano-drugs and its API. In addition poor rate of API release reduces availability of the API to the tumor cells. More advanced systems are therefore required to meet the high expectation of the performance of nano-drugs. Our current research is aimed to fill this gap. My presentation will focus on pegylated liposomal doxorubicin (PLD) including Doxil the first nano-drug Doxil® (we developed) that was approved by the FDA on 1995 and is in broad clinical use world-wide since then (more than 600,000 patients were treated so far)

The above described knowledge and our own extensive experience in this field has led us to hypothesize that by improving the cross talk between the unique tumor microenvironment, interstitial fluid and metabolome on one hand and of the nano-drug physico-chemical features, we can improve the therapeutic efficacy of anti-tumor liposome-based nano-drugs. We further hypothesize that radiotherapy (RT), radiofrequency (RF), or high intensity focused ultrasound (HIFU) can effectively re-modulate the tumor microenvironment to enhance nano-drug accumulation and drug release and work in synergy with nano-drug delivered chemotherapeutics. We will demonstrate how unique tumor metabolites (especially ammonia) in the tumor interstitial fluid induce doxorubicin controlled (non-burst) release at the tumor "as is" allowing for the selective doxorubicin release in the tumor. Also how modifying and re-modulating tumor microenvironment by exposing it to radiofrequency (RF)is improving Doxil therapeutic. We will show that slow doxorubicin release is therapeutically advantageous over very fast drug release.

EDUNANO: EDUCATION, TRAINING AND CUTTING EDGE KNOWLEDGE SHARING ON NANO TECHNOLOGIES FROM HIGH SCHOOL THROUGH HIGHER EDUCATION AND ON TO INDUSTRY

JACK BAROKAS

The EDUNANO project aim is to adapt and modernize existing Israeli curricula in Nanotechnologies; to develop new certified courses, to test innovated curricula and to disseminate the results. With this it fits into the development strategies of the country in both curriculum modernization and high-tech production stimulation.

The key to development of nanotechnology-based industry in Israel is promotion of academia–industry collaboration.

In the framework of the project partners will develop and test a complete responsive eco system in which the changes in the needs of the industry to employees with new competencies and skills will be monitored and relayed to academy and later on the high schools hence reducing the system respond time to emerging technologies. That is why a new and expended syllabus of studies at the universities was established with emphasis on gaining knowledge on broader range of areas that can complement and strengthen the nanotechnology now and in the future and lead to the continues growth and expansion of the nanotechnology in Israel.

This project focus is on common courses development for the new skills needed for the new jobs in the multidisciplinary nanotechnologies. As a complement to Israeli development strategies, the needs in European level are best identified in[2]: "In knowledge-intensive and growing sectors such as nanotechnology, there will be even greater demand for scientists skilled in more than just one area of research. " and "The studies in this area (nanoelectronics) point to the urgent need to further develop scientific education and training with a particular stress on interdisciplinary."[3] And the conclusions of DG EMPL project: "For some job functions special courses are needed. It is necessary to strike a balance between what is offered in the educational system and what is needed in the sector."

There are few individual research teams, laboratories or companies that can reasonably claim to be able to respond to the technological challenges. Even the big companies in the sector work with a common use of R&D resources (as Motorola & ST Microelectronics etc.). No one university can afford the necessary infrastructure, clean rooms, technology and experts in all fields of the multidisciplinary nanotechnology.

This project aims at transferring knowledge between EU higher education institutions and institutions in Israel and between Israeli institutions to modernize university curricula in nanotechnologies. The project builds upon the results of three previous Erasmus and Leonardo da Vinci projects in the framework of the EU's LLL program: NanoTrain, NanoSkills and NanoEI.

The planned curricular reform will focus on content, structure, teaching methods and the use of new teaching materials with regard to the European modernization agenda for higher education. Newly developed courses will be structured according to the three cycle system. Recognition arrangements between higher education institutions in the EU and in Israel will be established.
There is research and development in nanotechnologies in pharmacology, medicine, electronics, chemistry, and physics. So, not all courses will be implemented in each university but only those corresponding to the scientific area of the corresponding curriculum.
The teaching of new and updated courses will start during the life time of the project with at least 200 students and retrained teachers. Part of the second and the whole third project year are devoted to exploitation of courses.
The primary target groups are: university students and teachers of VET schools, professionals from SMEs in nanotechnologies.
This project will have an impact on the participating Israeli institutions with regard to the priorities of the country.
- new opportunities for cooperation between universities and enterprises, high schools and VET institutions in the sharing of knowledge and educational resources - a distributed support system in Israel and three European countries;
- up-to-date courses in the most rapidly developing sciences available for university students in a job-linked training environment will prepare better the future specialists for their job;
- Satisfied training needs of the staff in SMEs in the sectors of nanotechnologies with accessible on-line and on-the-job training courses.

[1]www.nanoisrael.org

[2]Communication from the Commission to the European parliament. An Agenda for new skills and jobs: A European contribution towards full employment, Strasbourg, 23.11.2010, COM(2010)

[3]Investing in the Future of Jobs and Skills. Scenarios, implications and options in anticipation of future skills and knowledge needs for the Computer, Electronic and Optical Products Sector. Policy Summary, DG EMPL report VC/2007/0866, Lot 7, 05.2009shown to promote the invasiveness of many solid tumors, including glioblastoma. A preclinical study showed that treatment with

TISSUE DERIVED NEO-ANTIGENS FOR T CELL-BASED CANCER IMMUNOTHERAPY

MICHAL BASSANI-STERNBERG

Cancer immunotherapy amplifies or reprograms the inherent capacity of immune cells to recognize molecular entities expressed specifically on tumor cells and to eliminate them. Tumor associated antigens that are presented as human leukocyte antigens (HLA) binding peptides on the surface of cells, namely the immunopeptidome, serve as the leading targets and they may be derived from tumor-associated (over-)expressed self-proteins, from oncogenic viruses, endogenous retroviral elements, or mutated tumor proteins. Recent data show that activation of the immune system by immune checkpoint blocking therapies leads to tumor rejection and that recognition of mutated antigens, known as 'neo-epitopes' plays a key role. So far, discovery of neo-epitopes relies mainly on prediction-based interrogation of the 'mutanome'. Tumor associated antigens have been regularly discovered in the last two decades by mass spectrometry (MS) based immunopeptidomics. However, it has been questioned whether MS would be sensitive enough to detect neo-epitopes, especially as a discovery approach.
With the aim to identify the in-vivo presented neo-epitopes from human melanoma tumors, we applied our recently developed in-depth and streamlined MS-based immunopeptidomics approach. We extracted HLA-I and HLA-II peptidomes from melanoma primary tissues, separated the peptides by a nanoflow HPLC and sprayed them directly into a Q Exactive HF mass spectrometer. We developed a new module in the MaxQuant computational environment that integrates next generation sequencing data and generates customized patients' specific reference databases that contain nonsynonymous somatic mutations for the direct identification of neo-epitopes.
We accurately identified the most comprehensive melanoma immunopeptidome, comprising almost 100,000 unique peptide ligands among them more than 300 known and novel peptides derived from known melanocyte-associated differentiation and cancer testis antigens. Using our discovery approach we identifed 11 neo-antigens from three patients. Four of eleven mutated ligands proved to be immunogenic by antigen-specific T-cell responses, hence are confirmed neo-epitopes. Moreover, tumor-reactive T-cells with specificity for two of the neo-epitopes identified by MS were detected in the patient`s peripheral blood and among the tumor infiltrating T cells.
Taken together, we established a comprehensive resource of the melanoma in-vivo immunopeptidome with a high number of attractive novel targets. Most importantly, we showed the feasibility of identifying the in-vivo and immunogenic neo-epitopes, directly from tumor tissues. This is a promising approach for the identification of new targets for the development of immunotherapies.

"SPIEGELMERS®" – THE ADOLESCENCE OF A NOVEL COMPOUND CLASS

MATTHIAS BAUMANN, CMO, NOXXON Pharma AG, Berlin

Pre-clinical and clinical development of three Spiegelmer drug candidates targeting

- SDF-1/CXCL12 in hematological and solid tumors (Olaptesed pegol/NOX-A12)
- MCP-1/CCL2 in diabetic nephropathy (Emapticap pegol/NOX-E36)
- Hepcidin in anemia of chronic disease (Lexaptepid pegol/NOX-H94)

SPIEGELMERS®

NOXXON focuses on the discovery and development of a new proprietary class of drugs called "Spiegelmers" which combine the benefits of biological drugs and small chemical molecules, namely the affinity and specificity of biologicals, with the ease of chemical synthesis of small molecules. Spiegelmers are chemically synthesized and, like antibodies, act by binding and neutralizing their targets, which are usually small extracellular proteins, by virtue of their distinct shape and physical and chemical properties. Spiegelmers are a variant of a drug class called oligonucleotide aptamers. Whereas aptamers are mainly built from the building blocks that occur naturally in RNA and DNA (D-stereoisomers), Spiegelmers are oligonucleotides that are built on a backbone of mirror-image RNA or DNA (L-stereoisomers). By leveraging this "mirror-image chemistry", Spiegelmers aim to solve two key problems that have limited the development of aptamers made with building blocks from natural D-stereoisomers: Spiegelmers have enhanced biological stability and are immunologically passive. Because they are mirror images, Spiegelmers are not recognized as RNA or DNA by enzymes found throughout the body called nucleases, and as such are not as easily degraded in the blood. For similar reasons, the components of the immune system that normally react to foreign RNA or DNA do not recognize Spiegelmers and as such do not activate the immune system in response to their administration.

NOXXON has brought three Spiegelmer drug candidates to clinical phase 2 stage:

NOX-A12/OLAPTESED PEGOL TARGETING SDF-1/CXCL12 IN HEMATOLOGICAL AND SOLID TUMORS

CXCL12 is a major retention factor for hematopoietic cells in the bone marrow. NOX-A12 blocks and detaches CXCL12 from the surface of bone marrow stromal cells, thereby neutralizing the local chemokine gradient in a dual way. As a result, NOX-A12 mobilizes hematopoietic stem cells as well as white blood cells from the bone

marrow into the periphery. This was demonstrated in mice as well as in a proof-of-mechanism Phase 1 study in healthy volunteers. The complex interactions between hematopoietic cells and the local bone marrow microenvironment support growth, development and function, and this can also be attributed to cancer cells. By modulating the microenvironment of the tumor in the bone marrow niche through inhibition of CXCL12 with NOX-A12, tumor cells can be mobilized to peripheral blood where they can be targeted more effectively by chemotherapy and biologics. In this context, NOX-A12 can be differentiated from CXCR4-blocking agents because it neutralizes CXCL12 action on its two receptors: CXCR4 and CXCR7, the latter being responsible for growth, migration and metastasis in some types of cancer. Functional proof for effects on cancer cell distribution has been obtained by inhibiting homing of human chronic lymphocytic leukemia cells to bone marrow in mice. Further preclinical data also showed that NOX-A12 in combination with the proteasome inhibitor bortezomib significantly reduced tumor load of human multiple myeloma cells in mice. Additionally, homing of a multiple myeloma cell line to bone marrow niches in mouse was reduced in the presence of NOX-A12. CXCL12 has been shown to promote the invasiveness of many solid tumors, including glioblastoma. A preclinical study showed that treatment with NOX-A12 in combination with radiation, leads to tumor shrinkage

LEARNING OUT OF THE FAILURES OF THE PAST – A NEW STRATEGY FOR TREATING CANCER

SHMUEL (MULI) BEN-SASSON

Cancer research is undergoing groundbreaking transformation in recent years. This revolution is recognized by the code name TCGA (The Cancer Genome Atlas/International Cancer Genome Consortium) and set new standards and new dimensions. It replaces fragmented information coming from individual labs, each working on selective signaling pathways, in a limited number of tumors or cancer cell-lines. Instead, a concerted international effort leads to the industrialization of cancer genome profiling, on unprecedented scale and in an unbiased manner. At present, tumor samples were collected from around 11,000 patients, across 33 tumor types. Each sample was comprehensively dissected regarding DNA mutations, mRNA and miRNA expression, DNA copy number, DNA methylation, protein expression and more. In short, by now, we intimately recognize the majority of molecular tricks played by cancer genomics.

The assumption behind the TCGA project was that it will enable the distillation of a short list of therapeutically manageable thematic pathways which govern multiple malignancies. The result, however, was surprising to an extent that requires a paradigm shift. Highlighting just a few: (i) there are around 500 driver mutations and the majority of them are not drugable (e.g. tumor suppressor genes). (ii) The involved driver genes are scattered among all major cellular pathways and activities. (iii) Samples obtained from different regions of the same tumor, display different sets of driver mutations and other genomic changes. (iv) This heterogeneity persists also among different metastasis in the same patient and, of course, malignant tumors of the same tissue are completely different from one patient to the other, in the composition of their genomic alterations. In other words, in the majority of cases, cancer cells are “moving targets” due to their continuously changing genomic makeup.

Due to TCGA we have for the first time such a clear and encompassing overview and far reaching conclusions should be drawn from it, regarding cancer treatment. Mainly, that in the majority of cases, even if we identify a dominating drugable mutation in tumors of a particular lineage (e.g. distinct bRaf mutation in melanoma), we can expect a regression or delay in tumor growth only for several months and never expect a cure.

The common denominator of classical chemotherapy and of newly developed targeted therapy (=cancer personalized medicine) is that they both adopt a direct strategy; namely, attack the tumor genome or its products. However, in light of the above, an alternative approach should be considered: i.e. instead of direct, “precise” attack of cancer cells, use an indirect strategy that bypass tumors and their roulette-like genome, altogether. There are three therapeutic avenues that follow such indirect strategy: (1) anti-angiogenic therapy; (2) immunotherapy related to tumor microenvironment and (3) cancer-epigenome targeting. The following elaborates on the utility of this third option.

Common to almost all tumors, regardless of their tissue of origin, is an epigenetic chaos.

It can be manifested by changes in DNA methylation, histone modification, nucleosome composition etc. Moreover, as the importance of chromatin 3D organization in the control of gene expression is becoming apparent, changes in DNA copy number and aneuploidy, which are very common in cancer cells, should be considered also as part of the epigenetic chaos. It was shown that such epigenetic changes occur very early during the course of malignant transformation and might even constitute a founding event.

On the other hand, normal cells have stable epigenome and diploid karyotype, making them a distinct entity, compared to cancer cells. In other words, while the introduction of further epigenetic instability could collapse tumor cells, normal cells will be refractory to it. From a chemical point of view, the toolbox of epigenetics is quite simple: amino acids acetylation, methylation or phosphorylation, and DNA methylation. Therefore, a careful manipulation of the epigenome by small-molecules is feasible; various labs around the world, including ours, are currently working on it.

It should be noted that cytotoxic agents, by virtue of their mechanism of action, causes also an increase in cell epigenetic chaos. Indeed, such agents lead to cancer cell apoptosis, despite of their indiscriminate mechanism of action. Thus, the introduction of transient epigenetic disturbance, which is well-tuned, could cause selective apoptosis of cancer cells while sparing normal cells which able to recover from it. This novel cancer treatment can be done under conditions much milder than conventional chemotherapy.

SMART AND LIVING IMPLANT EQUIPPED WITH ACTIVE THERAPEUTICS AND STEM CELLS FOR REGENERATIVE NANOMEDICINE

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Recently, We have reported an active nanostructured collagen implant reinforced with human stem cells for bone regeneration. In our group, we have reported a “Smart Hybrid Materials Equipped with Nanoreservoirs of Therapeutics and stem cells “. This unique nanotechnology strategy is used to entrap, protect, and stabilize therapeutic agents into polymer coatings acting as nanoreservoirs enrobing nanofibers of implantable membranes. Upon contact with cells, therapeutic agents become available through enzymatic degradation of the nanoreservoirs. As cells grow, divide, and infiltrate deeper into the porous membrane, they trigger slow and progressive release of therapeutic agents that, in turn, stimulate further cell proliferation. This constitutes the first instance of a smart living nanostructured hybrid membrane for regenerative medicine. The cell contact-dependent bioerodable nanoreservoirs described here will permit sustained release of drugs, genes, growth factors, etc., opening a general route to the design of sophisticated cell-therapy implants capable of robust and durable regeneration of a broad variety of tissues.

CLINICAL TRIAL: PHASE 1/2 HORIZON 2020, (FR, UK, SP, SW):

Feasibility, safety /efficacy assessment of a therapeutic implant based on a bioactive collagen membrane and autologous mesenchymal stem cells derived from bone marrow for the treatment of femoral cartilage isolated lesions

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NANOMEDICINE IN BRAINDISEASE

New Strategies using Local Brain-nano-intervention at the interface between Nano-science and Neurosciences-

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Targeted therapies and so-called precision medicine have been highly successful in oncology. However strong limitations are now emerging by the induction of molecular resistances, side effects related to systemic diffusion, tumor heterogeneity and the need for new peritumoral microenvironment targets. These bottlenecks are major in the brain tumor field were targeted therapies remains desperately inefficient in human patients contrasting by the cure published in mice models. Similarly, neurodegenerative disease as well as brain-post traumatic handicap did not benefit at all from these progress.

Lack of adequate technology to explore these inaccessible territories and to modulate the correlated targets with high precision and safely inside the brain circuitry is a medical priority. Deciphering the mechanisms of inaccessible Cancer/brain pathological areas and treating them locally with nonlesional technologies is mandatory. Beside drugs, neurostimulation paved the way toward alternative modalities to modulate and treat human brain diseases using a non-destructive physical intervention in the brain. It paved the way for more elaborated devices benefiting from the miniaturization provided by the exponential development of micro-nano-technologies and electronics.

The availability of a non-lesional micro-nano-invasive strategy to annotate and monitor tissue response to therapy is a crucial issue. In the medico-biological state of the art, this is performed by invasive lesional biopsies. In this context, the development of micro/nano-tools devoted for intra-tissular characterization is indispensable to annotate these inaccessible territories. We introduced several micro-nano "BioImprint" devices devices, which should provide a micro-nano-invasive non lesional access to the brain.

We optimized the tool by the addition of specific chemical and micro/nano-structuration modifications. Integration of a in situ intra-brain confocal strategy associated to the bioharvesting device defines a new opto-biomolecular device that will be crucial to explore the brain microenvironment in conjunction with the development of clinical molecular optical dyes. At the end, a local delivery strategy has been developed, providing a multimodal opportunity to modify the local microenvironment using drug or local physical modulation using polymers as well as brain-computer interfaces. Again, nano-devices provide an innovative strategy to make them more biocompatible and integrated inside the brain circuitry. A new precision medicine is emerging associating nano-electronics to theranostic brain-technology interfaces. All these technologies strongly question major ethical issues including their non-medical us in the transhumanist humanist area, that should be strongly prohibited.

CLINICAL OUTCOME AND REGULATORY APPROACHES FOR NANOMEDICINE AND ADVANCED DRUG DELIVERY PRODUCTS

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President, EUFEPS European Federation for Pharmaceutical Sciences, Stockholm, Sweden

Nanomedicines have emerged since 1990s as innovative drug delivery systems benefiting from targeting of cancer tissues as a result of leaky vasculature that is typical of cancer tissues. As nanoparticles tend to accumulate at tumor rather than healthy tissues, it is possible to avoid toxicity of conventional chemotherapeutics and to obtain similar efficacy with significantly lower systemic drug dose. Nanomedicines can be defined within a range of different dosage forms including nanoconjugates, gold nanoparticles, nanocrystals, nanoemulsions, fullerenes, nanotubes, polymeric nanoparticles, modified nanoparticles. These nanomedicines can also be actively targeted to tumor cell surface receptors such as folate, integrin or transferrin as well as to blood vessels feeding the tumor for inhibiting angiogenesis.

As of 2015, a total of 52 nanomedicines are on the pharmaceutical market for therapy and even more are in the pipeline for diagnostic purposes.

Regulatory approaches towards nanomedicines and their follow-on products referred to as nanosimilars are based on totality of evidence. This is a stepwise approach comprising full physicochemical characterization of the test and reference products, non-clinical and clinical studies to demonstrate the bioequivalence of the generic nanomedicine to the originator in terms of pharmacokinetic and pharmacodynamics parameters. FDA seems to have a more adaptive and generalized approach for the regulation of nanosimilars and EMA has established product-specific guidelines for nanomedicines such as iron carbohydrate nanoparticles, glatiromoids and liposomes.

This lecture will cover the regulatory landscape regarding nanomedicines and nanosimilars summarizing the market status of nanomedicines and what is to be expected in the coming years for these innovative advanced drug delivery products. Case studies of design and development of nanomedicines will also be discussed in this frame based on projects our group carries on nanomedicines administered through oral, parenteral or mucosal routes.

TISSUE PHENOMICS – PROFILING THE LOCAL TUMOR ECOSYSTEM OF CANCER PATIENTS

GERD BINNIG

The Local Tumor Ecosystem (the heterogeneous tumor plus its heterogeneous micro-environment), LTE, is complex and different for different patients. For prognosis as well as for predicting the best treatment for individual patients the evaluation of the LTE turns out to be very powerful. In Tissue Phenomics this evaluation is represented by analyzing and quantifying structures of the LTE within tissue slides. In particular for immunotherapy the interactions of the tumor cells with the immune cells play an important role. Immunotherapy is an exciting and fast emerging field in oncology enabling highly effective treatments of cancer patients. Selecting patients, however, for a specific cancer therapy is not trivial today and will become increasingly more complex. The number of drugs will increase drastically mainly due to the large amount of potential combination therapies in this field and it has to be decided, which of those treatments is best for a specific patient at what stage of the patient's disease. Tissue Phenomics is based on digital pathology, image analysis and data mining, and is more and more evolving into a comprehensive big data approach. The benefit of this technique is most obvious for oncology and more specifically for immunotherapy. Quantified morphological structures combined with the local co-existence of certain cell types carry biological meaning. Profiling in a quantitative form the local tumor ecosystem in a big data approach leads to the discovery of novel histological phenotypes even if only relative small samples like needle biopsies are available.

THE EUROPEAN NANOMEDICINE CHARACTERIZATION LABORATORY (EUNCL)

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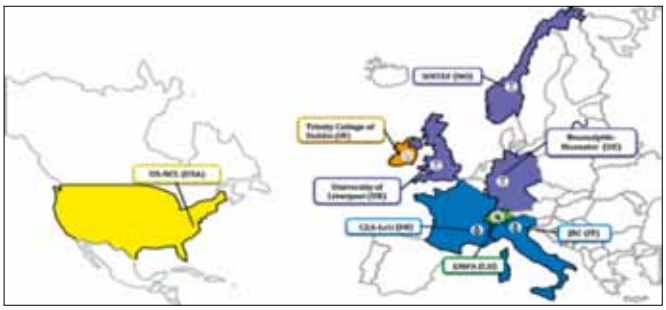
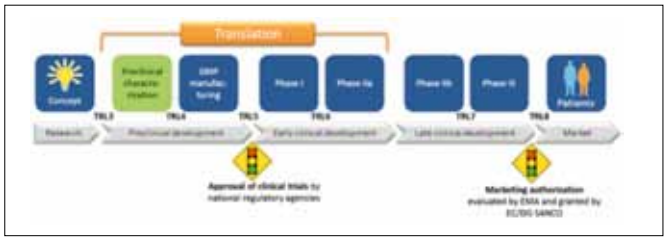
EUNCL is a European Infrastructure funded from the European Union's Horizon 2020 Research and Innovation Programme under grant agreement n°654190 programme. 9 partners from Europe and the USA are joining their forces, skills and resources to fulfill a common mission.



Its mission is:

- To provide a trans-disciplinary testing infrastructure covering a comprehensive set of preclinical characterisation assays (physical, chemical, in-vitro and in-vivo biological testing) allowing researchers to fully comprehend the biodistribution, metabolism, pharmacokinetics, safety profiles and immunological effects of their Med-NPs.
- To foster the use and deployment of standard operating procedures (SOPs), benchmark materials, and quality management for the preclinical characterisation of Med-NPs (nanoparticles used for medical applications).
- To promote inter-sectorial and inter-disciplinary communication among key drivers of innovation, especially between developers and regulatory agencies.



To fulfill its mission EU-NCL aims to achieve 4 major objectives:
Objective 1: To qualify a comprehensive portfolio of Med-NP preclinical characterisation assays (more than 40 assays) within an efficient collaborative environment over the first year of EU-NCL.
Objective 2: To provide preclinical characterisation of 15 Med-NPs to researchers from academia and industry developing Med-NPs by opening trans-national access (TNA) the second year of EU-NCL.
Objective 3: To constantly refine and upgrade the assay portfolio and processes of EU-NCL.
As nanomedicine is a fast evolving field of research, it is a key objective for EU-NCL to constantly refine and adapt its assay portfolio and processes in order maintain the provision of state-of-the-art TNA to the scientific community. Therefore, we will progressively implement additional assays to increase our characterisation capacity, for instance in terms of medical application or route of administration.
Objective 4: To disseminate the EU-NCL findings to the nanomedicine stakeholders in order to strengthen the innovation potential in that field.
The emphasis of the EU-NCL is to serve as a nexus for trans-disciplinary research, development and clinical applications of nanotechnology. Therefore, lessons-learned, best practices, knowledge, tools and methods will be made available to the scientific community such as academic researchers, industry, regulatory bodies, metrology institutes and others. However, care will be taken to ensure that proprietary information and materials disclosed to the EU-NCL by the TNA users are protected.
EUNCL was established in May 2015. Its first open call for application has been released on February 2016.

MICRO DISPENSING OF DRUG-NANOSUSPENSIONS FOR PERSONALIZED MEDICINE

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Personalized medicine, which has received intense attention in recent years, is a multidisciplinary field, which is aiming to optimize medical treatment for each individual patient.1 One important area is the optimization of dose strength based on e.g. family history or genetic information, which has the potential to increase medication performance and reduce side effects.
The manufacturing of personalized solid oral dosage forms close to the point-of-care is a promising alternative to partitioning and accumulating dosing approaches e.g. counting drops of liquid or splitting tablets. These methods are usually applied at home and show risk of administration errors, low dose accuracy and partly limited flexibility[2]. For the flexible manufacturing of personalized solid oral dosage forms very different dispensing technologies are considered, e.g. 3D printing[3], inkjet printing[4], flexographic printing[4] or micro-dispensing[5].
This study focusses on micro-dispensing technology using a piezo-actuated micro-valve. The ability to precisely and reliably dispense and deposition volumes from the nanoliter to microliter range per single dispensing event makes micro-valves particular interesting for the manufacturing of middle to large dose strength. The working principle is straightforward (see Figure 1).

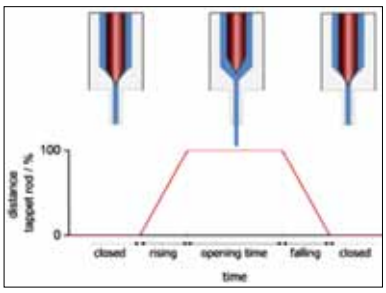


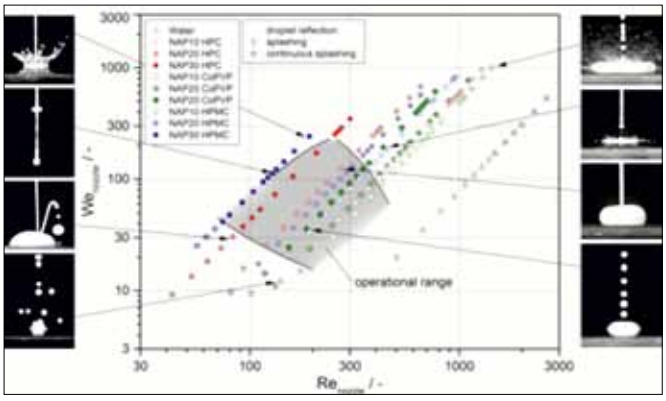
Figure 1: Working principle of the micro-valve

The micro-valve is opened and closed by moving a tappet rod via a piezo-electrical trigger pulse. The amount of dispensed liquid can be varied primarily by adjusting the reservoir pressure and valve opening time. While micro-valves can process various fluids e.g. solutions, suspensions or melts, in this study drug-nanosuspensions with different drug loads and stabilizer systems were considered for experimental trials.

All investigated drug-nanosuspensions, which show drug loads of up to 30% w/w and a wide range of material properties, e.g. viscosity, could be successfully processed by the micro-valve with high accuracy. Dose strengths from 10 µg to 100 mg per single dispensing event have been achieved showing a high dynamic working range. Relative standard deviations were found to be generally below 1%. A correlation between the steady state mass flow, material properties and dispensing parameters could be established, which enables the prediction of the steady state mass flow for low viscosity nanosuspensions with reasonable accuracy.

High speed camera analysis was used to qualitatively characterize the dispensing process regarding the behavior of the dispensed liquid jet after exit from the valve nozzle. The distance from the nozzle, at which the liquid jet breaks up into single droplets (breakup length)6, has been experimentally determined and could be successfully correlated using available equations from literature. Furthermore, the high speed camera was used to characterize the impact behavior of the liquid jet onto a smooth, solid substrate. Based on the individual videos, phase diagrams could be established considering the dimensionless Weber (We) and Reynolds (Re) number, which characterize fluid flow (see Figure 2).

Figure 2: We-Re phase diagram showing the impact behavior of drug nanosuspensions onto a glass plate with 20 mm nozzle-substrate distance. The grey area shows the operational range where a reliable depositioning is achieved.



Specific outcome mechanisms[7], e.g. depositioning, splashing or non-coalescence can be found in certain We and Re number regions. Based on the experimental results a systematic guidance was established, including a safe operational range for the reliable depositioning of drug nanosuspensions onto a substrate with high accuracy. A variation of the nozzle-substrate distance revealed a notable growth of the operational range with decreasing nozzle-substrate distance.

In summary, the reliable application of the investigated micro-dispensing system could be demonstrated for drug-nanosuspensions, including the development of a profound understanding for the flexible and precise dispensing and reliable depositioning onto substrates.

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COMPENDIAL INITIATIVES FOR NON-BIOLOGICAL COMPLEX DRUGS (NBCDS)

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High molecular weight drugs of biological origin such as therapeutic antibodies are of a complex structure, which determines their activity as well as safety in terms of immunogenicity. The manufacturing of such therapeutics requires highly complex and meticulously controlled up- and downstream processes. Still, the final drug product whose properties are determined by the process is characterized by a certain degree of “microheterogeneity”, i.e. the presence of several isomers of the active pharmacological principle (API).

Complex drugs of non-biological origin (NBCDs) share aspects of complex structure, potential immunogenicity and impossibility of full characterization by physicochemical methods alone with biological complex drugs. Examples for these complex drugs include “nanomedicines” such as glatiramoids (Copaxone®), liposomal formulations (Doxil®), and nanoparticles such as iron-carbohydrate particles (Venofer®). Their size and attributes at the molecular scale confer these systems certain properties to interact with their biological environment.

Nanoscale drug delivery systems have been under investigation for several decades, yet only very few have actually matured to clinical application. While analytical techniques describing the physicochemical properties of these systems are being constantly refined, we had to understand that these systems need a multi-pronged analytical approach to describe their physicochemical properties.

The situation is rendered even more complex by the appearance of intended copies of NBCDs. Some of those, e.g., iron sucrose products (“iron sucrose similars, ISSs”), and “generic” Doxil (approved by FDA in 2013 in view of drug shortages in the US) have entered the market under the generic paradigm, partially due to the absence of a more suitable regulatory evaluation process. Therefore, an effort is needed to discover these correlations between nano-properties and biological activity, develop suitable analytical techniques and define specifications, establish clinical protocols and, last not least, integrate this knowledge in a science-based regulatory approach to nanomedicines.

In the context of ensuring the quality and safety of medicines, pharmacopoeias, as standard references for pharmaceutical drug specifications in the form of monographs, play a pivotal role. Having the common goal to assure access to good quality medicines, the organizational forms of these pharmacopoeias and the procedures followed to introduce new monographs differ largely. This presentation will give a brief overview on such issues, and analyze the level of awareness for NBCDs at the European and US pharmacopoeias.

THE NEXT FRONTIERS IN NANO-CHARACTERIZATON

SVEN EVEN BORGOS

Characterisation of nanomedicines comes with a number of unique challenges, depending on the analytical endpoint. For physicochemical characterization, standard imaging techniques with sufficient resolution (electron microscopy) are generally vacuum-based can be complicated by the presence of aqueous or semivolatile components, whereas optical imaging is hindered by the diffraction limit. Physical and chemical composition is generally inhomogeneous, and the specific localization of functional groups (e.g. PEGs) has impact on the overall properties of the nanomaterial. For *in vitro* and vivo characterization, calculating the dose becomes challenging when nanoparticles distribute inhomogeneously in dispersion, e.g. by agglomeration, sedimentation or adhesion, and the presence of biomolecules in complex media can affect the nanoparticle–cell interaction in significant ways. Furthermore, nanoparticle-specific toxicity mechanisms can exist, demanding special attention.
Part of the EU-NCL project is to identify, assess and implement novel characterization techniques for nanomedicines, improved in terms of specific parameters like speed, cost, sensitivity, robustness – or that can provide new data types to improve the scientific foundation used to draw conclusions on nanomedicine safety (and, if possible, efficacy) in the preclinical setting. This will aid nanomedicines developers, regulatory authorities and other stakeholders in bringing more nanomedicine candidates into clinical trials. All major parts of the current characterization cascade (physicochemical, *in vitro* and *in vivo*) will be considered for improvement. The use of computer modelling also holds promise to generate high-quality data in a more cost-effective manner, while reducing the number of animal experiments. The current lecture will review novel analytical technologies and identify some still unmet demands for characterization of nanomedicines.

CONNECTING THE EU-NCL WITH REGULATORS

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The establishment of a two-way dialogue between the EU-NCL and regulatory bodies aims to translate the agencies opinion on data needs into practice in order to offer novel methodologies backing upsupporting the quality and safety assessments of the “next generation” nanomedicines and “nanosimilars” entering into clinical trials or seeking for market authorisation. The strong collaboration between the EU-NCL and the American NCI-NCL will supportcontribute to the mutual acceptance of data on both sides of the Atlantic as both NCLs are harmonising their test methods, information requirements and operational procedures as much as possible.
However, novel applications of nanotechnology to health care products can also span the regulatory boundaries between e.g. the legislative frameworks of medical devices and medicinal products and might create an uncertainty for product developer. Such so-called borderline products can be aof great challengeconcern to Member States, the European Commission (EC) and other stakeholders since they can lead to different interpretations within the Community. Currently some of these products are discussed on a case-by-case basis in an EC coordinated Borderline and Classification working group. The EU-NCL will serve as a communication platform for regulators of different sectors and discuss their information needs including the most suitable test method to obtain the requested information. Relevant methods willcan be taken up in the EU-NCL testing cascade. As such the EU-NCL is prepared to provide information to product developer independent of regulatory path the product will follow at a later stage.
As a first step aiming to get a better understanding on the information needs, the EU-NCL has performed a questionnaire addressed to members of the “nanomedicines” working group of the International Pharmaceutical Regulatory Forum (IPRF) (https://www.i-p-r-f.org/index.php/en/working-groups/nanomedicines-working-group/)international pharmaceutical regulatory forum which is currently under the chair of the European Medicine Agency. The survey addressed the regulatory experience with nanomedicines, information needs of regulators for the characterisation of nanomaterials and further steps that can support the uptake of nanotechnology based products in health care.

WHAT ETHICAL QUESTIONS WILL NANOMEDICINE FACE NEXT?

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In the nine years of Clinam we have seen the focus of nanomedicine gradually moving along the road from concept and laboratory science to clinical trials and to the clinic. It is a long journey and an exciting one for some destinations already reached, and very many more which can be seen ahead. We are perhaps less worried about defining what is exactly ‘nano’ and what is not. Our focus is rightly more on what combinations of techniques and enabling technologies can do in addressing some of those many unmet needs in the clinic, which Clinam is so good at showcasing for all of us, year by year. But in this exciting phase of translation, some of the old ethical questions are still there, in some new guises.
EC FP7 NanoAthero project is creating nanosystems suitable to locate, and either image or treat the vulnerable, unstable atherosclerotic plaque in arteries, or the blood clots that are a heart attack or stroke waiting to happen. I work as an ethicist within the project. How do we test these innovative nanosystems for use in humans, in an area of nanomedicine less developed than cancer treatments? We cannot try these directly in humans without some intermediary which we hope will show us if our methods are along the right lines, or if they are likely to cause harm. But most animals don’t show atherosclerosis. It has to be induced. We are using ApoE mice and balloon injured rabbits fed on a very bad ‘western diet’, to try to mimic the human expression of atherosclerosis, but it is inevitably somewhat artificial. The question is how well do these models reflect the real situation in the diversity of human patients who would present with a potentially dangerous heart condition?
One example is a test to find out if any proposed nanosystem would trigger the complement activation system, in this case using pigs. This can have a dramatic effect, indicating which nanosystems would not be suitable, if a small proportion of patients might have a similar response. But what about more subtle differences between mouse and human in the complex processes of plaque development? How good do we need to be at getting this right?
For people involved in medical research, we know that that such models can only be an approximation. There will always be a gap between what they can tell us and the response in real human patients. Very rarely, this gap has show up in the tragic deaths that have occurred in clinical trials in UK and France in recent years. There remains much public goodwill because we are seeking to cure diseases. They expect us to learn the lessons make changes, but still to carry on with the medical research. We all have an interest, after all.
But what if one of these deaths was in a ‘nano’ clinical trial? Once it was announced, would there be a public perception, perhaps fuelled by hostile NGOs or even by the media, that this strange

technology with these scary but powerful particles was the cause, rather than that such risks exist in most clinical trials? While we work to avoid that such a tragedy would ever happen, we should also prepare ourselves for how we would address that eventuality, and learn lessons from industries like nuclear power and GM food about how not to respond.

Part of the answer is to learn and to do still more excellent and careful science. But part is also to engage the public about what we are doing in nanomedicine. Perhaps we need to lose something of the artificial 'nanoaura' we have generated. The downside of promoting nanotechnology as a revolutionary new idea is that in saying how different it is, you also communicate unfamiliarity and, potentially, risk. Perhaps we should rather seek to present it more like normal medicine? It is to address questions such as these that we have developed the Democs card game for NanoAthero, to let people find out and explore for themselves, to overcome the unfamiliarity, and to appreciate that all medical advances carry risk.

But why are we using animals at all, if they are just an approximation? Human cells should do the job much better, animal advocates say. In principle, yes, but often there are not enough of the right sorts of cells. The FP7 ESNATS project sought to see if potentially unlimited supplies of differentiated cells derived from human embryonic stem cells could be used as alternatives to animals for toxicity testing of pharmaceuticals. Technically it proved more difficult than expected but gave some encouraging outcomes. But it was problematic ethically in that the solution was just as controversial for some people as animal research was for other (different) people. Induced pluripotent cells (iPS) may get round that ethical problem, but neither method solves the problem that cell cultures do not readily represent the behaviour in a whole organ or the whole organism, whereas a whole animal test can.

To what extent can the rapidly advancing field 3-dimensional tissue printing address this? It is early days yet, but, as the strengths of this new methodology are established, so no doubt will its limitations. Ethically speaking, using human cells in something like their real environment seems intuitively better than using animals, but it is not a silver bullet. It does not get over the basic question : 'how good is our approximation to the real human patient?' and the supplementary question : 'how do we verify that our 3D-tissue is a good model, without cross-correlating with (imperfect) animal test systems?'

Would a last step be to go straight to humans, using CRISPR-Cas or related genome editing technologies, to achieve what gene therapy hoped to do but has usually not succeeded? In somatic cells, possibly, but what about embryos? Ethical concerns have been raised by the recent granting of a license by the HFEA regulatory body to UK researchers to do four gene deletions on about six embryos, to see what effect these have on early embryo development. For some this is unacceptable at all. Even for those who would admit research with human embryos under some circumstances, some question it as a premature speculative application, which does not at this point in time justify the very large conceptual step of modifying the genes of human embryos. The licence application was also submitted only two weeks after the two main medical research funding bodies called for public debate on the issue, which has now been somewhat pre-empted by HFEA's granting of a first-of-a-kind licence.

Some talk of a far future step of performing gene editing on embryos to remove heritable diseases. This involves the very serious ethical issue of making inherited interventions in the human genome. But it also raises the question of how one would ever test such an intervention without treating the first babies as experimental subjects. We are back to the question of how to verify a medical technique, and in that case, it may not be possible.

NEGLECTED DISEASES, CURRENT STATUS AND FUTURE NEEDS

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Neglected tropical diseases (NTDs) represent a health burden to a significant part of our world's population. Diseases caused by viruses, bacteria, protozoa and helminths kill millions of people each year and are responsible for vast morbidity and disability. The existing diagnostic tools and medications are inadequate for most of these diseases, especially drugs which often lack efficacy and safety or require long and complicated application. Product-Development-Partnerships i.e. the Foundation for Innovative New Diagnostics (FIND)[1], the Medicines for Malaria Venture (MMV)[2] or the Drugs for Neglected Diseases initiative (DNDi)[3] closed the R&D gap for NTDs and took over the role the pharmaceutical industry played before. The main goal is to bring new products that are safe, effective and affordable to patients in resource poor countries.

Good progress could be reached for the hemoflagellate disease human African trypanosomiasis or sleeping sickness. Current drugs are either inadequate, e.g. the arsenical melarsoprol, or require a long and complicated treatment, e.g. the combination of oral nifurtimox and intravenous eflornithine. DNDi is developing two oral molecules which are in clinical trials. The first one is fexinidazole, a nitroimidazole with acceptable side effects that has to be taken as tablets for 10 days[4]. Hundreds of patients were treated and cured so far. The second one is the benzoxaborole SCYX-7158, another oral drug with excellent pharmacokinetic properties[4]. It passed safety in humans and will soon be tested in patients.

Several NTDs are ear-marked for world-wide elimination by WHO and the international community. According to the roadmap guinea worm disease, leprosy, lymphatic filariasis, blinding trachoma and African sleeping sickness are targeted for elimination while for schistosomiasis, river blindness, Chagas disease and visceral leishmaniasis control is in the focus for the year 2020 to 2030[5]. Elimination of sleeping sickness seems realistic with the number of reported cases at a very low level (<4000/year) and two new oral drugs in the development pipeline[6]. Efforts to eliminate NTDs can greatly benefit from improved PoC diagnostics and new effective and safe oral drugs. In the case of vector borne diseases control strategies for the insect vector or intermediate hosts (e.g. in schistosomiasis) are also crucial elements for elimination.

Nanotechnology has great potential for rapid diagnostic tests and new medications especially to target drugs to the parasite or to reach infected cells or organs.

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MODIFIED APTAMERS AS REPLACEMENT FOR ANTIBODIES IN DIAGNOSTICS AND THERAPEUTICS

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In today's society, there is an ever increasing desire to understand the world in which we live. This desire largely stems from a general concern for our own wellbeing and therefore extends to many areas ranging from the more obvious, such as human health or threat detection, to the more subtle, such as food safety or environmental monitoring.
There is also a continued need for improved therapies, with reduced side effects: often achieved by more specific delivery of the therapeutic to its specific site of action. All of these areas have at their heart, the need for an ever increasing 'tool kit' of reliable and specific target binding reagents.
Until recently, the majority of this need has been met by affinity reagents such as antibodies. While they have their uses, there are still a number of drawbacks which can be addressed by other technologies. Nucleic acid aptamers are now widely recognised as a viable alternative with the potential to fulfil many of these unmet needs. Aptamers are synthetic oligonucleotides, specifically isolated for their ability to recognise a given target with high affinity and specificity that is rarely matched by other means. In this regard, they are often thought of as nucleic acid equivalents of antibodies. Here, we will present a general introduction to nucleic acid aptamers and explain some of the key benefits that are enabling researchers to address targets where other technologies such as antibodies are unavailable or underperform. We will also highlight a few key applications of aptamers as novel diagnostics, as well as their use in therapeutic application.

ON-CHIP BIOCHEMICAL SENSING USING SI NANORIBBON FIELD-EFFECT TRANSISTORS

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Using a top-down approach applied to Silicon on insulator (SOI) wafers, we fabricate Silicon nanoribbons field effect transistors (ISFETs) and investigate their properties as bio-chemical sensors. ISFETs with high-k gate oxide layers (Al2O3 or HfO2) exhibit a very good sensitivity towards protons due to the high density of hydroxyl groups at their surface. The maximum pH response for an ideal oxide surface reaches approximately 60mV/pH (Nernst limit). Using a dual-gate approach, we demonstrated a pH response at the Nernst limit for both oxide surfaces[1]. To help establishing the detection limit of the sensors, we have characterized their low-frequency noise[2, 3].
Going beyond pH sensing, we have demonstrated the specific detection of ions (typically K , Na) by functionalizing the nanoribbons surface with e.g. polyvinyl chloride (PVC) membranes with embedded ionophores[4] or via ion-binding linkers covalently anchored to gold-coated nanoribbons[5]. We emphasize the importance of functionalization schemes leading to a dense, compact layer to avoid the influence of competing reactions taking place at unfunctionalized sites and model this competition effect[6]. We further illustrate this effect on an experimental system by investigating the influence of residual pH sensitivity on the response of nanoribbons functionalized for the specific detection of ions. Recently, we have started to investigate the possibilities of our sensors for the detection and binding kinetics characterization of small sugar binding proteins[7] and for the monitoring of ions diffusion through artificial membranes.

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HIS-TAG-BASED CONTROLLABLE RELEASE OF PEPTIDE AND PROTEIN DRUGS

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Keywords: Protein drug delivery; oral administration; controlled release; His-tag; Silica Nanoparticle.
Advances in genomics and proteomics researches show great promise for the development of peptide and protein drugs for many diseases. Current biotechnologies also enable the massive production of peptides and proteins of any sequences. However, the clinic use of peptide and protein drugs is hampered by several problems such as the difficulty for oral administration. Oral administration is the most preferred drug delivery method for its convenience and high degree of patient acceptability and compliance, but oral administration of peptide and protein drugs is still impractical, due to their poor intrinsic permeability, enzymatic degradation, rapid clearance, and chemical and conformational instability. Reversible non-covalent encapsulation of peptide and protein drugs by nanoparticle is an effective way to solve the above problem. For example, silica nanoparticles have been widely used to encapsulate proteins. Study showed that the efficiency of the protein encapsulation in silica NPs depends critically on the pI value of the protein. Due to the negative charge of silica NPs, negatively-charged proteins (pI < 7) are difficult to be encapsulated and easy to leak out. We developed a facile and general method to encapsulate His-tagged proteins and peptides in silica NPs, including the negatively-charged ones. The key factors in this method are taking advantage of the widely-used His-tag for protein purification, and using a small amount of calcium ions to conjugate His-tagged protein to silica shell (Fig.1). The negatively-charged enhanced green fluorescence protein (EGFP) can be effectively encapsulated in

silica NPs, and the silica encapsulation shows substantial increase of fluorescence intensity and stabilities against denaturants, protease and high temperature, making the EGFP-encapsulated silica NP a potential robust fluorescence probe.[1] Interestingly, this His-tag-based encapsulation method also provide a new controllable release mechanism for protein and peptide drugs. As shown in Fig. 2, the released of fluorescence dye-labelled his-tagged peptide for silica NPs shows a pH-responsive released behavior, i.e. normally released at physiological pH (pH 7-8), and hardly released at acid pH (pH2) corresponding to the pH value at stomach. Thus, the silica NPs could protect the encapsulated protein and peptides from metabolic digestion in stomach, demonstrating its application potential applications as an oral drug delivery system for peptide and protein drugs.

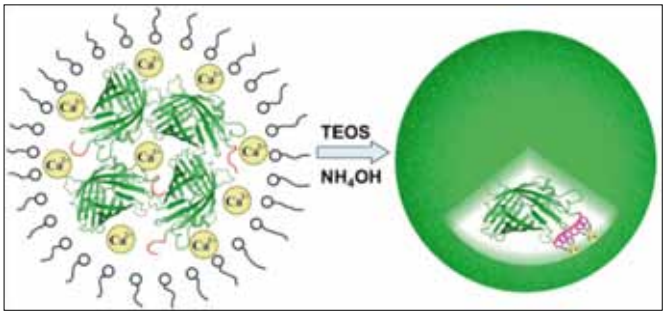

Fig. 1. Reverse micro-emulsion method for encapsulating His-tagged proteins. EGFPs (green) with His-tag (red) are anchored to the silica shell through coordinate bonds between Ca2+ (yellow) and the histidine residues of the His-tag.

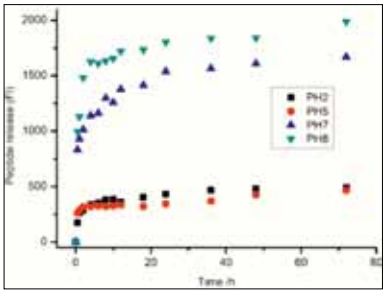

Fig. 2. The release of His-tagged peptide from peptides@Silica NPs at different pH conditions.

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THE CHALLENGES OF PEDIATRIC CANCER AND THE OPPORTUNITIES TO OVERCOME THEM WITH NANOMEDICINES

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Most pediatric tumors are characterized by unique genetic or biomarker alterations. Examples of pediatric tumor specific alterations are the EWS/FLI1 fusion gene of Ewing sarcoma, the high expression of the disialogangloside (GD2) in embrional cancers (neuroblastoma, retinoblastoma, rhabdomyosarcoma, Ewing sarcoma and brain tumors), or the H3 histone mutations of diffuse intrinsic pontine gliomas. Nanotechnology could take advantage of such homogeneous features of pediatric diseases to enable active drug targeting to specific pediatric tumor cells.

To validate candidate nanomedicines for pediatric cancer, a significant challenge is provided by the scarcity of pediatric solid tumor models to conduct accurate preclinical studies. The translational research program at Hospital Sant Joan de Déu Barcelona contributes to generate clinically relevant models of pediatric solid tumors, including patient-derived xenografts, which reproduce accurately the specific genetic or biomarker alterations of pediatric cancer patients. The new models developed are useful for the validation of novel nanotechnology approaches, the identification of biomarkers and the design of clinical trials.

In addition to the mentioned biological properties, pediatric oncology patients present unique medical needs that could be fulfilled with nanotechnology approaches. One of them is the need of local treatments that replace radiotherapy (radiation could be exceedingly toxic during the normal development of tissues). With this motivation we have developed a novel drug delivery system (DDS) consisting of matrices made of poly(lactic acid) electrospun polymer nanofibers loaded with SN-38 microcrystals for local release in difficult-to-treat pediatric solid tumors. To model the clinical scenario, we conducted extensive preclinical experiments to characterize the biodistribution of the released SN-38 using microdialysis sampling *in vivo*. We observed that the drug achieves high concentrations in the virtual space of the surgical bed and penetrates a maximum distance of 2 mm within the tumor bulk. Subsequently, we developed a model of subtotal tumor resection in clinically relevant pediatric patient-derived xenografts and used such models to provide evidence of the activity of the SN-38 DDS to inhibit tumor regrowth. We propose that this novel nano-DDS could represent a potential future strategy to avoid harmful radiation therapy as a primary tumor control together with surgery.

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SYNTHETIC VACCINE PARTICLES (SVP™), EFFECTIVE MESSAGING WITH THE IMMUNE SYSTEM

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Precision in Communicating with the Immune System.

Synthetic Vaccine Particles (SVP™) technology is a highly flexible platform, capable of incorporating a wide range of antigens and immunomodulators, allowing the development of SVP™ products for either inducing antigen-specific tolerance or activating the immune system.

SVP™ are made of biodegradable PLGA and are designed to remain intact after injection until they are taken up by dendritic cells. The content of the SVP™ is released inside the endosome of dendritic cells to deliver a precise message of immune tolerance or immune activation directed against a specific antigen. SVP™ are optimized for effective messaging, and the dendritic cells will process the antigen(s) in the context of the immunomodulatory for which load and release rates are optimized. Using this mechanism SVP™ induce antigen-specific T cells.

SVP™ are applied to the development of differentiated therapies that are designed to modulate the immune system to effectively and safely treat rare diseases by mitigating the formation of anti-drug antibodies (ADAs) in response to life-sustaining biologic drugs. Tolerance inducing SVP™ products also have potential applications in the treatment of allergies and autoimmune diseases. SVP™ products that stimulate the immune system can potentially prevent or treat cancer, infections and other serious diseases.

Examples of the application of SVP™ in the different therapeutic area will be presented.

ANTIGEN-SPECIFIC IMMUNE TOLERANCE IN THE CLINICAL TREATMENT OF SERIOUS AND RARE DISEASE

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Selecta Biosciences is advancing therapies based on its proprietary Synthetic Vaccine Particle (SVP™) platform to induce antigen-specific immune tolerance to mitigate the formation of anti-drug antibodies (ADAs) that may severely compromise the efficacy of life-sustaining drugs. Many rare and life-threatening diseases are treated with biologic therapies that are foreign to the patient's immune system and elicit an undesired immune response, such as ADA formation. The induction of ADAs can lead to neutralization of efficacy, modification of pharmacokinetics and pharmacodynamics, as well as allergic responses. Drug immunogenicity is a significant hurdle for the development of safe and effective biologic treatments and has become a key concern for regulators, as evidenced by over 100 approved biologics that describe immune responses on their labels. Drug immunogenicity may be a cause of treatment failure for patients and product development failures for the pharmaceutical industry. Immunogenicity may also represent a significant hurdle for the clinical development of novel technologies, such as gene therapy and gene editing, antibody-drug conjugates and others.Selecta's antigen specific tolerance programs utilize SVP™ Rapamycin, the company's biodegradable nanoparticle encapsulating the immunomodulator rapamycin. SVP™ Rapamycin is co administered at the beginning of therapy with a biologic drug to mitigate the formation of ADAs without altering the drug or its dose regimen. The technology was optimized in a number of preclinical models of protein and enzyme replacement therapies (FVIII deficient mice, uricase deficient mice, non-human primates) and mAb therapy with TNFα. First clinical trials were initiated with SEL-212 , the lead immunotherapeutic product candidate from Selecta's SVP™ platform, which combines its tolerogenic nanoparticles with a proprietary enzyme therapy to treat refractory and chronic tophaceous gout. In gene therapy, Selecta is applying the SVP platform to mitigate the undesired immunogenicity to therapeutic transgenes and vectors based on Adeno-Associated Virus (AAV), with a goal of enabling new applications based on the ability to repeatedly and effectively administer gene therapies.
Preclinical data leading to the first clinical data will be presented.

ANTI-INFLAMMATORY DENDRIMERS WITH POTENTIAL USE IN THE TREATMENT OF MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) affecting mainly young people aged between 20 and 40 at disease onset. It affects 2.5 million people worldwide (one million in the EU), with more than double incidence in women than in men and the symptoms include visual disturbances, paraesthesias, ataxia and impaired mood. The costs of the disease in the European Union has been estimated to be around 20 billion euros per year including direct (medical and non-medical) and indirect costs according to the European Brain Council. The life expectancy of patients with multiple sclerosis might be reduced by up to 10 to 12 years. In the initial stages, the marked inflammation in the white matter of brain and spinal cord represents the most relevant anatomical feature. Approximately 50% of these patients evolve to secondary progressive MS within 10 years of onset where the relevant lesions include oligodendrocyte damage, myelin destruction and section of axons, responsible for the progressive neurological deficits and the disability of the patients. Dendrimers are highly-branched, multivalent nanoparticles with a globular in shape and a particular architecture formed by three distinct domains: a) A central core that is either a single atom or a group of them having at least two chemical functionalities that facilitates the linkage of the branches, b) Branches emanating from the core composed of repeat units having at least one junction of branching, whose repetition is organized in a geometric progression that results in a series of radially concentric layers named generations (G) and c) Terminal functional groups, located in the exterior of the macromolecule, which play a key role in their gene-complexing or drug-entrapping ability. The presence of these numerous terminal groups facilitates interactions with solvents, surfaces or other molecules. In general, dendrimers tend to show high solubility, reactivity and binding. New phosphorus dendrimers have been tested for its anti-inflammatory activity to be considered as the base for the development of novel treatments for multiple sclerosis. The new molecules did not produce any toxicity on mouse cortical neurons and astrocytes even at concentrations of 10 µM indicating that no toxicity might be expected in healthy central nervous system when used as a potential treatment for multiple sclerosis. Moreover, the dendrimers did not show any toxicity on macrophages isolated from mice peritoneum or lymphocytes isolated from mice thymus. To test the anti-inflammatory properties of the dendrimers we used the LPS-stimulation of macrophages paradigm. Macrophages were isolated from the peritoneum of thyoglycolate-treated mice and stimulated with bacterial LPS (100 ng/mL). This stimulation induced a time-dependent increase in nitric oxide (NO) production due to the induction of the expression of the enzyme nitric oxide synthase (iNOS). Both iNOS induction and NO production were blocked in a dose-dependent way by the phosphodendrimers. LPS induces iNOS by promoting the translocation of the transcription factor NFkB from the macrophage cytosol to the nucleus where it promotes the expression of the iNOS gene. The phosphodendrimers markedly block LPS-induced NFkB translocation from cytosol to the nucleus in mice macrophages providing a mechanism of action for its anti-inflammatory activity. Moreover, the phosphodendrimers inhibit LPS-induced macrophage secretion of the pro-inflammatory cytokines Tumor Necrosis Factor α (TNFα) and IL-1 while preserving secretion of the anti-inflammatory cytokines IL-4 and IL-10. Taking together, these results support a potential therapeutic use of anti-inflammatory phosphorous dendrimers in multiple sclerosis.
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ENGINEERED LIVER TISSUES FOR DEVELOPING PRECISION CANCER NANOMEDICINES

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Antibodies are actively used for a wide array of therapeutic applications because of their intrinsic specificity of antigen recognition, avidity, tolerability, and well-characterized pharmacokinetic profiles. However, antibodies are bivalent with single specificities. By using the concepts of human antibody-based self-assembly, the bispecific agents have been made to greatly expand the repertoire of therapeutic applications. One of the first applications of bispecific agents has been to develop novel cancer therapies based T-cell redirection. These bispecific agents that have dual specificity for CD3, an antigen on the T cell and target cell antigen. Hence these proteins can bring T cells to be in close proximity to the target tumor cells for cell lysis. Although having some anti-tumor activity, the first generations of such bispecific agents had limitations in their pharmacokinetic and safety profiles as well as manufacturability. To broaden the therapeutic profile, antibody engineering based on the controlled Fab arm exchange (cFAE) generates a fully human bispecific antibody having two binding specificities: one arm that recognizes a CD3 domain on T cells and the other arm that binds to a target cell antigen. The cFAE process is robust and is being used to generate drug substance for clinical trials. A brief review of the applications and promise of such bispecific antibodies will be presented.

ENGINEERED LIVER TISSUES FOR DEVELOPING PRECISION CANCER NANOMEDICINES

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A major challenge for studying authentic liver cell function and cell replacement therapies is that primary human hepatocytes rapidly lose their features of advanced differentiation soon after plating into standard two-dimensional culture. Here we describe the fabrication of three-dimensional hexagonally arrayed lobular human liver tissues inspired by the liver's natural architecture. These tissues exhibit key features of advanced differentiation such as human-specific cytochrome P450 mediated drug metabolism and the ability to support efficient infection with patient-derived inoculums of hepatitis C virus. They permit the assessment of antiviral agents and maintain their advanced functions for over five months in culture. This extended functionality enabled the prediction of a fatal human specific hepatotoxicity caused by FIAU, that had escaped detection by preclinical models and short term clinical studies. We anticipate that these engineered human liver tissues can provide a new system to study other heretofore difficult to model important human liver diseases such as primary or metastatic liver cancer and improve regulatory assessment of candidate drug safety.

ARTIFICIAL SPORES

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Nature has developed a fascinating strategy of cryptobiosis ("secret life") for counteracting the stressful, and often lethal, environmental conditions that fluctuate sporadically over time. For example, certain bacteria sporulate to transform from a metabolically active, vegetative state to an ametabolic endospore state. The bacterial endospores, encased within tough biomolecular shells, withstand the extremes of harmful stressors, such as radiation, desiccation, and malnutrition, for extended periods of time and return to a vegetative state by breaking their protective shells apart when their environment becomes hospitable for living. Certain ciliates and even higher organisms, e.g., tardigrades, and others are also found to adopt a cryptobiotic strategy for their survival. A common feature of cryptobiosis is the structural presence of tough sheaths on cellular structures. However, most cells and cellular assemblies are not "spore-forming" and vulnerable to the outside threats. In particular, mammalian cells, enclosed with labile lipid bilayers, are highly susceptible to *in vitro* conditions in the laboratory and daily-life settings, making manipulation and preservation difficult outside of specialized conditions. The instability of living cells has been a main bottleneck to the advanced development of cell-based applications, such as cell therapy and cell-based sensors.

Recent studies have sought to chemically control and tailor the metabolic behaviors of non-spore-forming cells, as well as enhancing their viability against adverse environmental conditions, by forming thin (< 100 nm), tough artificial shells. These living "cell-in-shell" structures, called artificial spores (chemically-formed spore-like structures), enable control of cell division, protection against physical and chemical stresses, and cell-surface functionalizability, as well as providing the cells with exogenous properties that are not innate to the cells but are introduced chemically, such as magnetism, heat-tolerance, and UV-resistance. In addition, recent developments in the field have further advanced the synthetic tools available to the stage of chemical sporulation and germination of mammalian cells, where cytoprotective shells are formed on labile mammalian cells and broken apart on demand. Based on these demonstrations, the (degradable) cell-in-shell hybrids are anticipated to find their applications in various biomedical and bionanotechnological areas, such as cytotherapeutics, high-throughput screening, sensors, and biocatalysis, as well as providing a versatile research platform for single-cell biology.

TARGETING METABOLIC SYMBIOSIS TO OVERCOME RESISTANCE TO ANTI-ANGIOGENIC THERAPY

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We have developed new, anti-angiogenic nanoparticles (immunoliposomes, ILs) directed against tumor-associated vascular cells. Pegylated liposomal doxorubicin (PLD) was directed against VEGFR2 and VEGFR3-expressing cells by inserting anti-VEGFR2 and/or anti-VEGFR3 antibody fragments into the lipid bilayer membrane of PLD. These constructs were tested *in vitro*, and *in vivo* in mouse models of cancer. Anti-VEGFR2-ILs-dox and anti-VEGFR3-ILs-dox both reduced tumor burden more efficiently than PLD. Additionally, when applying the ILs in combination, an additive effect was observed. Detailed analysis of tumor-associated vessel-specific apoptosis revealed a specific increase upon therapy with anti-VEGFR2 or 3 ILs. The combination treatment with anti-VEGFR2-ILs-dox and anti-VEGFR3-ILs-dox provides a highly efficient approach to selectively deplete tumor-associated vasculature leading to tumor starvation and hence tumor reduction.

However, despite the approval of several anti-angiogenic therapies, clinical results remain unsatisfactory, and transient benefits are followed by rapid tumor recurrence. We have studied the anti-angiogenic efficacy of the multi-kinase inhibitor nintedanib in a mouse model of breast cancer. Nintedanib demonstrates a potent anti-angiogenic effect. However, after an initial repression, tumors resume growth in the absence of active tumor angiogenesis, suggesting a novel mechanism of therapy resistance whereby tumor cells proliferate without proper vascularization. Gene expression profiling analysis of flow cytometry-sorted tumor cells reveals a metabolic reprogramming towards anaerobic glycolysis. Indeed, combinatorial treatment with a glycolysis inhibitor (3PO) efficiently inhibits tumor growth. Moreover, alternation between hypoxic, glycolytic areas and normoxic areas in the nintedanib-treated tumors suggests the establishment of metabolic symbiosis, further illustrated by the differential expression of MCT1 and MCT4, monocarboxylate transporters implicated in lactate exchange in glycolytic tumors. Accordingly, ablation of MCT4 expression surmounts resistance to anti-angiogenic therapy. Our results suggest a mechanism of glycolytic symbiosis to overcome a potent anti-angiogenic therapy with a multi-kinase inhibitor. Hence, targeting metabolic symbiosis may be an attractive avenue to avoid resistance development to anti-angiogenic therapy in patients.

INTRODUCTION

DAAN CROMMELIN

Non-biological complex drugs (NBCDs), including many nanomedicines, are a class of medicinal products that cannot be fully quantitated and characterized by physico-chemical analytical means. They share that characteristic with other complex drugs belonging to the class of biologicals. Examples of NBCD products are glatiramoids, iron-carbohydrate complexes, polymeric micelles, complex ocular emulsions and liposomes. The complex nature of NBCD products means that minute variations in the manufacturing process can substantially change the composition of final products and their profile. Are the existing regulatory protocols indeed able

to assess equivalence of these NBCD products or should a nano-similar approach (cf. biosimilars) be pursued? As patents of the first generation of "futuristic" drugs are expired and authorized follow-ons have demonstrated non-comparability in clinical studies, the importance of appropriate science-based approval standards is evident.

LIPOSOME ENCAPSULATED ANTI-INFLAMMATORY AGENTS PROTECT CARDIAC FUNCTION AFTER MYOCARDIAL INFARCTION: FIRST PRECLINICAL RESULTS

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Heart protection after ischemia represents a major challenge in cardiology, with myocardial infarction being the major cause of mortality in industrialized countries, Research on cardiac protection has a long history with successes but also disappointments.

After myocardial infarction (MI) the inflammatory process plays an important role in the healing process occurring after the acute ischaemic event. Inflammation participates in the physiological process of myocardial scar formation. In the case of an exuberant inflammatory reaction though, the extent of the damage in the myocardial tissue can increase strongly. The delicate equilibrium between the loco-regional myocardial inflammatory response, and the post-MI systemic inflammatory reaction, is most critical for the healing process post-MI. As a measure of therapeutic efficacy of a certain treatment post-MI, the ejection fraction can be used. A reduction in ejection fraction can reflect adverse left ventricle remodelling potentially leading to congestive heart failure, which is a main determinant of mortality and morbidity after myocardial infarction. Berberine is an isoquinoline alkaloid extracted from barberry that has anti-inflammatory and anti-oxidant activities. Pretreatment with long-term administration of high doses of berberine has shown beneficial effects in experimental diabetes and cardiac ischemia reperfusion injury. However, its poor solubility and short half-life in the circulation have impeded the clinical use of berberine. We hypothesized that encapsulation of this drug into long-circulating liposomes could improve its therapeutic availability and efficacy to protect cardiac function *in vivo*.

For *in vivo* efficacy, $C^{57}Bl/6J$ mice subjected to myocardial infarction via permanent ligation of the left anterior descending artery were intravenously injected with empty liposomes, free berberine or liposome-Berberine (1.5 mg/kg). Ejection fraction was assessed by echocardiography at baseline, 7 and 28 days after MI. The liposome-encapsulated berberine significantly preserved ejection fraction after 28 days of MI while free berberine did not show any preservation of ejection fraction. In this study we also analyzed cell viability, reactive oxygen species production and cytokine secretion in lipopolysaccharide (LPS) activated mouse macrophages cell line after berberine exposition. The *in vitro* results show that berberine improved the viability of LPS-insulted macrophages, reduced production of reactive oxygen species and inhibited the secretion of inflammatory mediators including IL-6 and TNFα.

In conclusion, our results indicate that liposome-encapsulated berberine reduces adverse ventricle remodeling post-MI. Similar remodeling activity was seen when the liposomes contained corticosteroids. This outcome suggests that liposomal delivery of berberine significantly improves its therapeutic availability and therefore treatment efficacy *in vivo*.

BIOLOGICAL IDENTITY AND RECOGNITION ON THE NANOSCALE

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The endogenous (intrinsic) machinery of biology is defined and operates on the nanoscale. When it does so, it acts with a level of discernment and precision that has not been fully understood until now, within the context of bionanoscience. For example, the interaction of various biomolecular recognition elements derived from biomolecules interact with cells via receptors, and thereby dominates interactions with clearance mechanisms such as in the liver, drug targeting, and immune reaction. Now we see the need to have a detailed molecular understanding of the interface between nanomaterials and biological interactions, to the level appropriate for the questions.

Beginning with how interactions between nanoscale objects and living organisms occur, and their governing principles[1,2], we argue that the future lies in pressing forward to develop a truly microscopic (molecular scale) understanding between the nanoscale and living organisms[3,4]. We show new techniques that allow us to map out the nanoparticle interface in situ with molecular detail, and confirm the receptor interactions in detail, allowing for an increasingly predictive view of bionanoscience[5,6]. We have been surprised as a result, to find that some targeting strategies no longer considered very optimistic may have had trouble simply because of the lack of detailed picture.

More of the original aspirations within nanomedicine and beyond could now be realized by the disciplined linking to detailed molecular recognition design and fabrication enabled by these methods.

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6. Unpublished

NANOCONSTRUCTS FOR THERANOSIS: FROM IN SILICO/IN VITRO STUDIES TO PRECLINICAL MODELS

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Most nanoparticles for biomedical applications originate from the self-assembling of individual constituents through molecular interactions and possess limited geometry control and stability. Here, discoidal polymeric nanoconstructs (DPNs) are demonstrated by mixing hydrophobic and hydrophilic polymers with lipid chains and curing the resulting paste directly within silicon templates. The size, shape, surface properties and mechanical stiffness of DPNs can be precisely, and independently, tailored during the synthesis process. Specifically, by changing the paste composition, soft- and rigid-DPNs (s- and r-DPNs) are synthesized exhibiting the same ge-

ometry, a moderately negative surface electrostatic charge (–14 mV), and different mechanical stiffness (~ 1.3 and 15 kPa, respectively). These multifunctional nanoconstructs are used as carriers of imaging and therapeutic agents for cancer theranostics. Upon injection in mice bearing brain or skin cancers, s-DPNs exhibit ~24h circulation half-life and accumulate up to ~20% of the injected dose per gram tumor, detecting malignant masses as small as ~ 0.1% the animal weight via PET imaging. In silico simulations and *in vitro* microfluidic testing are used to elucidate the mechanisms regulating the *in vivo* vascular behavior of DPNs and their biomedical performance. The unprecedented behavior of DPNs is ascribed to the unique combination of geometry, surface properties, and mechanical stiffness which minimizes s-DPN sequestration by the mononuclear phagocyte system. Our results could boost the interest in using less conventional delivery systems for cancer theranosis.

ABRAXANE IN COMBINATION WITH IMMUNE CHECKPOINT INHIBITORS

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nab-Paclitaxel (ABRAXANE®) is a albumin-bound nanoparticle form of paclitaxel, which has been approved for the treatment of metastatic breast cancer (MBC), for the first-line treatment of locally advanced or metastatic non-small cell lung cancer (NSCLC) in combination with carboplatin, and for the first-line treatment of metastatic pancreatic ductal adenocarcinoma in combination with gemcitabine.

Recently the immune checkpoint inhibitors such as nivolumab, pembrolizumab and atezolizumab have demonstrated impressive efficacy in a number of cancer types leading to several breakthrough therapy designations from the FDA. To investigate potential synergy, there are multiple clinical studies ongoing to evaluate the safety and efficacy of nab-paclitaxel in combination with immune checkpoint inhibitors

In a phase 1b study in patients with metastatic triple-negative breast cancer (TNBC), 32 patients received concurrent treatment with nab-paclitaxel at 125 mg/m2 and atezolizumab (anti-PDL1, Roche) at 800 mg. In 24 patients evaluable for efficacy, the confirmed objective response rate (ORR) was 66.7%, 25%, and 28.6% for first-line, second-line, and third-line and beyond settings, respectively. The overall ORR was 41.7%. Overall, there were no treatment-related deaths observed in the study. After a median follow-up of 5.2 months for all 32 patients, grade 3/4 adverse events (AEs) had occurred in 56% of patients, including neutropenia (41%), thrombocytopenia (9%), and anemia (6%). Five patients discontinued nab-paclitaxel as a result of an AE.

The encouraging safety and efficacy results support further development of combination regimens of nab-paclitaxel and immune checkpoint inhibitors. In the phase 3 IMpassion130 trial, patients (target enrollment goal: 350) with previously untreated metastatic TNBC will be randomized to nab-paclitaxel plus placebo or atezolizumab.

In addition, the phase 3 IMpower 131 trial will evaluate atezolizumab in combination with carboplatin + paclitaxel or carboplatin + nab-paclitaxel compared with carboplatin + nab-paclitaxel in patients with Stage IV squamous NSCLC.

Separately, a phase 1 clinical trial is ongoing to evaluate the safety, tolerability and preliminary efficacy of a combination regimen of nab-paclitaxel and nivolumab (OPDIVO®, anti-PD1 immune checkpoint inhibitor, Bristol-Myers Squibb). Multiple tumor types will be explored in the study, including HER-2 negative metastatic breast cancer (nab-paclitaxel and nivolumab), NSCLC (nab-paclitaxel, carboplatin, and nivolumab), and pancreatic cancer (nab-paclitaxel, gemcitabine, and nivolumab).

Taken together, combination regimens of nab-paclitaxel and immune checkpoint inhibitors have shown early promise and can potentially become a viable choice for the treatment of different solid tumors in the near future.

NEW CLINICAL DIRECTIONS WITH ABI-009, A TARGETED NANOPARTICLE MTOR INHIBITOR

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ABI-009 (nab-rapamycin) is a novel albumin-based nanoparticle version of sirolimus (rapamycin) with a mean particle size of approximately 100 nm, that can target various tissues based on mechanisms of albumin transport. This technology has previously achieved significant commercial success through marketing approval of albumin-bound paclitaxel for several oncology indications.

Based on its highly favorable pharmacological and pharmacokinetic profile relative to other known mTOR inhibitors, after completion of phase 1 studies, ABI-009 is being investigated in both cancer and non-cancer indications based on the recently demonstrated biological relevance of mTOR activation in these diseases. A phase 2 trial has been initiated in an extremely rare form of sarcoma called perivascular epithelioid cell tumor (PEComa). These tumors are almost exclusively driven through mutations or deletions of TSC2 in the mTOR pathway. Another phase 2 trial has been initiated in a series of solid tumors driven by mTOR activation. Hyperactivation of mTOR pathway has also been found in human bladder cancer. and a multi-center phase 1/2 study to investigate the intravesical use of ABI-009 to treat early stage (non-muscle invasive) bladder cancer was initiated. These and other clinical applications of ABI-009 will be discussed.

COMPARISON OF THE ANIMAL MODELS OF COMPLEMENT ACTIVATION RELATED PSEUDOALLERGY

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INTRODUCTION

Intravenous injection of a variety of nanomedicinal (liposomal, micellar, polymer conjugated) and protein-based drugs (antibodies, enzymes) can lead to hypersensitivity reactions. These nanoparticles are recognized by the immune system as foreign that leads to the activation of the complement system. These hypersensitivity reactions were recently described as complement activation-related pseudoallergy (CARPA). The mechanism of CARPA involves the activation of macrophages and the release of vasoactive and inflammatory mediators, e.g. TXA2, PAF, histamine, etc. CARPA is characterized by cardiopulmonary changes including arrhythmia, pulmonary edema, hypotension, airway occlusion, respiratory distress and cardiac arrest. The clinical symptoms of CARPA greatly vary among patients and the outcome can be severe or even fatal. CARPA is considered as a safety issue and its preclinical assessment has been recommended by the European Medicines Agency in the development of liposomal drugs. Therefore, there is increasing interest in the prediction and prevention of CARPA using laboratory assays and animal models. CARPA can be tested in mice, rats, dogs and pigs but the methods are not yet standardized. The major advantage of the porcine model is its high sensitivity and the predictability of human CARPA. However, it is cost and labor demanding, therefore not suitable for routine screening. Rodents can be an alternative but literature data in rats and mice are sparse. For this reason, the aim of our Laboratory in recent years has been the development of rodent CARPA models. We now describe our novel mouse model, the recently developed rat model, and compare the hemodynamic and hematological results in these species with those obtained in pigs.

MATERIALS AND METHODS

Pigs: Domestic male Yorkshire pigs (20-25 kg) were anesthetized with isoflurane (2-3% in O2) after ketamin/xilazine induction (n=2-3/group). Pulmonary arterial pressure (PAP) was measured using

a Swan-Ganz catheter, systemic arterial pressure (SAP), and heart rate (HR) was measured in the femoral artery. Blood was taken from the femoral vein and bolus injections were given via the left external jugular vein. Hemodynamic changes were continuously monitored and evaluated using AD Instruments (ADI) PowerLab system.

Rats: Male Wistar rats weighing 400-600 g (n=8-10/group) were anesthetized with thiobutabarbital (Inactin, 120 mg/kg i.p.). The left common carotid artery (for blood sampling), the left femoral artery (for recording of SAP and HR), the left femoral vein (for bolus injections) were cannulated.

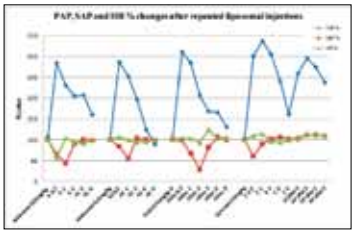
Mice: Male NMRI mice (29-35 g) were anesthetized with Na-pentobarbital (60 mg/kg i.p.) and the left common carotid artery (recording of SAP and HR), and left jugular vein (for bolus injections) were cannulated. In parallel, groups of mice were injected by nanodrugs via the tail vein (n=4-6/group) and blood of mice were obtained in isoflurane anesthesia.

Blood sampling: Blood samples of 0.5 ml and 2 ml, were collected into Hirudin or K2-EDTA Blood Tubes, or Eppendorf tubes containing 10 µl lepirudin (Refludan, 1mg/ml) before (time 0), and at different time points (1-3-5-10- 30 min) after treatments. Blood was centrifuged at 1500 rpm for 10 min at 4°C, and plasma was stored at -80°C until analysis.

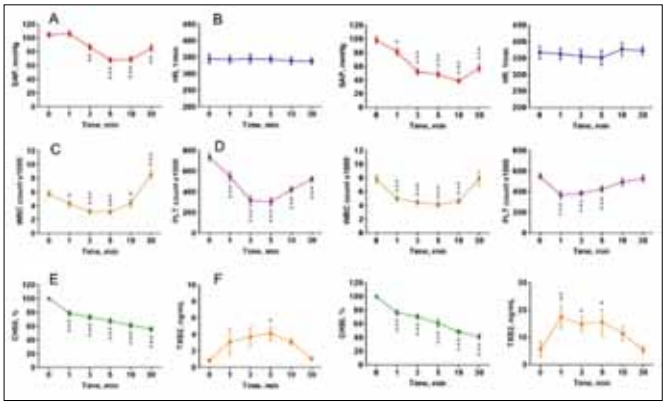
Complement activation: The total complement hemolytic activity (CHA) was measured as the capacity of test serum to lyse antibody-coated sheep RBCs. A fixed volume of sensitized sheep RBCs was added to serum, the mixture was centrifuged and hemolysis was quantified by measuring free hemoglobin absorbance at 540 nm.

Thromboxane B2 levels: Plasma TXB2 (the stable metabolite of plasma TXA2) levels were measured with an ELISA kit (Cayman Chemicals).

RESULTS



Pigs (Fig. 1): *Cardiopulmonary effects of repetitive, bolus administration of 0.01 mg/kg AmBisome lead to similar; 3-fold (280%) rises in PAP with a 50% drop in SAP. Doxil gave similar results. The effect of Ambisome is not tachyphylactic, which is the opposite to that of Doxil. AmBisome did not desensitize the animals against the effects of Doxil, i.e. no cross-tolerance developed. A CARPA reaction similar to that caused by AmBisome could be elicited by a 50-fold higher dose of zymosan.*



Rats (Fig. 2): *Administration of 22 mg PL/kg AmBisome (left) lead to gradual decrease in SAP while the HR did not change. Significant leukopenia, switching to leukocytosis, thrombocytopenia, and a parallel reduction in hemolytic activity (CH50) was observed. Plasma TXB2 rose only minimally. The effect of 10 mg/kg zymosan (right) was very similar to that seen with AmBisome, except for smaller hematological and larger TXB2 changes.*

Mice: *Zymosan (30 mg/kg) induced CARPA in NMRI mice. After an initial SAP increase (15%) hypotension and hypotensive shock was seen. Trombocitopenia was also observed. The lipid complex Abelcet (30 mg/kg) behaved differently: it resulted only in 30% SAP increase, but no later hypotension occurred. For details see our CLINAM 2016 poster (Őrfi et al.).*

CONCLUSIONS

This study confirmed that the porcine model is highly sensitive to liposomes and zymosan to produce CARPA. Rodents also show CARPA at high doses. Although rats or mice are not so CARPA-reactive than pigs, physiological changes in both species are essentially the same as seen in pigs and men. Considering lower costs and other advantages we propose our new rodent models for immune toxicity screening, as well as to study the mechanism of CARPA.

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NOVEL PEPTIDE-BASED DELIVERY FOR POLYNUCLEOTIDES BYPASSING THE ENDOSOMAL UPTAKE PATHWAY

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We have designed the ADGN-technology based on short amphipathic peptides that form stable positively-charged nanoparticles with a wide range of nucleic acids through non-covalent electrostatic and hydrophobic interactions. Self assembly of these peptide molecules around nucleic acids leads to peptide/nucleic acid complexes in the form of stable nanoparticles.

These nanoparticles promote efficient targeted-delivery of siRNA or small oligonucleotides into a wide variety of cell lines *in vitro* and in animal models *in vivo*. Investigation of the cellular uptake mechanism of the peptide/siRNA nanoparticles reveals that they enter the cell via translocation through the cell membrane and bypass the endosomal pathway. Systemic administration by intravenous or subcutaneous injections in mice does not induce a non-specific inflammatory or cytokine response. The potency of ADGN-technology has been demonstrated *in vivo* via delivery of peptide/siRNA nanoparticles in several tumor models targeting major cell cycle regulatory proteins, as well as liver based targets. We demonstrated that ADGN-mediated delivery of Cyclin B1 siRNA inhibits tumor growth *in vivo* following intravenous injections.

Given the robustness of the biological response achieved through this approach, ADGN-technology holds a strong promise for therapeutic administration of oligonucleotides.

CHALLENGES AND CONSIDERATIONS FOR THE DETECTION OF ENDOTOXIN IN NANOMEDICINES

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Nanoparticle contamination with endotoxin is one of the grand challenges of nanomedicine. Endotoxin is a component of the cell wall of gram-negative bacteria. In contrast, to control standard endotoxin (CSE) used in analytical assays to quantify endotoxin contamination in the pharmaceutical products and medical devices, naturally occurring endotoxin is a very stable molecule. Due to its inherent stability and presence virtually everywhere, endotoxin is a potent biological contaminant in bio- and nanotechnology products. The danger of endotoxin contamination is that it induces inflammation at low (picogram level) concentrations, and may lead to serious health conditions such as septic shock and endotoxin tolerance. Moreover, some nanomaterials are not inflammatory

themselves but exaggerate endotoxin-mediated inflammation. Many engineered nanomaterials interfere with the LAL methods traditionally used for the detection and quantification of endotoxin. Assorted challenges also exist with alternative endotoxin detection methods. The mechanisms of the interference vary and depend on the nanoparticle physicochemical properties. In addition to assay interferences, some nanoparticles may entrap endotoxin and thus make it unavailable to the test. Translational challenges, case studies, and solutions will be discussed.

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CYTOKINES AND NANOPARTICLES: A TRANSLATIONAL CASE STUDY

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Many nanotechnology carriers, formulation excipients, and drugs are not immunologically inert and can either stimulate, modulate or inhibit the immune system function or affect its structure. Cytokines are important markers of the immunotoxicity and were also shown to be helpful in identifying immunoreactive nanoparticles.

Approximately one-tenths of the nanomaterials evaluated by the NCI Nanotechnology Characterization Lab induced pro-inflammatory cytokines. Over 60% of these materials induced pro-inflammatory chemokine IL-8, and more than 50% of IL-8 triggering nanoparticles did so exclusively, i.e. without induction of TNFα and IL-1β. The exclusive IL-8 inducers were typically liposomes and emulsions. The mechanism of such exclusivity is not entirely understood but includes an involvement of an oxidative-stress-mediated stabilization of the pre-synthesized IL-8 mRNA maintained by the mononuclear cells. Implications of this finding for the development of nanotechnology-formulated drugs will be discussed.

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HUMAN MULTI-ORGAN-CHIP TECHNOLOGIES – A STEP TOWARDS EMULATION OF SYSTEMIC ASPECTS OF HUMAN BIOLOGY IN VITRO

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Present *in vitro* and animal tests for drug development do not reliably predict the human outcomes of tested drugs or substances because they are failing to emulate the organ complexity of the human body, leading to high attrition rates in clinical studies. Various organ-on-a-chip systems have emerged over the last years to support predictive substance testing and disease modelling *in vitro* at relevant throughput. Fluid dynamics and fluid-to-tissue ratios fully emulating human biology remain a major challenge in such systems to overcome the current over-reliance on animal studies and static cellular assays in pharmaceutical and substance testing. Here we present a universal microfluidic chip platform the size of a microscopic slide, consisting of an on-chip micro-pump and, capable to interconnect up to four different organ equivalents. The micro-pump ensures stable long-term circulation of media through the tissue culture compartments at variable flow rates, adjustable to physiological mechanical stresses of the respective tissues. The tissue culture compartments and the connecting channels are optically accessible, thus supporting life tissue imaging. The layout supports both flexible integration of conventional miniaturized tissue culture formats, such as Transwell® inserts, special organotypic matrices or tissue exposed directly to the fluid flow. This Multi-Organ-Chip is capable of maintaining 3D tissues derived from cell lines, primary cells and biopsies of various human organs. Furthermore the connecting channels could be covered with human endothelia mimicking blood transport vessels. System layout and chip design support repeated substance exposure for safety or efficacy test assay development. Results from several case studies with different organ combinations on the Multi-Organ-Chip platform are shown: Skin – Liver, Liver – Neuronal tissuse, Intestine – Liver, Intestine – Liver- Skin – Kidney. In addition, a strategy to integrate vasculature into the Multi-Organ-Chip will be presented. Results from the presented studies show that the Multi-Organ-Chip technology can be a promising tool for the exposition of tissue cultures to pharmaceutical substances, cosmetic ingredients and chemicals at regimes relevant to respective guidelines, currently used for sub-systemic substance testing in animals. Building up on the expertise gained on the 2-Organ-Chip and 4-Organ-Chip platform a Mini-Organism-Chip containing 10 or more organs is currently being developed. This new Chip generation should then ultimately be able to not only reduce but also replace most of the animal studies currently required for safety and efficacy evaluation of substances.

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CRISPR BASED SYNTHETIC LETHALITY SCREENING TO IDENTIFY NOVEL ANTICANCER TARGETS

BASTIAAN EVERS

Mutated genes, inherited or somatic, that lead to dysfunctional proteins underlie many human diseases such as cancer and several developmental disorders. Genes and their products, however, do not function in solitude but in complex networks with functional redundancy and interdependency among the constituent nodes. When taking cell viability as a functional read-out, these genetic interactions lead to the existence of so-called synthetic lethal and synthetic fit phenotypes, where the simultaneous abrogation of two genes leads to a different phenotype than expected from the phenotypes of the individual genotypes.

These combinatory phenotypes have gained much attention due to their potential application in the development of novel therapies. Targeting the synthetic lethal partner of any gene mutated in cancer cells, or the synthetic fit partner of a gene mutated in patients with a developmental disorder may lead to therapies with unprecedentedly large therapeutic windows. Despite the huge promises of these genetic interactions, only few have thus far been discovered in human cells.

We have developed technology to uncover genetic interactions on a large scale and subsequently develop these into new targeted therapies for personalized medicine. In addition, (chemo)-genetic interaction networks may help in identifying targets of new drugs as well as functions of newly discovered genes, by comparing their interaction patterns to those of known drugs/genes.

GRAPHENE OXIDE INTERACTIONS WITH INNATE IMMUNE CELLS: NEUTROPHIL BIODEGRADATION AND INFLAMMASOME ACTIVATION

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The biocompatibility of graphene oxide (GO), especially its interactions with the immune system, should be controlled for successful applications in medicine. The innate immune system rapidly detects invading foreign materials and eliminates them, and neutrophils act as the first line of defense. Neutrophils can engulf and digest microbes or can release neutrophil extracellular traps (NETs) to digest microbes extracellularly. Here, we asked whether neutrophils also are capable of 'sensing' GO. To this end, we studied the interaction of freshly isolated primary human neutrophils with GO produced by a modified Hummers method. GO with small or large lateral dimension was produced and was shown to be endotoxin-free. The effect of GO on cell viability of freshly isolated neutrophils was determined by measuring intracellular ATP. Using transmission electron microscopy, we noted that GO flakes were aligned with the cell membrane leading to membrane stripping, and this effect was more pronounced for the larger flakes. Furthermore, GO was found to trigger NET formation in a size-dependent manner as evidenced by using scanning electron microscopy and confocal microscopy. GO was also shown to be degraded by myeloperoxidase (MPO) present in the NETs and this biodegradation was size-dependent. We also evaluated the interaction of GO with primary human monocyte-derived macrophages and found that GO with small or large dimensions were readily internalized yet were non-cytotoxic for these cells. However, GO triggered inflammasome activation in LPS-primed macrophages. The latter effects were similar for small and large GO. Overall, our work has provided new insights regarding the interaction of GO with innate immune cells and has revealed cell type-specific differences.

Further reading: Bhattacharya K, Mukherjee SP, Gallud A, Burkert SC, Bistarelli S, Bellucci S, Bottini M, Star A, Fadeel B. Biological interactions of carbon-based nanomaterials: From coronation to degradation. Nanomedicine. 2016 Feb;12(2):333-51.

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"POLYMERIC NANOPARTICLES: TUMOR MICROENVIRONMENT AND IMPLICATIONS FOR NEW NANOPARTICLE DESIGN AND DEVELOPMENT"

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A variety of organic and inorganic materials have been utilized to generate nanoparticles for drug delivery applications, including polymeric nanoparticles, dendrimers, nanoshells, liposomes, nucleic acid based nanoparticles, magnetic nanoparticles, and virus nanoparticles. The two most commonly used systems are polymeric nanoparticles and liposomes. Controlled release polymer technology has impacted virtually every branch of medicine, including ophthalmology, pulmonary, pain medicine, endocrinology, cardiology, orthopedics, immunology, neurology and dentistry, with several of these systems in clinical practice today such as Atridox, Lupron Depot, Gliadel, Zoladex, Trelstart Depot, Risperidol Consta and Sandostatin LAR. Polymeric nanoparticles can deliver drugs in the optimum dosage over time, thus increasing the efficacy of the drug, maximizing patient compliance and enhancing the ability to use highly toxic, poorly soluble, or relatively unstable drugs. These systems can also be used to co-deliver two or more drugs for combination therapy. The surface engineering of these nanoparticles may yield them "stealth" to prolong their residence in blood and the functionalization of these particles with targeting ligands can differentially target their delivery or uptake by a subset of cells, further increasing their specificity and efficacy. The successful clinical translation of therapeutic nanoparticles requires optimization of many distinct parameters including: variation in the composition of the carrier system, drug loading efficiency, surface hydrophilicity, surface charge, particle size, density of possible ligands for targeting, etc., resulting in a large number of potential variables for optimization which is impractical to achieve using a low throughput approach. Combinatorial approaches have been developed to precisely engineer nanoparticles and screen multiple nanoparticle characteristics simultaneously with the goal of identifying formulations with the desired physical and biochemical properties for each specific application. Despite these advances, therapeutic nanoparticles have shown heterogeneous responses in human clinical trials, raising questions of whether selection of patients with a higher likelihood of nanoparticle accumulation and thus therapeutic response is the key missing step. In parallel it is increasingly clear that enhanced permeability and retention (EPR) is extremely variable; yet, little experimental data exist to predict the clinical utility of EPR and its influence on therapeutic nanoparticle efficacy. The goal of this talk is to review our efforts in the design and optimization of polymeric nanoparticles for medical applications, which formed the foundation for the clinical translation of two first-in-kind targeted and controlled-release nanoparticles, and to discuss the lessons learned in this process.

DENDRONIZED SUPERPARAMAGNETIC NANOPARTICLES AS TOOLS FOR MRI, EFFICIENT *IN VIVO* CANCER TARGETING AND MAGNETIC HYPERTHERMIA TREATMENT

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An important area of nano-scale science is the development of nano-structured carriers for medical applications. Various colloidal inorganic nanoparticles that exhibit unique inherent properties such as fluorescence properties (e.g. semiconductor quantum dots (QDs) up-/down-conversion nanoparticles), magnetic properties (e.g. metal oxide nanoparticles) and plasmonic properties (e.g. noble metallic nanoparticles) have been widely explored, particularly for biological and medical applications. For instance, various types of magnetic nanoparticles have a widespread range of applications such as in magnetic resonance imaging (MRI) as T1 and T2 contrast agent, magnetically guided drug/gene delivery, magnetic hyperthermia and magnetic biosensors; up-/down-conversion nanoparticles and QDs and their niche in biological and medical imaging; noble metallic nanoparticles can be used for photothermal therapy and bio-sensing. A proper surface coating can stabilize particles and avoid agglomeration, which hence may increase the sensitivity of

NPs based sensor. Coating is also an effective manner of preventing the dissolution and release of core materials that may cause toxicity to biological system. Furthermore, the steric hindrance of coating can affect the fate of NPs in biological system, such as cellular uptake and accumulation, circulation and clearance from body. In addition, the surface can affect the maintenance of the intrinsic nanocrystal properties such as fluorescence and magnetic behaviour. Moreover, appropriate surface functionality is the perquisite for conjugating biomolecules to NPs for biomedical applications. A dendritic approach as a coating strategy for the design of functional nanoparticles is particularly interesting in the field of cancer diagnostics (Figure 1). The appeal of such strategy is due to the unique properties of the dendritic structures which can be chemically tuned to reach ideal biodistribution or highly and efficient targeting efficacies. Indeed, dendrimers are macromolecules consisting of multiple perfectly branched monomers and this architecture makes them versatile constructs for the simultaneous presentation of receptor binding ligands and other biologically relevant molecules. Additionally, dendrimers might serve as promising molecular scaffolds containing a number of ligands thereby inducing an apparent increase of ligand concentration and increasing the probability of statistical rebinding. Alternatively, dendrimers may align these ligands and induce multivalency when receptor clustering occurs or is initiated after initial monovalent binding.

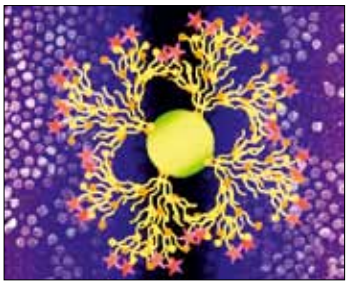

Figure 1 Dendronized iron oxide NPs as highly efficient in vivo cancer targeting probes

To improve tumour targeting efficacy and to obtain better *in vivo* imaging properties, our studies explored the multivalency effect of dendrimers or of a dendritic surface functionalization of nanomaterials. Iron oxide NPs synthesized by thermal decomposition were coated with functional oligoethyleneglycol dendrons to improve colloidal stability, graft fluorophores and melanocyte ligands to develop MRI probes allowing for an early spread-over melanoma metastases diagnosis. The size distribution, colloidal stability in isoosmolar media, nature of surface complex, biodistribution and contrast enhancement properties evaluated through *in vitro* and *in vivo* MRI experiments (Figure 2) were compared as a function of the nature of both dendrons and nanoparticles. Cell interactions and *in vivo* tumour targeting studies after iv injection of the dendritic probes were conducted (Figures 3 and 4).All functionalized nanoparticles display good colloidal stability in isoosmolar media. The contrast enhancement properties of all dendronized nanoparticles (Figure 2) were found higher than those of commercial products (polymer-decorated). Moreover, no evident adverse effect was observed in rat after injection, even at high concentrations and a long time after injection. The biodistribution of such nanohybrids was also studied by optical imaging thanks to Alexa labelling at the dendron periphery. In this case, a fast hepatobiliary, together with a low urinary, elimination was observed. Luckily, no RES uptake could be highlighted.

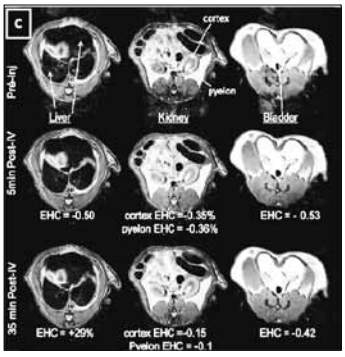

Figure 2 In vivo spin echo T2 weighted axial images before and after IV injection of PEGylated dendronized iron oxide NPs

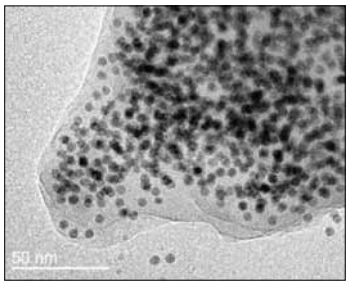

Figure 3 Ex vivo Transmission Electron Microscopy image of calcinated melanoma tumour after IV injection of dendronized iron oxide nanoparticles bearing a melanocyte-targeting ligand.

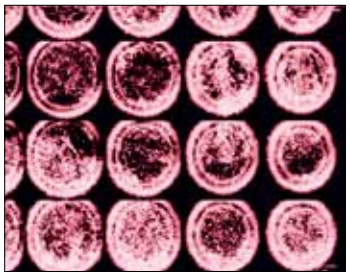

Figure 4 Ex vivo confocal images of a melanoma tumour taken at 658 nm + reflectance, showing the co-localization of the melanocytes and the dendronized iron oxide NPs, IV injected.

Intravenously injected Melanin-targeting dendronized iron oxides probes showed efficient *in vivo* targeting capacities studied by confocal microscopy. In this regard, multivalent but small core-shell nanoprobes (< 30 nm) may have a bright future not only for MRI imaging sensitivity enhancement but also for *in vivo* hyperthermia for cancer treatment.

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LOADING ANTIMALARIAL DRUGS INTO NON-INFECTED RED BLOOD CELLS: AN UNDESIRABLE ROOMMATE FOR PLASMODIUM

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The malaria parasite, Plasmodium spp., is a delicate unicellular organism unable to survive in free form for more than a couple of minutes in the bloodstream. Upon injection in a human by its Anopheles mosquito vector, Plasmodium sporozoites passing through the liver desperately try to invade hepatocytes. Those which succeed spend inside their host cell a recovery time before replicating and entering the blood circulation as fragile merozoites, although their exposure to host defenses is extraordinarily short. A quick invasion of red blood cells (RBCs) in a process lasting just a few minutes allows the parasite to escape immune system surveillance. For most of its erythrocytic cycle the pathogen feeds mainly on hemoglobin as it progresses from the early blood stages, termed rings, to the late forms trophozoites and schizonts. Early stages are ideal targets for antimalarial therapies because drugs delivered to them would have a longer time to kill the parasite before it completes its development. However, only six hours after invasion does the permeability of the infected erythrocyte to anions and small nonelectrolytes, in-

cluding some drugs, start to increase as the parasite matures. During this maturation process the parasite hydrolyzes hemoglobin in a digestive vacuole, which is the target of many amphiphilic drugs that freely cross the RBC membrane and accumulate intracellularly. As a result, most antimalarials start affecting the infected cell relatively late in the intraerythrocytic parasite life cycle, when their effect is probably often too short to be lethal for Plasmodium.

MALARIA-INFECTED ERYTHROCYTES: AN ELUSIVE TARGET

Several strategies to improve the activity of antimalarial drugs concern their encapsulation in nanocarriers specifically targeted to parasitized RBCs (pRBCs), an approach that requires the existence of specific pRBC markers. 200-nm liposomes studded with heparin or specific antibodies raised against pRBCs have been shown to bind late forms with high specificity, improving the activity of encapsulated antimalarial drugs up to tenfold. In addition to the inconvenient late-stage targeting, such liposomal delivery models will also have to overcome the obstacle of timing nanocarrier administration to the precise moment of the parasite's life cycle when trophozoites and schizonts are present. The relatively short blood half-life of liposomes (in the best case, <10 h for polyethylene glycol-coated stealth liposomes) guarantees that if injected at the wrong moment (too soon or too late), they will not last the 48h needed to ensure that they are present for the next pathogen's cycle. In another display of cunningness, Plasmodium leaves virtually no external signal on the parasitized cell, and only after spending half its life inside the erythrocyte does the parasite export a significant number of receptors and transporters to the host cell plasma membrane. Most of these externally recognizable clues are present in the parasite genome as multiple variants that can be clonally expressed, which further complicates delivery approaches designed to specifically target pRBCs. A receptor-independent alternative for the nanovector-mediated delivery of antimalarial drugs to Plasmodium blood stages can be provided by the tubulovesicular network (TVN) induced in the host cell by the pathogen during its intraerythrocytic growth, which confers pRBC accessibility to a wide range of particles up to diameters of 70 nm. Indeed, polymeric nanovectors were observed to penetrate trophozoites and schizonts, possibly in a significant fraction through the TVN, although entry of nanoparticles into early ring stages has not been observed so far.

IS THERE AN IDEAL CARRIER FOR BLOOD-CIRCULATING DRUGS?

Antimalarial drug carriers should provide optimal drug half-lives in circulation, adequate clearance mechanisms, restriction of unintended drug effects in non-target cells, specific delivery to the correct tissue, and a timely initiation and termination of the therapeutic action. Considering the need to target intraerythrocytic Plasmodium as early in its life cycle as possible and the lack of strategies currently out there for shuttling drugs into pRBCs, it is imperative that these issues are addressed and that alternative approaches are explored. A solution to the aforementioned problems in the design of pRBC-targeted nanocarriers can perhaps be provided by one of the most adequate vascular carriers, red blood cells themselves. Human erythrocytes have a life span in the blood of up to 120 days, which makes them attractive carriers for intravascular delivery because they prolong drug circulation. In addition, their large size (ca. 7 µm across and around 2 µm thick) significantly restricts unintended extravasation and in principle allows for a much larger encapsulation capacity than liposomes. Other interesting features of RBCs as drug carriers are their biocompatibility and the existence of natural mechanisms for their safe elimination from the body. In fact, delivery of antimalarials to non-infected red blood cells has been previously carried out in chemotherapeutic investigations, in order to examine the effects on later invading parasites. In one such study, red blood cells were pretreated with the drugs halofantrine, lumefantrine, piperaquine, amodiaquine, and mefloquine, which were observed to diffuse into and remain within the erythrocyte, inhibiting downstream growth of Plasmodium. However, it should be noted that the loading of drugs into non-infected RBCs has not yet been explored in detail as a clinically feasible therapeutic strategy against malaria, in part because of a number of restrictions that must be taken into consideration.

WHICH ARE THE LIMITATIONS OF ERYTHROCYTES AS DRUG CARRIERS?

A significant limiting factor for the use of RBCs as antimalarial carriers is that when present at therapeutically active concentration, the drug has to be innocuous for the cell physiology, which might not be an unsurmountable obstacle given the reduced metabolic activity of eryrthrocytes. However, loading of some antimalarial drugs like clotrimazole had been observed to predispose RBCs to oxidative damage, an undesirable scenario since oxidized RBCs are rapidly taken up by hepatic reticuloendothelial system macrophages. Another obstacle for the incorporation of antimalarial drugs into RBCs is drug loading itself, since most currently available protocols use a harsh ex vivo isolation of erythrocytes followed by drug loading by diffusion. In a clinical setting, perhaps RBC-targeted immunoliposomes can come to rescue, although the incapacity of mature erythrocytes to endocyte calls for the development of specific targeted drug delivery strategies independent from the receptor-mediated endocytic pathway. Moreover, the physicochemical properties of each particular antimalarial drug will constrain the nanovector composition and the corresponding drug delivery mechanism. As an example, immunoliposomal fusion with the RBC membrane would be the optimal approach for the delivery of membrane-impermeable hydrophilic drugs such as fosmidomycin, which requires the incorporation of special fusogenic agents into highly fluid vesicles. Including negatively-charged phospholipids in the liposome formulation has been found to be crucial for the delivery of trehalose into RBCs *in vitro*, but nanovector fusion can be inhibited by components found in plasma, and charged vesicles are quickly complexed by serum proteins that target them for clearance from circulation. A possible solution consists of incorporating stealth agents onto the nanovector surface like polyethylene glycol chains or gangliosides, which neutralize vesicle charge and significantly reduce unspecific interaction events, although they can also interfere with fusion if excessive amounts are used.

The capacity of amphiphilic antimalarial drugs (which comprise the extensive aminoquinoline and artemisinin drug derivative families) to easily cross lipid bilayers demands a careful design of their targeting liposomes. Active loading techniques based on pH gradients across liposome membranes are required to efficiently encapsulate the fully ionized species of amphiphilic drugs, in combination with a saturated lipid-enriched bilayer capable of maintaining a proton gradient. As a consequence of the reduced fluidity of the resulting membrane, fusion events with targeted cells are significantly inhibited, and sustained drug delivery, while the liposome is docked onto the RBC, would be the most likely mechanism through which such nanovectors operate. This process would be mediated by a depletion of the liposomal proton gradient by means of temperature, liposome-cell interaction events and lipid transference to plasma components, and might be highly effective for the delivery of weak basic drugs such as those from the aminoquinoline family. These compounds, positively charged at neutral pH, will theoretically accumulate inside the cell and become entrapped by virtue of the electrochemical gradient created by the phospholipid asymmetry in RBC membranes, which maintains a negatively charged intracellular membrane lining. Liposomal nanovectors are also efficient carriers for hydrophobic drugs like lumefantrine and halofantrine, which can be delivered to RBCs following a sustained release process by an exchange mechanism of hydrophobic material between the apposed membranes of liposome and erythrocyte. Since the liposomes adsorbed on RBC surfaces would probably sufficiently modify cell shape to target it for removal through spleen filtration, a compromise between stable drug containment and lipid bilayer fusion will have to be reached through the adequate liposome formulation, with the objective of achieving liposome-RBC merging before spleen removal while avoiding rapid drug leaking from liposomes.

It is reasonable to predict that the nanovector design limitations exposed above can be satisfactorily dealt with, and that some of the future antimalarials yet to be discovered will be harmless for

erythrocytes, thus allowing for the loading in these cells of drug amounts that are lethal for Plasmodium. If so, the pathogen might encounter its enemy at home, right at the very moment of entering the host cell, which would have devastating effects for the parasite and significantly compromise its survival capacity. Such a strategy could be likely developed into a prophylactic treatment against erythrocyte infection.

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PROLONGED INTRACELLULAR ACCUMULATION OF LIGHT-ACTIVATABLE NANOPARTICLES IN LEUKEMIC CELLS ALLOWS REMOTE ACTIVATION

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The differentiation of leukemic cells is a therapeutic platform very often used in the clinic to eradicate blood cancers, being the concentration of the inductive agent and the spatio-temporal control of its application very important variables for the success of the therapy1. Induction of leukemic cell differentiation by RA is a therapeutic strategy that has been used with great success in the treatment of acute promyelocytic leukaemia (APL)[2, 3]. RA activates nuclear RA receptors (RARs) that induce cell growth arrest and differentiation4. Despite its clear therapeutic efficacy, approximately 25% of patients receiving RA will develop serious complications including the "differentiation syndrome"[5], and thus the need for more effective formulations to deliver RA into leukemic cells while preventing RA side effects. In addition, leukemia-maintaining cells that resist to therapy reside in microenvironmental niches at the bone marrow that are difficult to reach by conventional therapy6. Therefore, news strategies are required to tackle this problem.
NPs that disassemble in response to light7[-9] might address both issues. Recent light-activatable NPs have been reported to target solid tumors based in the accumulation of the NPs in tumor vasculature after intravenous injection10. Yet, such approach is not extensive to leukemias. The hypotheses of the current work are: (i) light-activatable NPs containing RA might be a more effective strategy to differentiate leukemic cells because they release high and more effective concentrations of RA in a short period of time (minutes range) after NP disassembly, and (ii) light-activatable NPs containing RA accumulated in the cytoplasm of leukemic cells might offer a unique opportunity to differentiate remotely these cells in leukemic niches at the bone marrow, which in turn might interfere with the differentiation profile of the leukemia-maintaining cells by paracrine factors.
Here, we describe light-activatable polymeric NPs that are very effective in accumulating in the cytoplasm of leukemia cells and to induce cell differentiation either *in vitro* or *in vivo* after light activation. To prepare light-activatable polymeric NPs, poly(ethyleneimine) (PEI) was initially derivatized with 4,5-dimethoxy-2-nitrobenzyl chloroformate (DMNC), a light-sensitive photochrome. PEI was selected as initial NP block because it facilitates the cellular internalization of NPs and subsequent escape from endosomes11, 12, while DMNC was selected because responds rapidly to light and the degradation products are relatively non-cytotoxic13. PEI-DMNC was then added to dextran sulfate (DS) to form NPs by electrostatic (PEI:DS) and hydrophobic (DMNC:DMNC) interactions. To stabilize the NP formulation, zinc sulfate was added12, 14. NPs with an average diameter of 108.1 ± 9.9 nm and a zeta potential of 27.4 ± 1.6 mV were obtained. Our results show that light-activatable NPs rapidly (minutes range) release retinoic acid (RA) when exposed to a blue laser/UV light. These NPs reduce more efficiently the clonogenicity of bone marrow tumor cells from patients with acute myeloid leukemia (AML) and induce more efficiently the differentiation of RA-low sensitive leukemia cells than NPs without light activation (Figure 1). Further, we show that leukemia cells transfected with light-activatable NPs containing RA can engraft into the *in vivo* bone marrow, in the proximity of other leukemic cells, be differentiated after blue laser activation, and release paracrine factors that modulate leukemic cells in the vicinity.

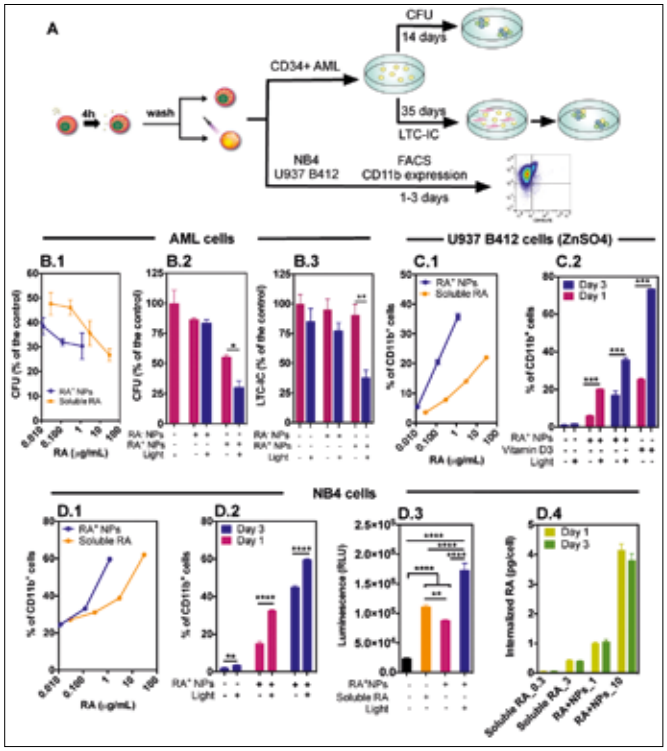

Figure 1- Effect of RA⁺ NPs on human leukemia cells. (A) Schematic representation of the methodology used. Cells were treated with RA⁺ NPs for 4 h, washed, activated or not with UV light (365 nm, 100 Watts) for 5 min, and then cultured for a certain period of time. In case of cells treated with soluble RA, cells were cultured in media containing soluble RA for the entire period of culture. (B.1) Differentiation of CD34⁺ AML cells isolated from the bone marrow aspirates of patients with AML cultured with light-activated RA⁺NPs or soluble RA. Cell differentiation was evaluated by a colony forming unit (CFU) assay. (B.2) Differentiation of CD34⁺ AML cells with RA⁺NPs (10 µg/mL) or blank (RA-NPs) NPs (10 µg/mL), exposed or not to UV light, by a CFU assay. (B.3) Differentiation of CD34⁺ AML cells with RA⁺NPs (10 µg/mL) or blank NPs (10 µg/mL), exposed or not to UV light, by a long-term culture-initiating cell assay. Results are expressed as a mean percentage of control plates containing only AML cells. (C.1) Myelocytic differentiation of human Zn-induced U937-B412 cells cultured with light-activated NPs or soluble RA at day 3. (C.2) Myelocytic differentiation of human Zn-induced U937-B412 cells cultured with RA⁺NPs with or without light activation. Cells cultured with 10^{-7} M of vitamin D3 were used as positive controls. (D.1) Myelocytic differentiation of human NB4 cells cultured with light-activated NPs or soluble RA. NB4 cells were cultured for 3 days. (D.2) Myelocytic dif-

*ferentiation of human NB4 cells cultured with RA⁺NPs with or without light activation. (D.3) Intracellular release of RA as evaluated by a RARE luciferase cell line. NB4-RARE cells were cultured with soluble RA (3 µg of RA per mL) for the entire duration of the experiment, or light-activatable RA⁺ NPs (5 µg/mL; 0.6 µg of RA per mL). Cells were exposed to NPs for 1 h, washed with PBS, and resuspended in cell medium. Some samples were exposed to UV light for 5 min. The cells were then cultured for 24 h before luciferase luminescence reading. (D.4) [³H]-RA uptake by NB4 cells. NB4 cells were cultured with soluble ³H-RA (0.3 and 3 µg/mL) for the entire duration of the experiment, or light-activatable ³H-RA⁺ NPs (1 and 10 µg/mL) for 4 h and then the cells were washed and cultured in cell medium for additional 20/68 h before scintillation counting. In B.1, B.2, B.3, C.1, C.2, D.1, D.2, D.3 and D.4, results are expressed as Mean ± SEM (n=3). *P<0.05, **P<0.01, ***P<0.001.*

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MULTICOLOR SUPER-RESOLUTION STED MICROSCOPY FROM ITS INVENTORS-TURN-KEY, LIVE CELL, 30NM RESOLUTION

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Stimulated Emission Depletion (STED) microscopy invented by Abberior-Instruments' co-founder Stefan W. Hell in 1994 is a microscopy method that extends the resolution of conventional light microscopes to length scales far below the diffraction limit of light. STED microscopy has thus become a powerful tool that allows for imaging of biological samples at spatial scales that were not accessible before. STED microscopy and other super-resolution microscopy techniques have matured from laboratory curiosities to commercial products that are being actively developed and are getting more powerful and user-friendly every day.

Owing to this fast-paced development we are updating our technology in fast development cycles and as a result, we offer a platform for STED and RESOLFT super-resolution microscopy that delivers cutting edge performance while being highly customizable in order to meet the customers' experimental requirements.

In our easy3D STED microscopes, a liquid-crystal device (spatial light modulator) is employed to generate the STED focus. This active optics device allows the user to choose between purely lateral resolution enhancement ("2D STED mode") and a combined resolution enhancement in all three spatial directions ("3D STED mode") with a single mouse click. Easy3D STED is compatible with a variety of objectives – including water-immersion objectives – making it highly attractive for live-cell imaging. Moreover, optical aberrations induced to the STED focus by refractive-index mismatches within samples can be corrected in our easy3D STED microscope. The resolution of our microscopes routinely reaches 30nm x 30nm x 500nm in 2D STED mode and 80nm x 80nm x 90nm in 3D STED mode, compared to 250nm x 250nm x 500nm in a regular confocal microscope.

Live cell imaging is one of the most important applications of light microscopy in biology and medicine. To facilitate live cell STED microscopy we have launched a novel microscopy technique called RESCue (REduction of State transition Cycles) STED. In this technique lasers are switched intelligently on and off during the image acquisition. Thereby the applied light dose is reduced by a factor of 5 as compared to regular pulsed STED microscopy and a factor of 25 as compared to gated STED with continuous wave depletion lasers – without any loss in resolution. This leads to considerably lower photobleaching and lower phototoxicity. Hence, it enables long term observations of living cells, the use of less photostable dyes, and the acquisition of large 3D volume stacks.

In combination with the development of new bright, live-cell compatible, red-emitting dyes, we expect live-cell RESCue STED to become a powerful routine application in biological imaging.

COMPUTATIONAL DESIGN OF PEPTIDE BASED ARCHITECTURES FOR BIOMARKER RECOGNITION

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An ideal binder should be capable of capturing with high affinity, sensitivity, and specificity a target molecule, such as an organic molecule or a biomolecule. Examples include antibodies and minibodies typically optimised *in vivo*, as well as DNAs and RNAs based aptamers evolved *in vitro*, and peptides optimised in silico. This latter opportunity offers invaluable advantages as peptides small number of amino acids allow for their optimisation by exploiting current advances in computing power together with the development of new and powerful algorithms for protein folding, docking, and structure prediction. Computer optimized peptides have already been successful in the framework of drug recognition. In particular a recent algorithm based on replica exchange Monte Carlo proposed by Laio and co-workers, is able to optimize simultaneously the sequence and conformation of small peptides in order to reach a high binding affinity to a target organic molecule [1]. The same algorithm has been recently adapted for the generation of peptides for protein recognition (Fig.1). Our results have shown how computationally-generated peptides can bind with nM affinity a target protein[2]. Recent algorithm developments allow generating peptides with an even higher affinity towards their targets or even picking a surface exposed non-druggable binding site and design ex novo a binder capable of immobilizing a protein on a surface.

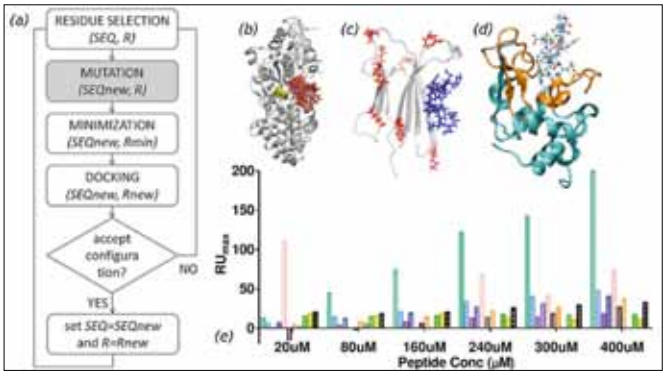

Fig.1: (a) The algorithm we use to optimize sequence (SEQ) and conformation (R) of a random starting peptide of fixed length iteratively (i) mutates the peptide, (ii) minimises its structure, and (iii) docks the new structure to the target, (iv) accepts or rejects the mutation following a Monte Carlo based acceptance probability [1,2]. Peptides have been generated for a number of model systems: (a) maltose binding protein, (b) beta-2-microglobulin, and (c) lysozyme. (d) Surface plasmon resonance shows signal variation upon peptide-protein binding, showing the effectiveness of the computation protocol proposed.

Further, to push forward the binding affinity of a binder towards its target, we have studied the possibility of building a molecular probe consisting of two binding moieties linked through a flexible spacer (Fig.2). It is in fact well known in coordination chemistry that polydentate binders have enhanced affinity compared to the affinity of a collection of monodentate binders, the simplest example being that of dicarboxylic acids binding a metal with stronger affinity with respect to that of the corresponding uncoupled acids (the so-called "chelating effect"). Nature itself exploits the synergy amongst coupled multiple binding sites in biological systems. For instance antibodies are capable of binding their target thanks to a number of coupled loops. Here we propose to exploit this characteristic to design generic binders with enhanced affinity towards large macromolecules. The setup we propose, that we call "nanoheadphones", enables two binding moieties to reach two different sites on the same macromolecule. To optimise its design we make use of a minimal model and Monte Carlo simulations. We show that flexible linkers are a viable option to enhance the ability of two binding moieties to reach their target as the free energy cost of dissociating two coupled binders is higher than that of dissociating two monodentate binders (chelating-like effect). By optimizing the free energy gain, we then pinpoint the optimum nanoheadphones design strategies[3].

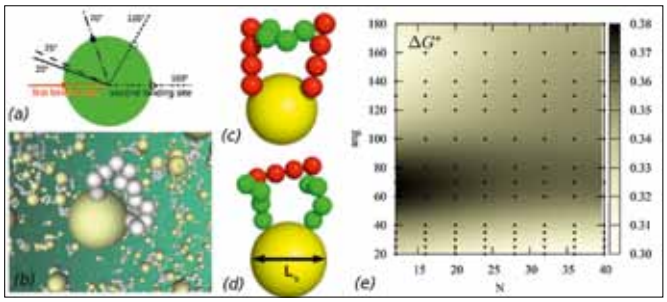

Fig.2: Bidentate polymers for protein biosensing are here modelled as a chain of beads interacting through their extremes with (a) a spherical target having two binding sites. Different polymer flexibility are taken into account: (b) fully flexible, (c) extreme-rigid, (d) center-rigid. Simulations reveals that fully flexible polymers with a number of beads N smaller than 10 and for binding angles between 60 and 80 degrees, the dissociating fee energy is maximised, making this setup the most promising for maximising the binding affinity of binders coupled through fully flexible linkers[3].

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NOVEL APPROACHES FOR INDUSTRY/ACADEMIA COOPERATION IN REGENERATIVE MEDICINE

RUDOLF FRYČEK, AMIRES/CONTIPRO, Dolní Dobrouč (CZ)

Translation of nanomedicine or novel medical devices from laboratory to clinical practice is a complex process, with multiple barriers, requiring specific knowledge and experience. Due to this complexity, many great inventions ends in proof of concept level, with no or limited effort towards commercialization. Additionally, an important ratio of European inventions are finally not manufactured in Europe and/or the integrating company is based outside of Europe, which actually strongly questions the effectiveness of public funding in this domain.

The presentation will propose a new cooperative model between inventors and medium-sized companies and various modalities will be explained. These models could be considered as exploitation strategies for public funded projects on various levels. They also present significant opportunity for inventors and innovators (enterprises) and it could effectively stimulate the European industrial environment in respective markets.

PRECLINICAL AND CLINICAL EXPERIENCE WITH PROMITIL®[1], A NANO-FORMULATION OF A LIPOSOMAL ANTI-CANCER PRODRUG

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1 Promitil® is a product of Lipomedix Pharmaceuticals Ltd.

Promitil, a pegylated liposomal (PL) nano-formulation of a lipid-based prodrug of mitomycin C (MLP) has recently entered clinical testing in cancer patients. We studied the preclinical and clinical pharmacology of PL-MLP. The stability, pharmacokinetics, biodistribution, and other pharmacologic parameters of PL-MLP were examined. Thiolytic cleavage of MLP and release of active mitomycin C (MMC) were studied using dithiothreitol (DTT), and by incubation with tissue homogenates. Pharmacokinetic data were collected from a dose escalation phase 1 study in cancer patients. *In vitro*, DTT induced cleavage of MLP with predictable kinetics, generating free mitomycin C (MMC) and enhancing pharmacological activity. A long half-life of MLP (10-15 hours) was observed in rodents and minipigs. Studies in mice with H3-cholesterol radiolabeled PL-MLP demonstrated relatively greater tissue levels of H3 than MLP. MLP levels were highest in tumor and spleen, and very low or undetectable in liver and lung. Rapid cleavage of MLP in various tissues, particularly in liver, was shown in ex-vivo experiments of PL-MLP with tissue homogenates. Urine from PL-MLP injected rats revealed delayed but significant excretion of MMC indicating *in vivo* activation of MLP. Therapeutic studies in C26 mouse tumor models demonstrated improved dose-dependent efficacy of PL-MLP over MMC.
The pharmacokinetics of PL-MLP in a first-in-man study showed a median $t_{1/2}$ of 23 hours, with no trend by dose or cycle, while C_{max} and $AUC_{0-\infty}$ increased linearly over the dose range 0.5-2.0 mg/kg, and greater than linearly from 2.5-3.5 mg/kg. No free MMC was detected. The human results were consistent with preclinical observations.
A phase 1A, dose-escalating study, with Promitil® in 27 patients with advanced cancer showed that the maximal tolerated dose of Promitil® (in mitomycin C-equivalents) is ~3-fold greater than the maximal recommended dose of MMC, indicating that toxicity is substantially reduced. The cumulative dose-limiting toxicity of Promitil® was found to be thrombocytopenia. Clinical benefit (stable disease, partial response) was noted in 11 patients. A phase 1B study of Promitil® in combination with capecitabine and bevacizumab in patients with advanced colon cancer is currently ongoing.
In conclusion, thiolytic activation of PL-MLP occurs in tissues but not in plasma. Liposomal delivery of MLP confers a favorable pharmacological profile and greater therapeutic index than MMC. Further clinical testing is ongoing.

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REDEFINING CANCER BY INTEGRATING THE IMMUNE SYSTEM: TRANSFERRING CUTTING EDGE MEDICINE TO THE PATIENTS

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To date the anatomic extent of tumor (TNM-classification) has been by far the most important factors to predict the prognosis of cancer patients. However, this classification provides limited prognostic information and does not predict response to therapy. We redefined cancer by integrating the immune system, to transfer cutting edge medicine to the patients. We showed that tumors from human colorectal cancer with a high-density of infiltrating memory and effector-memory T-cells (Tem) are less likely to disseminate to lymphovascular and perineural structures and to regional lymph-nodes. We demonstrated the critical tumor-microenvironment parameters determining the dissemination to distant metastasis. We showed that the combination of immune parameters associating the nature, the density, the functional immune orientation and the location of immune cells within the tumor was essential to accurately define the impact of the local host-immune reaction on patients prognosis. We defined these parameters as the "immune contexture". We characterized the immune landscape within human tumors, and showed the importance of adaptive immune cells including, cytotoxic T-cells, Th1-cells, B-cells and T-follicular-helper (Tfh) cells. We described the immunopenotype and antigenome associated with immune escape mechanisms and demonstrated mechanisms associated with pre-existing and proliferating intratumoral T-cells.
Based on the immune contexture, a standardized, simple and powerful digital-pathology-based immune stratification-system, termed "Immunoscore", was delineated having a prognostic power superior to that of the currently used cancer staging-system. Tumor invasion parameters were statistically dependent on the host-immune reaction. A worldwide consortium is validating the prognostic value of Immunoscore, using a standardized-assay. Recent data are supporting the significant role of Immunoscore within lung, liver, and brain metastases. Thus, tumor progression, invasion and recurrence are dependent on pre-existing immunity and on Immunoscore.

LIPOSOME DELIVERY OF M2 POLARIZING AGENTS TO MACROPHAGES

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Chronic inflammatory conditions are characterized by a sustained high number of active pro-inflammatory M1 macrophages combined with a lack of anti-inflammatory/pro-resolution M2 macrophages that actively support the resolution of inflammation. Glucocorticoids are well established anti-inflammatory agents driving M2 polarization, however, side-effects limit their use in systemic applications. Hence, delivery of such agents specifically to monocytes/macrophages e.g. via liposomes represents a new therapeutic avenue.
The objective of the present study was to compare liposome formulations encapsulating the pro-drug dexamethasone phosphate (DexP) with either 10% phosphatidylserine (PS) or polyethylene

glycol (PEG) in their lipid bilayer. These formulations were evaluated based on their ability to enhance the pro-resolution activity of human primary macrophages in terms of efficacy of delivery, cell polarization status and induction of pro-resolution functions.
Both types of DexP liposomes were able to drive a Dexamethasone-like M2 signature, with the upregulation of CD163, MERTK, IL1R2, ALOX15B in monocyte primary cultures grown for 1 day. A kinetic study suggested a more robust uptake of the DexP PS liposomes than the PEG liposomes, on short times (3-5hrs), but with similar activity of both formulations at 24hr. We could also show the induction of the Dexamethasone-like profile at the protein level, with the upregulation of MERTK after 3 days of treatment.
An additional characteristic of M2 macrophages is the reduced release of LPS-induced pro-inflammatory cytokines: treatment of monocytes for 24hr with DexP liposomes decreased IL6 and TNFa release. In mature macrophages, DexP PS liposomes (and with lower efficiency PEG liposomes) induced an M1 to M2 switch in INFg-induced pro-inflammatory macrophages and an anti-inflammatory cytokine profile in control macrophages.
We further investigated the impact of the DexP liposomes on the ability of the macrophages to take up apoptotic cells in an efferocytosis assay with daily addition of liposomes over 3 days. We found that both DexP formulations stimulate an increase in efferocytosis in comparison to the control liposomes and this directly correlated to MertK expression. Integration of PS in the liposomal bilayer, potentially mimicking the membrane of apoptotic cells, did not amplify the response of macrophages to the liposomes.
In summary, we have shown that DexP liposomes can induce an anti-inflammatory Dex-like phenotype, at the gene level, by cytokine profiling and in a functional assay. The formulations showed similar properties, except an interesting higher potency of PS liposomes in M1 macrophages. Although short term delivery of Dexamethasone is lower with liposome encapsulated DexP than with the free molecule, a longer treatment time shows that both DexP liposome formulations were able to achieved the efficacy of free Dexamethasone in the efferocytosis assay.

TAKING AFM TECHNOLOGIES TO THE CLINIC

CHRISTOPH GERBER

According to the American skin cancer foundation, there are more new cases of skin cancer than the combined incidence of cancers of the breast, prostate, lung and colon each year and malignant melanoma represents its deadliest form. About 50% of all cases are characterized by a particular mutation BRAFV600E in the BRAF-gene. Recently developed highly specific drugs are able to fight BRAFV600E mutated tumors, but require diagnostic tools for fast and reliable mutation detection to warrant treatment efficiency. We performed the first clinical pilot study based on nanomechanical microcantilever sensors demonstrating the identification of the BRAFV600E single-point mutation in total RNA extracted from surgical samples of metastatic melanoma of different origins and forms (frozen or formalin-fixed paraffin-embedded tissues). The method is faster than the standard Sanger or pyrosequencing methods and comparably sensitive as next-generation sequencing. Processing time from biopsy to diagnosis is below one day and does not require PCR-amplification, sequencing and labels.

ENATRANS – THE EU-FUNDED COORDINATION AND SUPPORT ACTION TO ENABLE NANOMEDICINE TRANSLATION

NICOLAS GOUZE

ENATRANS' main objective is to network and support SMEs in translation of nanomedicine in Europe by providing a one-stop-shop service to interact and share information, experience and advice with up-to-date information and interactive tools, but also enabling personal contacts.
To be successful SMEs need to understand the requirements of the complex ecosystem made up of regulatory and reimbursement agencies, as well as the requirements of large industrial companies and clinical needs. ENATRANS gathers and provides information about approval processes, regulatory authorities and agencies, clinical and market data, and specific nanomedicine value chain analysis, relevant SME support projects and organisations. ENATRANS converts this critical information in dedicated learning programs and tools dedicated to translation of nanomedicine.
As central tool of the action, ENATRANS implements the concept of a Translation Advisory Board (TAB) with senior experienced translation experts to guide R&D teams in SMEs and research institutes along the translation process to successfully make it to clinical trials and later to the market. Promising projects identified and supported by the TAB need access to clinical centers for first studies in patients, which are able to handle the specificity of new nanotechnology based therapeutics. In addition, financial resources are needed to go pass the regulatory and scale-up processes, which often require funding from investors and/or large companies. ENATRANS builds these bridges to clinical trial centers, investors and large companies with dedicated actions and events.
ENATRANS is coordinated by VDI/VDE-IT (Germany) and benefits from experience, capacities and competencies from following partners: Nanobiotix (France), CEA-Leti (France), bioanalytik-muenster (Germany), Tel Aviv University (Israel), Fondazione Don Gnocchi (Italy) and TecMinho (Portugal).

US-EU COOPERATION ON NANOMEDICINE CHARACTERIZATION

DR. JENNIFER GROSSMAN, Senior Scientist, Nanotechnology Characterization Laboratory (NCL), National Cancer Institute, Leidos Biomedical Research, Inc., Frederick, (USA)

The US NCL is a public-private partnership with the mission of accelerating the development of promising nanotech cancer therapies in order to bring more effective, affordable treatments to patients. The NCL was founded in 2004 as a partnership of the National Cancer Institute (NCI, one of the National Institutes of Health), the FDA, and the National Institute of Standards and Technology (NIST, part of the Department of Commerce). NCL's initial mission was to develop an "Assay Cascade" of scientific tests that would help determine the reproducibility, safety, and efficacy of cancer drugs and diagnostics involving nanotechnology. Once developed, the NCL Assay Cascade was used to generate data in support of regulatory filings, to help investigators get their promising nanotech cancer drugs into clinical trials, and to help spin-off companies garner investment in their technologies. The NCL has tested more than 350 nanomaterials, including almost every type of nanoparticle used in biomedical R&D: metalic, liposomes, polymers, proteins, micelles, DNA and RNA nanostructures, CNTs, etc. In addition, NCL has helped several nanomedicine formulations enter and progress through clinical trials.
As part of the transatlantic collaboration on nanomedicine, the NCL has been funded to host guest researchers from the EU-NCL for training, cross-validate methods, provide consultation, and facilitate transatlantic interlaboratory studies and round robin tests. The aim of the US-EU collaboration is to accelerate the establishment of the EU-NCL by sharing US NCL's 12 years of experience in developing and implementing processes for characterizing nanoparticles. Further objectives of the collaboration are to facilitate standardization, improve quality control, and leverage shared expertise toward the development of novel methods. This presentation will provide an overview of the ongoing US-EU cooperation to establish the EU-NCL, highlight lessons learned, new developments, and results thus far.

ORGANS-ON-CHIP FOR DRUG DEVELOPMENT

OLIVIER T. GUENAT[1,2,3], Colette Bichsel[1], Janick Stucki[1], Andreas Stucki[1], Marcel Felder[1], Artur Galimov[1], Yves Mermoud[1], Sean Hall[3], Ralph Schmid[3], Thomas Geiser[2]

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INTRODUCTION:

Organs-on-chips are believed to become the next generation of in-vitro models as they are expected to better predict the drugs' response in humans than standard in-vitro models. They make it possible to accurately reproduce important biophysical aspects of the cellular microenvironment, including dynamic factors, such as the shear stress induced by the blood flow and the mechanical strain generated by the respiratory movements. Here, we report on two lung-on-chip models that mimic key parameters of the air-blood barrier. A first model is a lung-on-chip that reproduces the thin alveolar barrier as well as the cyclic strain of the respiration [(1)] a second in-vitro model mimics a perfusable and functional lung microvasculature [(2)].

METHODOLOGY:

Both organs-on-chips are produced using standard soft lithography techniques, in particular PDMS casting on hard polymeric molds. The lung-on-chip is equipped with a 3um-thin, porous (3um pores) and flexible membrane in PDMS produced using a deep reactive ion-etching mold in silicon. Cell lines and primary cells are cultured on the chips on a coated membrane (fibronectin or collagen). A three-dimensional physiological mechanical strain – that corresponds to 10% elongation – is applied to the cells by cyclically deflecting the thin, porous membrane at a frequency of 10 breathings per minute. Two molecules (RITC-Dextran, and FITC-Na+) are used to assess the effects of the cyclic stress on their transport through the epithelial barrier. The epithelial microinjuries are induced by scratching the epithelial layer cultured on the porous membrane with a micropipette. Human primary endothelial cells and lung pericytes are cultured in a fibrin gel environment contained in microcompartments. Pericytes are obtained from patients following surgical resection for lung cancer.

RESULTS:

We show that the applied physiological three-dimensional mechanical strain importantly affects a number of biological processes regarding health and diseases. A study about the permeability of the epithelial barrier exposed to different molecules showed that the transport of the molecules through the barrier depends on the size of the molecules and on the cyclic stress, an effect also observed in-vivo. In a second study, the effect of a physiological mechanical stress on the wound-healing process of an alveolar epithelium has been demonstrated. Wound-healing is an important process in lung fibrosis, where repetitive lung alveolar epithelial microinjuries are thought to be at the onset of the disease. The developed lung-on-chip array not only accurately mimics the lung parenchymal environment, but is easy to handle and well suited for air-liquid interface experiments (Fig.1).

Fig. 1. Lung on chip that mimics the thin alveolar barrier as well as the cyclic mechanical strain induced by the respiration (from (3)). Scale bar: 5mm.

In the lung microvasculature-on-chip model, endothelial cells and pericytes self-assemble in a tight, stable and perfusable microvessel network within the microcompartment filled with fibrin gel. The role of the pericytes is crucial to the stability and tightness of the microvessels. In addition, their presence enables the restoration of one of the key functions of the microvasculature. Indeed upon exposure to a vasoconstrictor, phenylephrine, the lung capillaries contracted within a few minutes. In contrast, capillaries without pericytes do not contract when exposed to phenylephrine.

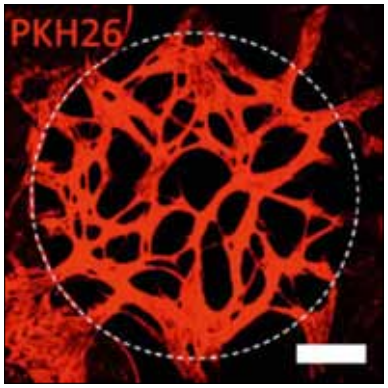

Fig. 2. Lung microvasculature on chip. Endothelial cells and pericytes form tight and stable microvessels (adapted from (2)). Scale bar: 500um.

CONCLUSION:

We demonstrate that by reproducing key biophysical parameters functional in-vitro models can be created. Such organs-on-chip systems have the potential to be used to screen new molecules in the drug discovery process for their efficacy in a very near future.

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RNA NANOMEDICINES FOR INDIVIDUALIZED TUMOR IMMUNOTHERAPY: PATIENT TRIALS

HEINRICH HAAS

Therapeutic vaccination against cancer represents a new approach for tumor therapy, complementary to classical options as chemotherapy, radiotherapy and surgery. Various products have reached clinical development or clinical trials.

Here, we report on the development progress of a new class of RNA pharmaceuticals for tumor immunotherapy, where tumor antigen-encoding mRNA is delivered into antigen presenting cells (APCs) in order to induce T-cell mediated antitumoral responses. This concept enables provision of truly personalized cancer vaccines for the treatment of patients. Several clinical trials have been initiated using synthetic RNA vaccines for local (intranodal) and systemic (intravenous) injection. For intravenous injection, a nanoparticulate RNA lipoplex product for specific APC targeting has been developed, which is the first in its class that has made its way from benchtop to bedside. A fixed set of four liposome formulated RNA drug products (DPs) each encoding one shared tumour antigen (Lipoplex Melanoma RNA Immunotherapy, "Lipo-MERIT") has been applied successfully to patients. In this presentation, the steps for translating these novel RNA nanomedicines into clinical trials, current status, and future perspectives are summarized.

CONTROLLING BIODISTRIBUTIONS WITH GLYCAN-TARGETED LIPOSOMES – AIMING FOR TUMORS AND BEYOND

STEFAN HALBHERR

Drug delivery with high target tissue-specificity has been subject of intense research over many decades. While liposomes gained broad acceptance as a platform for encapsulating and delivering drugs, it is yet largely unclear how exactly nanocarrier surfaces enable specific tissue targeting. Current attempts of active targeting generally rely on protein-protein interactions of either antibodies or peptide ligands with their corresponding receptors. Even though preclinical results were often promising, the clinical translation of these formulations has been difficult. We here propose a novel targeting system inspired by the migratory mechanisms of immune cells and body-own lipid nanoparticles. The approach makes use of surface-exposed glycan molecules as ligands with advantageous properties such as non-immunogenicity, high hydrophilicity, and molecular specificity to multiple different lectin targets with varying binding strengths. Up to now, numerous studies describe the pivotal role of the glycan-lectin interplay in the functionality of the blood system, inflammatory reactions, host-pathogen interactions of viruses and bacteria, as well as tumor differentiation and metastasis. In sum, there is a strong rationale that lectins can work as highly promising targets for specific drug delivery.
We showed that liposomal glycans displayed on the liposome surface could drastically influence biodistribution patterns. In recent studies, liposomes were labelled with near-infrared tracer dye for *in vivo* biodistribution analysis. Different glycan-targeted and non-targeted formulations were investigated and tissue distribution analysis was performed in mice carrying human ovarian xenograft tumors. While untargeted particles with surfaces comprised of either polyethyleneglycol (PEG) or human serum albumin (HSA) principally accumulated in liver tissue, several glycan structures that were responsible for tumor accumulation were identified. Moreover, targeting effects of glycans were not restricted to the underlying vesicle type but consistent for multiple vesicle types. Finally, the particles were loaded with doxorubicin and their ability to inhibit cancer growth *in vivo* was compared against doxorubicin as free drug and commercially available liposomal doxorubicin Caelyx/Doxil. InnoMedica's liposomes conferred markedly reduced side effects compared to both free drug or Caelyx/Doxil whilst greater anticancer efficacy was achieved. This represents a step forward towards the refinement of current state of the art chemotherapy, allowing for reduction of adverse drug reactions and opening ways to better therapeutic outcomes.

REPROGRAMMING THE IMMUNE SYSTEM – NOVEL PATHWAYS IN PHARMACEUTICAL INDUSTRY

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Modifying the body's own immune system has been a therapeutic approach to fight autoimmune diseases and cancer. Both approaches are related, basically the two sides of the same coin. Until now, autoimmune reactions were treated with anti-inflammatory medications and steroids. While they temporarily relieve symptoms, these drugs dampen the immune system at the same time and leave patients susceptible to infections. A new path forward is to use autoantigen-specific regulatory T cells (Tregs). With this technology native T-cells are differentiated into immunosuppressive Tregs. Once reprogrammed, these autoantigen-specific Tregs regenerate with ongoing disease remission in animal models. Another approach is to induce immune tolerance using allogeneic donor peripheral blood-derived bioengineered hematopoietic stem cells. Kidney transplant patients treated with this approach could be weaned off immunosuppressive therapies, avoiding their long time toxicities and ultimately preserving the function of the kidney graft.
Chemotherapy has been the traditional backbone of tumor therapy. The addition of tumor specific antibodies such as the CD20-targeting antibody rituximab or the HER2-targeting trastuzumab has significantly changed the prognosis of various cancers. The use of tumor specific antibodies mimics the body's normal immune response to foreign antigens. With the better understanding of mechanisms regulating the activity of immune cells, especially the so called immune checkpoint, various checkpoint inhibitors and immune activators have entered the clinical arena with very promising results, changing old paradigms.
At the cellular level adoptive transfer of T cells is being developed as promising treatment approach to fight cancer. One promising approach is to modify human T cells using viral and non-viral vectors to express engineered T-cell receptors (TCRs) or chimeric antigen receptors (CARs) to target specific antigens found on tumor cells. Several companies are now developing this technology for commercial use. Additional development programs are directed at modifying the immune checkpoint to enhance the activity of tumor-infiltrating lymphocytes (TILs) *in vivo* without the need for ex vivo processing of the patients cells.

OPTICAL MICROSCOPY: THE RESOLUTION REVOLUTION

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Throughout the 20th century it was widely accepted that a light microscope relying on conventional optical lenses cannot discern details that are much finer than about half the wavelength of light (200-400 nm), due to diffraction. However, in the 1990s, the viability to overcome the diffraction barrier was realized and microscopy concepts defined, that can resolve fluorescent features down to molecular dimensions. In this lecture, I will discuss the simple yet powerful principles that allow neutralizing the limiting role of diffraction1,2. In a nutshell, feature molecules residing closer than the diffraction barrier are transferred to different (quantum) states, usually a bright fluorescent state and a dark state, so that they become discernible for a brief period of detection. Thus, the resolution-limiting role of diffraction is overcome, and the interior of transparent samples, such as living cells and tissues, can be imaged at the nanoscale.

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UPDATE ON NANOPARTICLE TRACKING (NTA): QUANTIFICATION OF SUB-POPULATIONS WITH REGARD TO SIZE, CONCENTRATION, ZETA POTENTIAL AND FLUORESCENCE

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Nanoparticle Tracking Analysis (NTA) is a versatile method for the in situ characterization of nanoparticles: Particle size, concentration and Zeta potential is available within minutes. A laser beam illuminates the nanoparticle in the detection volume while undergoing Brownian motion. The scattered light is visualized directly on a digital camera chip. The camera records an image series which is fed into an image processing algorithm detecting the particle loca-

tions calculating particle size of individual particle traces using the Stokes-Einstein relationship.
When tagged with fluorescent markers, even specific surface characterization of bionanoparticles, such as extracellular vesicles (EVs), liposomes or virus like particles (VLP) can be performed. The principle of detection is shown in figure 1 and is termed fluorescent-NTA (F-NTA).

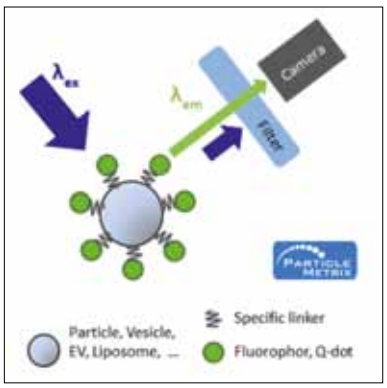

Figure 1: Principle of F-NTA, detection only fluorescent emission. When the filter is removed, the system detects unlabeled and labeled particles together.

Typically, a mixtrued of particles of different material but same size result in one peak in the particle size distribution. Figure 2 shows a 1:1 mixture of polystyrene and gold NP (both 60 nm). In figure 3 the particle size distributions shows two fractions; the tracks are separated according to intensity of objects. The bright objects correspond to gold as strong scatterer, the weak scattering objects are polystyrene (verified in single size measurement).

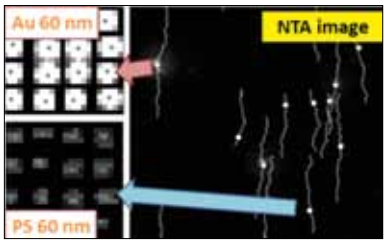

Figure 2: Mixture of 60 nm polysyrene and gold nanoparticles. Tracks contain intensity information of objects.

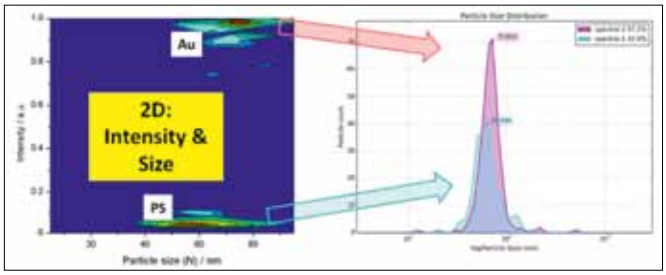

Figure 3: Extraction of intensity of objectes allows differentiation between gold and polystyrene particles of same size

By extracting additional information out of a typical NTA image sequence, sub-populations within the ensemble can be detected. Typical applications are detection and quantification of agglomeration or perhaps type and number of subpopulations after verification with the measurement of the single fractions, such as purified extracellular vesicles or drug carrier dispersions.

MAGNETIC BLOOD PURIFICATION REVISITED!

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The direct removal of disease-causing compounds from blood is appealing for the treatment of a number of medical conditions, including blood poisoning and autoimmune diseases. Magnetic separa-tion-based blood purification is especially attractive for the removal of high-molecular weight com-pounds, which are poorly removed by conventional blood purifications systems (e.g. dialysis, hemoad-sorption)[1]. In extracorporeal magnetic blood purification, functionalized magnetic (nano)particles bind to pathogenic substances circulating in the blood. Subsequently, the pathogen-loaded particles can be removed by magnetic separation before the blood is recirculated. However, despite promising *in vitro* and *in vivo* results, translation of such a process into clinics is not straightforward[1-3].
Major limitations of current systems include the risk of particles escaping magnetic separation and the resulting unwanted side effects (both short and long-term), as well as the need for prior identification of the disease-causing pathogen.
In this presentation, I will present a strategy on how to assess potential process-associated risks along with the results of a comprehensive risk assessment study[4]. I will then show a number of process modifications and carrier optimizations (Figure 1) that help to overcome most of the risks and lead to a favourable benefit-risk ratio.
Then, I will present a novel approach to exploit the theranostic potential of the magnetic blood pu-rification process. I will show how we assembled a magnetic capturing agent that enables rapid captur-ing and isolation of a wide range of pathogenic bacteria without the need for prior pathogen identifica-tion.

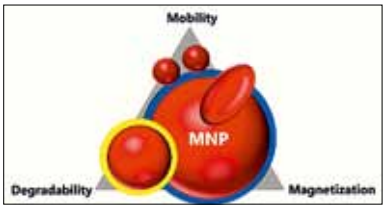

Figure 1: Magnetic capturing agents optimization based on mobility, magnetization and degradability.

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SIMPLE, SCALABLE NANOMEDICINE FORMULATION USING THE MICROFLUIDIC PLATFORM WITH IN VITRO AND IN VIVO APPLICATIONS IN GENE MODULATION – FROM CELL CULTURE TO THE CLINIC

GESINE HEUCK

Nanomedicines are potent carriers for gene delivery. When designed adequately, they allow protection of sensitive molecules such as oligonucleotides and enable control over release and biodistribution of their payload. This improves availability and transfection efficiency while reducing unwanted side effects. However, translation of nanomedicines from bench to bedside has been limited due to challenges, such as low reproducibility and inability to scale up in the manufacturing procedure, as well as poor biocompatibility *in vivo*. Microfluidics have emerged as a solution to overcome these challenges.
Here, we present a non-toxic nanoparticle design for use *in vitro* and *in vivo* prepared by a microfluidic method. We will introduce the principles of oligonucleotide lipid nanoparticle formation with microfluidics. The impact of this technology on therapeutic development will be highlighted through examples including targeted gene knockdown in CD4+ T-Cells, exploitation of the RNAi pathway in neuro-regeneration and *in vivo* mRNA delivery for RNA transcript therapy using lipid nanoparticles.

BIOMEDICAL SIMULATIONS IN THE DEVELOPMENT OF A MECHANO-SENSITIVE DRUG DELIVERY SYSTEM

SIMONE E. HIEBER

Atherosclerosis is the leading cause of death in the western world and it caused by stenosed vessels. Drugs for opening the vessel affect not only the affected vessel, but the entire vascular system. Thus, a targeted drug delivery system is highly desirable.
The vessel morphology dominates the changes in the vascular blood flow during the development of a stenosis. Within this context, nanocontainers have been proposed for the use in a drug delivery system that releases medication in the blood flow of high shear stresses. Here, flow simulations provides in-depth information in three-dimensional shear stress field complementary to experiments. For this purpose, the morphology of critically stenosed arteries has been acquired using synchrotron radiation–based micro computed tomography in high resolution. The lumen was segmented using a region-growing approach and extracted into a triangular surface. After smoothing the surface a volumetric tetrahedral computational grid was generated. The flow was governed by the incompressible Navier-Stokes and the continuity equations were numerically solved in ANSYS CFX (ANSYS). The results showed the velocity and pressure fields as well as shear stress profiles within the stenosed artery. The average wall shear stresses are low in the healthy part of the artery and more than double in the stenosed part. Thus, the high shear stress can serve as a trigger for drug release within the stenosed area using a mechano-sensitive nanocontainer.
Currently, the achieved shear stresses are mimicked in microfluidic devices to tailor the nanocontainer design. Numerical simulations of the blood flow will complement the findings of the experiments as well.

THE ZIKA VIRUS EPIDEMIC: WHY NOW, AND WHAT ARE THE DOES AND DON'TS?

HANS H. HIRSCH, MD, MSc, University of Basel & University Hospital Basel

The global expansion of Zika virus (ZIKV) has hit the world's attention only 6 month ago when increasing numbers of newborn children with microcephaly were reported in Northwestern Brazil, coinciding tantalizingly with the first time ever spread of ZIKV infections into the Americas. The dramatic pictures of the affected children, the overwhelming threat of transmission through prevalent day-active mosquitos of the Aedes family, in particular A. aegyptii, and the complete lack of herd immunity, vaccines, and antiviral drugs provoked a helpless plea by government and health agencies advising against pregnancies for an undefined time in the future, possibly years. Today, the evidence for causality of ZIKV infection and microcephaly is overwhelming and reflects the by now well-documented neurotropism of this virus. Ongoing tests with non-human primates are closing the missing links of Koch and Henle's postulates. In fact, a number of other neurological disorders have been attributed to ZIKV infection such as meningitis, encephalitis, myelitis, and Guillain-Barre syndrome. However, careful diagnostic and epidemiological studies now indicate that ZIKV infections are clinically asymptomatic in 80% of cases, whereas in the majority of the remaining 20%, a viral illness may occur, and only minority of mostly elderly persons suffer from neurological complications. In this sense, ZIKV differs little from other arthropod-borne viruses including tick-borne encephalitis and West-Nile virus. As more advanced molecular and diagnostics tools have become available, some peculiarities have been noted including the prolonged shedding of ZIKV in urine and the detection of ZIKV in sperm coupled to the risk of sexual transmission several weeks to months after primary infection. Also, transmission by transfusion has been documented akin to West-Nile virus, and a corresponding risk for transmission via organ transplantation is expected. These factors complicate the counseling of potentially exposed asymptomatic as well as previously symptomatic persons and their close contacts. By the same token, an enormous pressure is placed on specific and sensitive ZIKV diagnostics, which cannot be interpreted without medical and epidemiological competence: A moving target for both, clinicians and virologists as more and more information is emerging.
Why now? Unlike Ebolavirus, the ingredients of the ZIKV epidemic are the same as for other recent viral epidemics. Chikungunya virus spread around the Indian Ocean in 2006, and then to the Carribean islands and the Americas in 2013, and West-Nile virus spread from the Mediterranean to the East and then West coast of North-America in 1999. All three cases involved a viremic host, a blood-feeding vector, and transmission by re-feeding on susceptible host populations. Although in principle, only one viremic host is required for this scenario, several factors are critical for establishing an epidemic. Besides the biological miracle of a virus being able to equally well highjack the cellular machinery of insects and vertebrates including mammals and primates equally well, other factors are important such as the incubation time in the infected hosts, the level and duration of viremia, the vector density, and the life span and opportunity of the vector to re-feed on ZIKV-naïve hosts. Currently, there is no animal reservoir known in the Brazilian ZIKV epidemic, but absence of evidence is not yet evidence of absence. The ZIKV incubation period in man is between 1 and 3 weeks, and viremia in immunocompetent humans typically less than 1 week. The detection of ZIKV RNA by nucleic acid amplification testing (NAT) is therefore short and restricted to blood of symptomatic persons. Testing of urine and amniotic fluid is only advised after specific epidemiologic and clinical reasoning. Importantly, clinical symptoms and epidemiological exposure overlap with other viral diseases including Chikungunya and Dengue, which should be considered accordingly. Cross-reactivity between members of the flaviviruses e.g. tick-borne encephalitis, West Nile, and Yellow fever virus complicates serological testing leading to false-positive ZIKV diagnosis, Recently, a more specific ZIKV ELISA test has become available using NS1 protein from an African ZIKV.
ZIKV was discovered in 1947 in the Zika forest, Uganda, and the first human cases were noted in the 1950's in tropical Africa and then later in South-East Asia. The current epidemic in Brazil was preceded by outbreaks in Malaysia and since 2013 on the Pacific islands including French Polynesia. PROMED mail subscribers were well informed about Japanese travelers returning from Polynesia with ZIKV infection as early as 2013 followed by a syndromic network surveillance recording more than 6000 affected persons in Polynesia and new Caledonia in January and February 2014. However, these outbreaks went by little noticed, as most attention was given to the concurrent Chikungunya spread in the Americas and the emerging Ebola epidemic in West-Africa. Although it is not clear whether it took one or several viremic donors, recent genome sequencing studies from Brazil propose that, based on phylogenetic relationships and accumulated mutations, ("molecular genetic clock"), ZIKV was most likely introduced to Brazil as early as 2013 from the French Polynesian outbreak.
What now? The increasing scientific knowledge about ZIKV is central to rational approaches, of which the most important one remains prevention of transmission via vector control. This will allow reducing, but not completely abolishing exposure, but particularly susceptible populations required attentions such as pregnant women and those of childbearing age. However, asymptomatic course and persisting infections in certain body sites, and sexual transmission complicate host-centered prevention measures. Vaccine development is an alternative strategy, especially using recombinants of the yellow fever vaccine, but these are live attenuated, and until clinical phase III studies permitting broad coverage will not be available within the next 5 years. Similarly, research on neutralizing antibodies for passive vaccination seem promising for pre- and post-exposure and even therapeutic agent, but may also not be available within 5 years and faces the challenge of presumably little efficacy of clearing ZIKV from immune-privileged sites. Antivirals, especially those targeting conserved functions of the flaviviral RNA polymerase may be a rewarding step forward, especially in view of

the progress made with direct acting antivirals for the related Hepatitis C virus and some agents in development targeting respiratory RNA viruses. However, the safety concerns of antiviral drugs during pregnancy will be remarkable and reliable data are unlikely to be available for many years to come.
What not? WHO, CDC, ECDC, and various national health agencies advise pregnant women and immunocompromised persons against avoidable travels of into the epicenters of ZIKV epidemics. In areas of active transmission, avoidance of mosquito bites is key for persons and close contacts including children. Laboratory testing following abnormal ultrasound findings in pregnant women with epidemiological risk should not disregard other prevalent reasons for fetal head and neural malformations including cytomegalovirus, syphilis, rubella, and toxoplasmosis. Diagnostic ZIKV testing without special trained health care professionals providing counseling will be difficult to interpret due to the risk of false-positives and false-negatives.
Thus, a combination of the above steps seems to be the only feasible strategy. Regardless, the past decades have demonstrated that flaviviruses and vector expansion remain sources of repeating epidemic threats, which are unlikely to disappear in view of globalized travel and trade. Moreover, autochthonous transmission in formerly temperate climate zones is becoming a reality in the wake of climate change. The answers can only lie in dedicated surveillance, collaboration, integrated research, and better medical and public education.

IRON OXIDE NANOPARTICLES

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Superparamagnetic iron oxide nanoparticles (SPIONs) are recognised as promising advanced materials for various biomedical applications, such as targeted drug delivery, contrast agent for imaging, cell tracking, and transfections 1-6. Iron oxide, γ-Fe_2O_3, is of special interest because of the approved biocompatibility of these nanoparticles (NPs), including the well-understood metabolism of the NPs in the liver 7. Because of their special properties, these particles offer a variety of advantages compared to other tools: (i) the controllable sizes ranging from ~3 to several hundred nanometers (in beads), and (ii) the tailor-made surface coating, which can be adapted in a way so that the particles can selectively bind to a defined biologic entity (such as cells or degraded extracellular matrix molecules) or deliver molecules and drugs to specific sites. In addition, their outstanding magnetic properties makes them versatile candidates for molecular resonance imaging (MRI) or hyperthermia8. Most commercially available particles or beads with modified surfaces show sizes. 150 nm and are used for *in vitro* separation but are not designed for selective adsorption/uptake into cells or tissue. On the other hand, very small particles (diameter < 30 nm) are commercially available, but only with a limited number of functional surfaces, and were developed for liver and recently also for imaging metastases in lymph nodes by MRI9.
One of the promising minimally-invasive cancer treatments is magnetically-mediated hyperthermia, which is based on the sensitivity of cancer cells at temperatures above 41°C. Heat is generated by MNP placed in an external alternating magnetic field. In other words, part of the body or the whole patient, having MNP in the tumour, is placed in the coil providing the alternating magnetic field with frequency and amplitude. Due to both the maximal energy to which humans can be exposed and temperature fluctuations at high and/or non-uniform fields, there are limits for the applied frequency and magnetic field strengths. Therefore the nanoparticle properties has to be adapted so that the enough heat is generated by MNPs and dissipated in the surrounding cancerous tissue inducing cell death or tissue sensitisation to increase the efficacy of other anti-cancer therapies. The heating ability of MNPs is quantified by the rate of heat dissipation per unit mass of MNPs, called the specific absorption rate (SAR). It is still a need to develop MNPs having higher SAR values for improved treatment with reduced injected dose, but in allowed magnetic field conditions safe for the patients. SAR is also a complex non-linear function of the magnetic anisotropy constant, the magnetic volume and the saturation magnetization, where optimisation of the first two variables has the largest impact on the SAR increase. In this presentation, the different parameters which determine the SAR value were discussed. Finally the potential of this type of particles for clinical applications, especially as contrast agent for MRI and hyperthermia at the same time, theranostics, is highlighted and future needs and developments discussed.

Acknowledgements:
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STRUCTURE-FUNCTION RELATIONSHIPS OF NANO-BIO INTERACTION

PATRICK HUNZIKER

The number potential nano constructs created by synthesis of building blocks followed by composition of one or multiple types of such building blocks to supramolecular nano-constructs is huge. Likewise, the number of biological macromolecules that are derived from the human genome (~25'000 genes) which are in addition diversified by processes like somatic recombination, somatic hypermutation and posttranslational modification is quite large. For medical purposes, predicting nano-bio interactions of a newly designed nanomaterial with various relevant bio systems is critical for efficacy and safety, but the sheer number of potential interaction pairs renders this task daunting.
In this presentation, we discuss the combination of systematic synthesis, systematic bio-nano interaction study and computational modelling as a path towards a more mechanistic understanding with the ultimate goal of future predictive modelling of nano-bio interactions.

THE PRINCIPLES OF TRANSLATION IN NANOMEDICINE FOR PERSONALIZED MEDICINE

PATRICK HUNZIKER

Personalized medicine is the future paradigm of medicine, where patients and their diseases are no longer solely considered and treated as "statistical averages of large cohorts" but where individual aspects play a decisive role to achieve more efficient, less toxic and optimally cost effective management strategies.
Nanomedicine is an indispensable enabling technology of personalized medicine by combining advances in precision diagnostics and in individualized therapies.
Progress in medicine is thus critically dependent on progress in manufacture, in characterization, in regulatory science towards real-world translation of nanomedicine.
Nanomedicine, the application of the broad spectrum of nanoscience tools, materials and methods to the benefit of patients, encompasses diagnostic nanomedical tools for precise assessment of the genomic background of a patient and his disease, in combination with tools for personalized assessment of the phenotype across all size scales at a given timepoint in the disease course.
Nanomedicine also allows to deliver a variety of therapies that act with molecular precision (e.g., by receptor targeted delivery) and exhibit differential activity depending on the individual phenotype of the patient and a diseased organ, e.g. through novel functional materials.
This presentation exemplifies and emphasizes the critical importance of thorough understanding and the optimal matching of biomedical requirements, technical aspects, manufacturing issues, regulatory paths, and the wise choice of clinical trials strategy.
This talk will explore these challenges and delineate strategies towards clinical benefit of this truly interdisciplinary endeavour towards the medicine of the future.

ATHEROSCLEROSIS

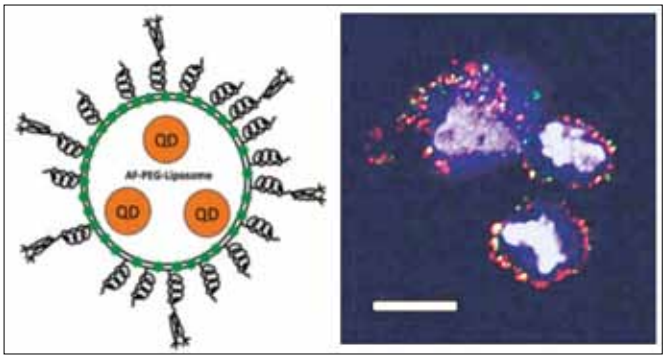
PATRICK HUNZIKER

A major development goal in the agenda of the medicine of the future is the eradication of atherosclerosis.
Atherosclerosis is a major cause of morbidity, a leading cause of death, and a major driver of healthcare costs, but progress in combatting this key disease has been rather slow in the last decade.
This presentation evaluates the state of the art 2016 in nanomedical diagnosis and management of atherosclerosis and discusses potential approaches to eradication.

DRUG TARGETING TO HEPATOCYTES USING ASIALOFETUIN-CONJUGATED LIPOSOMES

PATRICK HUWYLER

GRAPHICAL ABSTRACT:



INTRODUCTION:

During the last decades, the lack of efficient therapeutic options resulted in a strong increase in liver disease rates and mortality. In many cases, hepatocytes are the key pathogenic cell type. Hepatocytes are therefore an interesting pharmacological target for novel therapeutic strategies. Targeted nanomedicines have the potential to increase drug efficiency and decrease severe side-effects associated with conventional therapeutics. It was therefore the aim of the present project to develop a hepatocyte-specific targeting strategy using pegylated liposomes covalently coupled to asialofetuin (AF) or isolated antennary N-glycans. *In vivo* pharmacokinetic experiments in rats demonstrated specific uptake of targeted liposomes by liver parenchymal cells but no interactions with tissue resident macrophages (Kupffer cells).

RESULTS:

AF was conjugated to the distal end of polyethylene glycol–functionalized phospholipids. This was achieved by thiolation of proteins using Traut's reagent and coupling to pegylated liposomes through maleimide-functionalized lipids. Chemical modification of AF did not interfere with its receptor interaction. AF-liposomes had a size of less than 130 nm, were judged to be monodisperse and were labelled with fluorescent organic dyes or loaded with quantum dots. *In vitro,* binding and cellular uptake of fluorescent AF-liposomes by HepG2 hepatocellular carcinoma cells were reduced at low temperature and in presence of an excess of the unbound receptor ligand AF. Hepatocyte-specific targeting and internalization of AF-liposomes *in vivo* was confirmed in the rat and could be competitively inhibited by co-injection of unbound AF. Uptake by cells of the mononuclear phagocyte system such as hepatic Kupffer cells could be avoided (Figure 1).

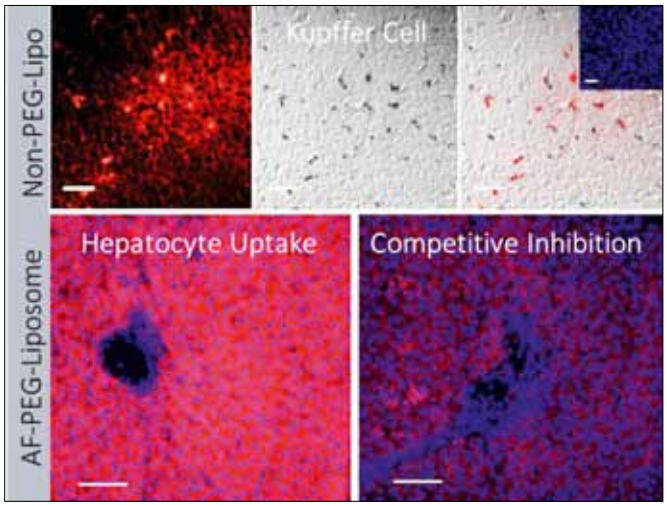


Figure 1: In vivo accumulation of non-pegylated liposomes in Kupffer cells and of AF-PEG- liposomes in rat hepatocytes. Upper panels: Non-pegylated liposomes (red signal) were administered together with colloidal carbon, a marker for Kupffer cells (black signal, differential interference contrast microscopy). Lower panels: Analysis by confocal fluorescence microscopy of representative rat liver cryosections 30 min after i.v. injection of rhodamine-labelled AF-PEG liposomes (red signal). Targeted AF-PEG liposomes accumulate in hepatocytes. Competitive inhibition in vivo was achieved by coadministration of free AF. Nuclei were stained with Hoechst 33342 (blue signal). Scale bars = 40 µm.

DISCUSSION:

We conclude that the use of AF-conjugated, pegylated liposomes is a promising strategy to avoid the reticuloendothelial system and to specifically target hepatocytes via the asialoglycoprotein receptor (ASGPR) *in vitro* as well as *in vivo*. However, the success of such technologies depends on the expression level of the ASGPR. In follow-up studies, we therefore evaluated the mRNA and protein expression level of the major subunit ASGR1 *in vitro* as well as *in vivo*. In hepatocellular carcinoma derived (HCC) HepG2 cells as well as in liver derived micro arrays from patients, mRNA levels highly correlated with protein expression. The human data showed variable ASGR1 expression between patients (Figure 2). In general, we observed a statistical trend towards a decrease in ASGR1 mRNA and protein expression in HCC, especially in poor differentiated (Grade

III/IV) HCC specimens. A surprising discovery was the increased ASGR1 protein expression in cirrhotic specimens.

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A BACTERIAL PLATFORM FOR TARGETED DELIVERY OF PROTEINS INTO CANCER CELLS

SIMON ITTIG

More than 100 years ago German physicians have made the observation that patients suffering from cancer can benefit from bacterial infections. Since then, many different bacteria have been found to preferentially grow within solid tumors.
We have further optimized bacteria by genetic engineering to be highly specific for different solid tumors. This preferential growth is based on the weakly active immune system in the solid tumor, allowing virulence-attenuated bacteria to colonize the tumor tissue. We have validated genetically engineered bacteria, Yersinia enterocolitica, as a highly specific tumor-targeting vehicle in murine allograft models of cancer, with a preference for solid tumors of more than one million fold compared to healthy tissue. Furthermore, the bacterial colonization of the tumor lasts for more than a week. Therefore, these bacteria offer an ideal vehicle to deliver therapeutic cargo to cancer cells.
For the purposeful delivery of therapeutic proteins by these bacteria, we have developed a technology allowing the delivery of cell death-inducing proteins produced by bacteria directly into cancer cells. To this end, we make use of a natural bacterial nano-machine, called type 3 secretion system, which is best described as a needle-like structure at the periphery of the bacteria. Using these needles, bacteria can inject proteins into cancer cells. Employing this technology, we have obtained very promising results in delivering toxic proteins to cancer cells leading to pronounced cell death of these cells.
Our aim is to optimize this system and to validate its efficacy in cancer treatment in murine models of cancer. Our vision is to bring this novel and attractive treatment strategy into clinical practice.

ROLE OF NANOBIOTECHNOLOGY IN PERSONALIZED MANAGEMENT OF INFECTIONS

KEWAL JAIN

Personalized medicine simply means the prescription of specific treatments best suited for an individual taking into consideration both genetic and environmental factors as well as the pathology or pathogenic agent that influence response to therapy. Omics technologies, eg, genomics/proteomics have facilitated the development of personalized medicines but other technologies such as nanobiotechnology have made significant contributions. Personalized medicine is the best way to integrate new biotechnologies into medicine for improving the diagnosis and understanding of pathomechanism of diseases as well as integrating it with therapy (Jain 2015). Role of nanobiotechnology in an integrated approach to challenges of antiviral diseases will be outlined. Nanobiotechnology facilitates personalized treatment of infections by:

1. Refinement of molecular diagnosis by detecting few or even single microorganisms.
2. Nanobiochips for fast, direct, detection of microorganosms at point-of-care.
3. Optical biosensors for direct detection of microorganisms
4. Nanowire biosensors for IVD and potentially *in vivo* diagnosis of infections.
5. Detection of genetic differences in infectious agents and genetic susceptibility of the patient.
6. Detection of biomarkers for monitoring the course of infection as well as effect of therapy.
7. Nanoparticles as antimicrobial agents
8. Nanovaccinology: development of novel personalized vaccines and methods of delivery.
9. Nanoparticle-based tailored adjuvants.
10. Nanobiotechnology facilitates integration of diagnosis with therapy

Examples will be given of the role of nanobiotechnology for the personalized management of HIV, hepatitis B&C, and meningitis
Jain KK. A Textbook of Personalized Medicine, 2nd ed, Springer, New York, 2015.

VARIOUS OPTIONS FOR TARGETING – A SHORT OVERVIEW

KEWAL JAIN

In pharmaceutics, active targeting by molecular recognition has refined applications ranging from diagnostics and drug discovery to drug delivery for targeted therapeutics. The latter are the most important with applications in various therapeutic areas such as neurological disorders and cancer. Along with other technologies, refinements in targeted drug delivery, many of which are based on nanobiotechnology, will play an important role in the development of personalized medicine. Objectives of targeted delivery are:

- Selective therapy to target a lesion or an organ and spare the normal tissues
- Reduction of drug dosage and toxicity
- Controlled passage through barriers
- Combination of diagnostics with therapeutics

Several options are available for targeting and those relevant to nanobiotechnology include the following which will be discussed in this session:

- Antibodies: nanobodies
- Monoclonal antibodies & antibody-drug conjugates
- Aptamers
- Nanoparticles: e.g., nanovesicles.

I will give example of strategy for targeting a brain tumor that will combine nervus system with cancer and crossing the blood-brain barrier.

BIOINSPIRED EXOSOME-MIMETIC NANOVESICLES FOR TARGETED DELIVERY OF CHEMOTHERAPEUTICS

SU CHUL JANG, Krefting Research Centre, Department of Internal Medicine and Clinical Nutrition, University of Gothenburg, Gothenburg 405 30, Sweden

After discovering the natural function of exosomes in transporting RNA and protein, exosomes have been highlighted as the drug delivery vehicles. However, low productivity of exosomes is one of the limitations for clinical use of exosomes. Therefore, the development of exosome-like vesicles with a substantially greater yield is attractive for future nano-sized drug delivery systems. Here, we developed artificial bioinspired exosome-mimetic nanovesicles (NV) that mimic exosomes with high production yield. NV were generated from monocytes/macrophages by serial extrusion through a series of polycarbonate membranes in the absence or presence of chemotherapeutics. NV had similar characteristics with that of exosomes, but had more than 100-fold higher yield than exosomes.

In addition, NV have natural targeting ability of cells by maintaining the topology of plasma membrane proteins. *In vitro*, chemotherapeutic drug-loaded NV induced TNF-α-stimulated endothelial cell death in a dose-dependent manner. *In vivo*, experiments in mice showed that the chemotherapeutic drug-loaded NV traffic to tumor tissue and reduce tumor growth without the adverse effects observed with equipotent free drug. Furthermore, compared with doxorubicin-loaded exosomes, doxorubicin-loaded NV showed similar *in vivo* anti-tumor activity. However, doxorubicin-loaded liposomes that did not carry targeting proteins were inefficient in reducing tumor growth. Importantly, removal of the plasma membrane proteins by trypsinization eliminated the therapeutic effects of the NV both *in vitro* and *in vivo*. Taken together, these studies suggest that the NV can serve as novel exosome-mimetics to effectively deliver chemotherapeutics to treat malignant tumors.

TARGETING STRATEGIES FOR THE DETECTION OF PROSTATE CANCER METASTASES TO LYMPH NODES

OLIVER JORDAN, Senior lecturer, School of pharmaceutical sciences, University of Geneva (CH)

Lymphangiogenesis and migration of tumour cells through the lymphatic system are key processes of early cancer metastatic spread [1]. In order to diagnose, and potentially treat early metastases of prostate cancer, we developed nanocarriers based on SPIONs (superparamagnetic iron oxide nanoparticles), decorated with PSMA (prostate surface membrane antigen)-targeting moieties. Such nanocarriers may be effective for MRI sensitive detection of the metastases and potential treatment by magnetic field-induced hyperthermia.

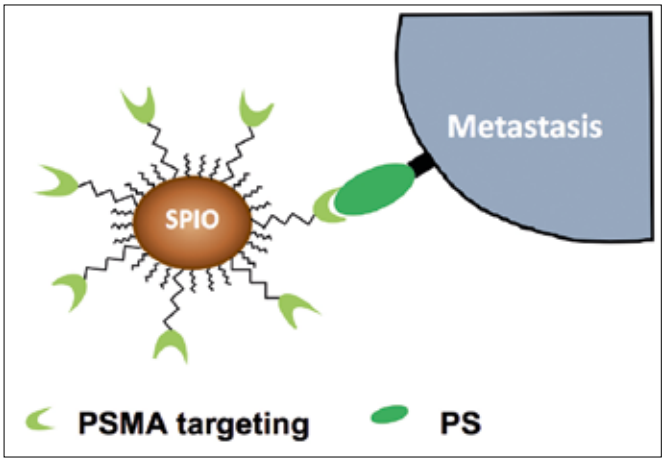

Figure 1: Principle of PSMA-targeted SPION for prostate cancer imaging and hyperthermia.

We present herein different SPION targeting strategies, based on a RNA aptamer[2] or a urea-like small molecule[3] as targeting moieties. Fluorescently tagged aptamer and urea small molecule were shown to bind to human LNCaP (PSMA positive) cells, but not to human PC3 (PSMA negative) cells, showing specific binding affinity. SPIONs optimized for hyperthermia were functionalized with the RNA aptamer and the urea small molecule using click chemistry. Crucial aspects of nanoparticle production, animal model development, clinical imaging, hyperthermia application and modelization have also been addressed, in the frame of a Nano-Tera Swiss consortium.

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ENGINEERING T CELLS FOR CANCER THERAPY: CHALLENGES AND OPPORTUNITIES

CARL H. JUNE

It is now well established that the immune system can control and eliminate cancer cells. Adoptive T cell transfer has the potential to overcome the significant limitations associated with vaccine-based strategies in patients who are often immune compromised. Application of the emerging discipline of synthetic biology to cancer, which combines elements of genetic engineering and molecular biology to create new biological structures with enhanced functionalities, is the subject of this seminar. Various chimeric antigen receptor designs, manufacturing processes and study populations, among other variables, have been tested and reported in recent clinical trials. Many questions remain in the field of engineered T cells, but the encouraging response rates pave a wide road for future investigation into fields as diverse as cancer and chronic infections.

LIPID NANOPARTICLE MEDIATED RNAI FUNCTIONAL DELIVERY: A PROOF OF CONCEPT FOR LEUKEMIA NANO-THERAPEUTICS.

NIDHI JYOTSANA[1], Amit Sharma[1], Anuhar Chaturvedi[1], Colin Walsh[2], Anitha Thomas[2], Michaela Scherr[1], Matthias Eder[1], Karin Battmer[1], Florian Kuchenbauer[3], Laszlo Sajti[4], Annette Barchanski[4], Kerstin Gorlich[1], Robert Lindner[5], Fatih Noyan[6], Sühs Kurt-Wolfram[7], Denis Grote-Koska[8], Korbinian Brand[8], Hans-Peter Vornlocher[9], Arnold Ganser[1], Euan Ramsay[2], Pieter Cullis[2], Michael Heuser[1]

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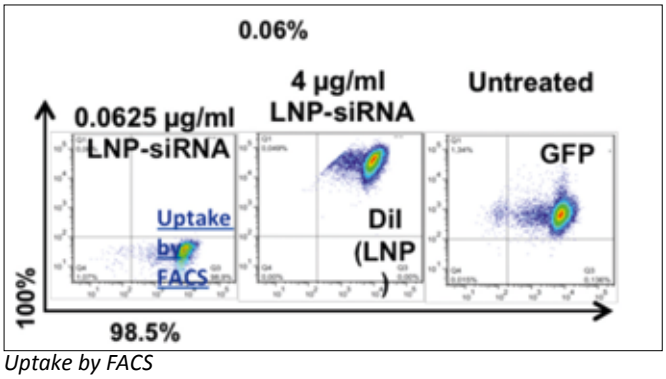
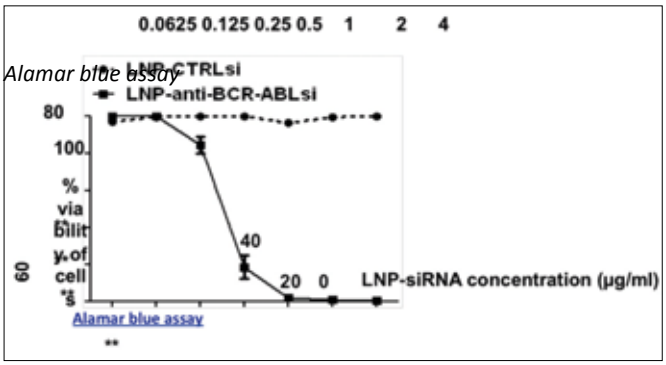
Leukemia is the tenth most frequently occurring type of cancer among all races. Current treatment regimens of chemotherapy and bone marrow transplantation lack specificity and are associated with severe adverse effects. Despite the wide potential of RNA interference (RNAi) for translational therapeutics, systemic application of siRNA is hampered by rapid renal clearance, degradation by serum nucleases or associated immune responses. The tunable size and biological behavior of lipid nanoparticles (LNPs) surmounts the barriers encountered by siRNA delivery *in vivo* and help improve treatment of diseases like cancer. Here, we established and employed the BCR-ABL dependent K562-CML xenotransplantation model as a proof of principle to validate LNP mediated siRNA functional delivery *in vivo*.
A microfluidic mixing technology was used to obtain reproducible ionizable cationic LNPs loaded with anti-BCR-ABL or CTRL siRNA. To determine the delivery efficiency of LNP-siRNA formulations, human leukemic K562 cells were incubated with siRNA-containing LNPs at various concentrations. Almost 100% of cells had taken up siRNA containing LNPs even at the lowest concentration of 0.0625µg/ml with stable uptake kinetics (Figure 1).



Uptake by FACS

Looking at the on-target functional efficacy of LNP-siRNA formulations, we observed a time and dose dependent increase in apoptosis and decrease in cell viability (Figure 2) of K562 cells treated with anti-BCR-ABL siRNA but not CTRL siRNA. A robust knockdown in BCR-ABL mRNA levels (65-90%) at 72 hours and protein at 96 hours was observed which confirmed that cell death was an on-target effect.



Alamar blue assay

To translate our findings *in vivo*, we evaluated the safety profile, delivery potential and functional efficacy of LNP-siRNA in mice. A total dose of 15mg/kg (3 injections of 5mg/kg at day 0, 1 and 2) in healthy NSG (NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ) mice resulted in 100% LNP positive cells in peripheral blood up to day 10. The formulations were highly tolerable *in vivo* with no significant differences in body weight and complete blood counts between treated and control mice. With a focus on hematopoietic tissues following systemic administration, NSG mice received transplants of human K562 cells (stably expressing GFP and luciferase) intrafemorally and were injected intravenously for 3 consecutive injections of LNP-siRNA (1 or 5mg/kg body weight) at 8 hours interval. Interestingly, almost 100% LNP uptake was observed in xenograft leukemic cells in bone marrow at 48 hours at both doses. The leukemic burden of luciferase expressing K562 cells in mice was quantified using *in vivo* imaging before and during treatment and was found to be decreased by 0.5 fold with anti-BCR-ABL siRNA treatment compared to control group.

CONCLUSION:

We show a highly efficient and non-toxic delivery *in vitro* and *in vivo* with nearly 100% uptake of LNP-siRNA formulations in bone marrow of leukemic mice. By inhibiting BCR-ABL we show a reduction of leukemic burden in our xenotransplant model, while leukemic cells expanded in CTRL siRNA treated mice. Our study provides a proof-of-principle that the combined use of lipid nanoparticles and RNAi technology can be used to target leukemia cells *in vivo* with promising therapeutic implications.

ALBUMIN AS NATURAL NANOPARTICLES FOR IN VIVO IMAGING AND DRUG DELIVERY

KEON WOOK KANG, Department of Nuclear Medicine & Cancer Research Institute, Seoul National University College of Medicine, Korea

Various nanoparticles have been tried for drug delivery system. However toxicity issues arising from nanoparticles are the major hurdles in translating into clinical applications. We studied targeted imaging *in vivo* to validate targeted drug delivery using albumin as a natural nanoparticle. We radiolabeled human serum albumin (HSA) with positron emitting ^{64}Cu for PET imaging. We used a click chemistry of azide (N3)- or DBCO-functional groups in room temperature and neutral pH to maintain natural properties of HSA. We investigated *in vivo* biodistribution using ^{64}Cu labeled HSA. Serial PET imaging revealed that heart-to-liver activity ratios were higher for the DBCO- and N3-HSA (HSA:DBCO(or -N3) = 1:5) than others. We also evaluated targeting ability of 64Cu labeled lactosaminated human serum albumin (^{64}Cu-L-HSA) to asialoglycoprotein receptor (ASGPR) which is overexpressed in hepatic cell in the liver and hepatocellular carcinoma *in vitro* and *in vivo* models. Human serum albumin was conjugated with various numbers of lactosamine residues (Ln; L0-, L8-, L26-, L56-HSA). Serial PET imaging revealed that HepG2 tumor uptake of ^{64}Cu-L26-HSA was 7.5, 7.9 and 7.3 %ID/g at 10, 22, and 46 h post-injection, respectively. Lactosaminated HSA enhanced the targeting ability to ASGPR positive cells by addition of 26 lactosamine residues on the surface of HSA. We propose human serum albumin is a promising candidate for *in vivo* imaging and drug delivery.

STEM CELL-BASED MICROTISSUE ENGINEERING FOR DRUG DEVELOPMENT

JENS M. KELM

Induced pluripotent stem cells (iPS) have become a valuable source for toxicological profiling of drugs pharmaceutical industry. However, they are also a valuable cell source to create 3D tissues *in vitro*, especially for organs from which it is difficult to obtain primary cells such as the heart and brain. Next to the use of iPS cells other stem cell types such as adult stem cells are also being used to re-create tissue structures in the petri dish e.g. the epithelial barrier of the intestine. Here we provide an overview how stem cells can be used to create physiological relevant microtissue models *in vitro*, show casing heart and brain.

MICROBUBBLES FOR DRUG DELIVERY TO TUMORS AND THE BRAIN

FABIAN KIESSLING

Since many years microbubbles (MB) are used as contrast agents in ultrasound imaging. These particles of 1-10 µm remain strictly intravascular after intravenous injection and thus are used to assess vessel morphology and function. Different designs of microbubbles have been developed and translated into clinical evaluation. Hard shell MB usually possess shells of denaturated protein, sugars or polymers, while soft-shell MB are stabilized with phospholipids. While air is the preferred core of hard shell MB, for soft shell MB usually heavier gases with lower solubility are chosen. Sonification of the MB leads to their oscillation or at higher ultrasound pressure to their destruction, which both lead to specific nonlinear signals that can be detected by ultrasound. Detection sensitivity is high and under ideal circumstances even single MB can be detected. Although MB are considered as considerably save contrast agents, physical forces emitted by oscillating and disintegrating MB are significant. In a therapeutic setting these physical forces can be used to open cell membranes, vascular barriers and to untighten tumor stroma. Our group and others could show that MB enhanced ultrasound can be used to effectively deliver drugs into the brain tissue and that the penetration depth of the drugs into the brain tissue is depending on the ultrasound settings. However, care has to be taken to prevent the development of brain edema and additional imaging biomarkers may be required to monitor the degree of vascular opening, which was done in our study by incorporating ultrasmall superparamagnetic iron oxide nanoparticles (USPIO) into the MB shell that were released after ultrasound-based destruction, then extravasated and generated an MRI signal that cor-

responded to the vascular permeation. Also in peripheral tumors sonopermeabilisation works and the delivery and penetration of liposomes to tumor tissues could be improved, an effect that was similarly strong for soft and hard shell MB. Alternatively to co-administration, drugs may also directly be loaded into the MB shell or connected to its surface, which might be a reasonable strategy for highly toxic drugs or in case of gene therapy. In this context, accumulation of the MB at the target site can be further enhanced by active targeting of the MB to the vascular wall.
In summary, MB-enhanced ultrasound can be considered a very effective tool to improve drug and gene delivery to the extravascular space, through the stroma and into the cell. However, much further research is required to optimize the focused ultrasound devices, the microbubbles and the settings to exploit the maximal effect and to render the method sufficiently safe.

INORGANIC NANOMATERIALS FOR IMAGE-GUIDED SURGERY

MORITZ KIRCHER

"Surface-enhanced Raman spectroscopy" (SERS) nanoparticles have gained much attention in recent years for in silico, *in vitro* and *in vivo* sensing applications. Our group has developed novel generations of biocompatible "surface-enhanced resonance Raman spectroscopy" (SERRS) nanoparticles as novel molecular imaging agents. Via rigorous optimization of the different variables contributing to the Raman enhancement, we were able to design SERRS nanoparticles with so far unprecedented sensitivity of detection (femto-attomolar range under *in vivo* imaging conditions). This has resulted in our ability to visualize, after intravenous injection with a single nanoparticle, many different cancer types in mouse models. The cancer types we have tested so far include brain, breast, esophagus, stomach, pancreas, colon, sarcoma, and prostate cancer. While this approach proved very promising, and relies solely on the "enhanced permeability and retention" (EPR) effect, we have also tested the effect of active targeting on the performance of these nanoparticles. All mouse models used are state-of-the-art, and usually genetically engineered, in order to mimic the human tumor biology as close as possible. In these studies, we were able to visualize not only the bulk tumors with high accuracy, but importantly also microscopic extensions and locoregional satellite metastases, thus delineating the true extent of tumor spread. Moreover, the Raman nanoparticles enable the detection of premalignant lesions. Given their inert composition they are expected to have a high chance for clinical translation, where we envision them to have an impact in various scenarios ranging from early detection to image-guidance in open or minimally invasive surgical procedures, to noninvasive imaging in conjunction with spatially offset (SESORS) Raman detection devices.

PHOSPHORUS DENDRIMERS IN PHOTODYNAMIC THERAPY

BARBARA KLAJNERT-MACULEWICZ[1], Monika Dabrzalska[1], Anna Janaszewska[1], Maria Zablocka[3], Serge Mignani[4], Jean Pierre Majoral[5]

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Photodynamic therapy (PDT) is an alternative to chemotherapy and radiotherapy treatment for tumors. It is based on the induction of cell death through the combination of a photosensitizer (PS) and irradiation. The photosensitizer selectively accumulates in malignant tissues and is activated by light of a specific wavelength, preferentially in the red region of the visible spectrum. Energy from the light-excited photosensitizer is transferred to O_2 to produce singlet oxygen ($^1O_2$) and highly reactive oxygen species (ROS). These cytotoxic products initiate a cascade of reactions that induces the death of tumor cells [1-3]. PDT was proposed as a useful oncology tool more than 30 years ago but it has limitations. The success of PDT depends on photosensitizers and development of effective ones is being continued. Ideal photosensitizers should be characterized by high quantum yield of singlet oxygen generation, no dark toxicity (no systemic toxicity without irradiation), high solubility in aqueous medium, specific tumor localization, long wavelength absorption. Furthermore, the efficiency of photosensitizers depends on local accumulation and specific cellular uptake, stimulating research concentrated on the development of delivery systems. Most known photosensitizers cause toxic effects including prolonged skin photosensitivity due to a lack of specificity towards tumors [4]. Dendrimers can help to overcome the above mentioned obstacles. Two types of phosphorus dendrimers: cationic (1cat) and anionic (1an) and two photosensitizers: rose Bengal (RB) and methylene blue (MB) (Fig. 1) have been studied.

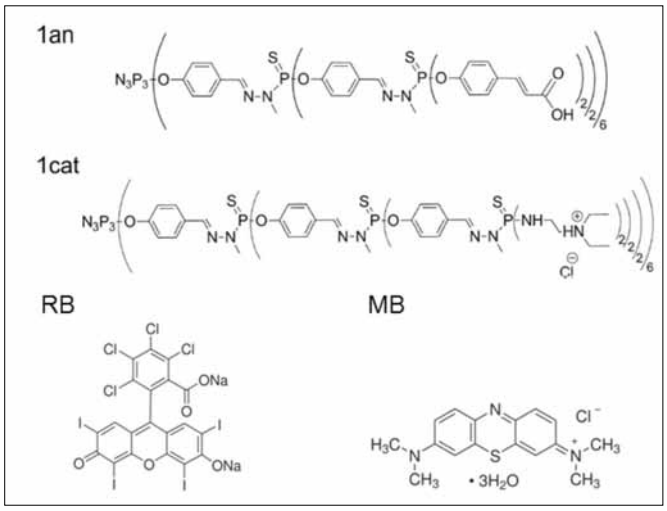

Figure 1. Chemical structures of anionic phosphorus dendrimer G2 (1an) and cationic phosphorus dendrimer G3 (1cat), rose Bengal (RB), and methylene blue (MB)

Using three complementary methods (UV-VIS spectroscopy, spectrofluorimetry, and FTIR spectroscopy) it has been demonstrated that 1cat dendrimers are able to form electrostatic complexes with RB, whereas 1an dendrimers encapsulate and electrostatically interact with MB. Molar ratios of dendrimer-photosensitizer complexes have been determined. The stoichiometry of the RB:1cat complex was estimated to be 7:1. In the case of the MB:1an complex the stoichiometry was 9:1 [4, 5]. It has been checked whether complexation does not negatively affect production of singlet oxygen by photosensitizers upon irradiation. Q-light Pro Unit lamp was the light source and Singlet Oxygen Sensor Green (SOSG) reagent was used. Complexes have not decreased $^1O_2$ concentration compared to free photosensitizers. Moreover, for the RB-1cat complex enhancement of $^1O_2$ production was observed (Fig. 2).

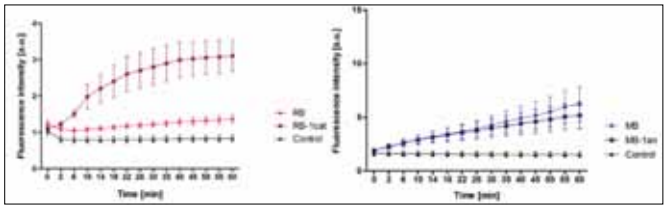

Figure 2. Changes in fluorescence intensity of SOSG in the presence of RB, RB-1cat, MB, and MB-1-an measured during irradiation

In vitro experiments were performed for murine basal carcinoma cell lines (ASZ1, BSZ, CSZ). It has been proven that cellular uptake is higher when photosensitizers are complexed with dendrimers, compared to free photosensitizers (Fig. 3)

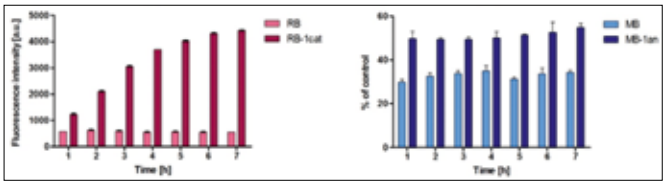

Figure 3. Cellular uptake of RB, RB-1cat, MB, and MB-1an

For all studied systems we have observed no significant "dark toxicity". After irradiation complexes were more toxic than free photosensitizers (Fig. 4, 5).

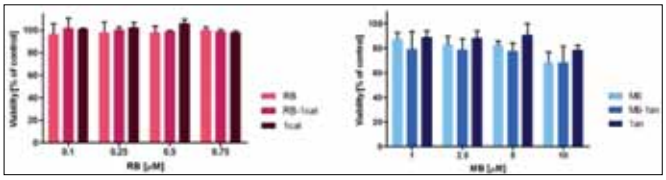

Figure 4. Viability of ASZ cells after incubation with RB, RB-1cat, MB, and MB-1an without irradiation ("dark toxicity")

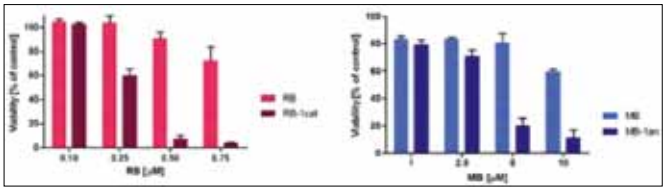

Figure 5. Viability of ASZ cells after incubation with RB, RB-1cat, MB, and MB-1an after irradiation ("phototoxicity")

SUMMARY

Dendrimer-photosensitizer systems are characterized by improved properties compared to free, unbound photosensitizers, especially in terms of improved cellular uptake and stronger phototoxic effect.

ACKNOWLEDGEMENTS

This study was funded by the project "Phosphorus dendrimers as carriers of photosensitizers in photodynamic therapy and its combination with hyperthermia in *in vitro* studies" operated within the Foundation for Polish Science VENTURES Programme (Project VENTURES number VENTURES/2013-11/3) co-financed by the EU European Regional Development Fund and by the grant HARMONIA "Studying phosphorus dendrimers as systems transporting photosensitizers" no. UMO-2013/08/M/NZ1/00761 supported by National Science Centre. Murine basal cell carcinoma cell lines were kindly provided by dr Ervin Epstein (Children's Hospital Oakland Research Institute).

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"THE INVENTION OF SPIEGELMERS TO OVERCOME STABILITY ISSUES OF OLIGONUCLEOTIDE APTAMERS"

DR. SVEN KLUSSMANN, Chief Scientific Officer, NOXXON Pharma AG, Berlin

Spiegelmers are mirror-image oligonucleotide aptamers that are exclusively developed by NOXXON Pharma AG, Berlin, Germany, for therapeutic uses in different indication areas. So far, three Spiegelmer drug candidates have been profiled to clinical Phase IIa stage: NOX-A12 (Olaptesed pegol) for mulitple myeloma and chronic lymphocytic leukemia, NOX-E36 (Emapticap pegol) for diabetic nephropathy, and NOX-H94 (Lexaptepid pegol) for anemia of chronic disease.

Spiegelmers are oligonucleotides that are built on a backbone of mirror-image RNA or DNA (L stereoisomers). By leveraging this "mirror-image chemistry", Spiegelmers solve two key problems that have limited the development of aptamers made with natural D stereoisomers: Spiegelmers have enhanced biological stability and are immunologically passive. This is due to the fact that the mirror-image L RNA and DNA of Spiegelmers is not recognized as

"THE INVENTION OF SPIEGELMERS TO OVERCOME STABILITY ISSUES OF OLIGONUCLEOTIDE APTAMERS"

SVEN KLUSSMANN, Chief Scientific Officer, NOXXON Pharma AG, Berlin

Spiegelmers are mirror-image oligonucleotide aptamers that are exclusively developed by NOXXON Pharma AG, Berlin, Germany, for therapeutic uses in different indication areas. So far, three Spiegelmer drug candidates have been profiled to clinical Phase IIa stage: NOX-A12 (Olaptesed pegol) for mulitple myeloma and chronic lymphocytic leukemia, NOX-E36 (Emapticap pegol) for diabetic nephropathy, and NOX-H94 (Lexaptepid pegol) for anemia of chronic disease.

Spiegelmers are oligonucleotides that are built on a backbone of mirror-image RNA or DNA (L stereoisomers). By leveraging this "mirror-image chemistry", Spiegelmers solve two key problems that have limited the development of aptamers made with natural D stereoisomers: Spiegelmers have enhanced biological stability and are immunologically passive. This is due to the fact that the mirror-image L RNA and DNA of Spiegelmers is not recognized as RNA or DNA by enzymes found throughout the body called nucleases, and are therefore not degraded in the blood. For similar reasons, the components of the immune system that normally react to foreign RNA or DNA do not recognize Spiegelmers and as such do not activate the immune system in response to their administration. In fact, Spiegelmers combine the benefits of biological drugs and small chemical molecules, namely the affinity and specificity of biologics in order to modulate the biological function of its target, with the ease of chemical synthesis.

Traditional aptamers have been validated as therapeutic drugs and drug candidates and have been approved for, or are under development in, ophthalmic indications. Since they are constructed from naturally-occurring nucleotide building blocks, known as D nucleotides, they require further molecular modification of their backbone in order to reduce susceptibility to degradation in the body by nucleases, which are certain enzymes that cleave nucleic acids. However, it is not always possible to modify the traditional aptamer's backbone without compromising the biological function of the molecule.

Spiegelmers are identified using an *in vitro* evolutionary screening process called SELEX (Systematic Evolution of Ligands by EXponential Enrichment) (Source: Tuerk, C. & Gold, L. Science 249.4968 (1990): 505-510). First, the mirror image of the intended target is chemically synthesized. Then, a mirror-image target-binding compound is selected from an oligonucleotide library containing approximately 1015 potential candidates through multiple rounds of selection and amplification. Third, single compounds, called aptamers, which bind to the mirror-image target are identified. Finally, the mirror image of the selected aptamer, i.e. the Spiegelmer, is chemically synthesized. This Spiegelmer will then bind to the intended target while retaining all the binding properties of the selected aptamer. Using this technology platform, it is possible to rapidly create additional Spiegelmers as potential drug candidates. Spiegelmers are chemically synthesized, rather than being manufactured by using a biological process that depends on living cells or organisms. We believe this is a clear advantage over the biological manufacturing methods used to make monoclonal antibodies and other biologic therapeutic agents, which are costly, complex and can be difficult to scale-up or to transfer manufacturing from one facility to another. Because they require the maintenance of populations of living cells, biological manufacturing methods are accompanied by the inherent risks of viral or bacterial contamination, as well as batch-to-batch variability in the manufactured product. By contrast, the Spiegelmer manufacturing process uses chemical synthesis that we believe is more amenable to stable commercial-scale production.

Spiegelmers are mainly identified to target extracellular signaling molecules, such as peptide hormones and chemokines. These molecules act as key regulators in various areas, such as in the tumor microenvironment, inflammation, tissue invasion and iron regulation, and are constantly replenished in the body. It is assumed that addressing imbalances in these types of molecules of a disease is an effective way to approach therapeutic intervention. However, peptide hormones and chemokines are difficult to address with small-molecule drugs because they lack the binding pockets that are necessary for small-molecule drugs to be effective. In addition, recent publications suggest that monoclonal antibodies may not be able to chronically suppress the biological activity of certain of these targets (Source: Haringman et al., 2006; Sandhu et al., 2013). Based on our preclinical and clinical experience, we believe that Spiegelmers will be able to address such peptide hormones and chemokines, making them ideal targets for development where small molecules and monoclonal antibodies have difficulties or have been unsuccessful. Finally, we are convinced that this platform is well-suited to identify additional product candidates in the area of cancer treatment and modulation of the tumor microenvironment.

NOVEL LINKERS FOR ANTICANCER PROTEIN CONJUGATES

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In oncology, protein-based drug conjugates incorporate an enzymatically cleavable, a reductive, a hydrolytic or an acid-sensitive linker that allows release of the anticancer drug at the tumor site. Aldoxorubicin, the (6-maleimidocaproyl)hydrazone derivative of doxorubicin is an acid-sensitive prodrug of doxorubicin that is rapidly and selectively bound in situ to the cysteine-34 position of endogenous albumin after intravenous administration. Uptake of albumin in solid tumors is mediated by (1) the enhanced permeation and retention effect and (2) the binding to proteins such as the endothelial gp60 receptor and SPARC (Secreted Protein, Acidic and Rich in Cysteine), a secreted glycoprotein with high binding affinity to albumin in the tumor interstitium[1].

Enrollment in a global phase 3 study in second-line soft tissue sarcoma was completed in November 2015 under a Special Protocol Assessment granted by the FDA comparing the efficacy of aldoxorubicin for patients with second-line soft tissue sarcoma with five optional anticancer agents. Meanwhile, in this pivotal, global phase 3 clinical trial with aldoxorubicin - in accordance with the statistical analysis plan - 191 events have been reached that allow to trigger the analysis of the primary endpoint of progression-free survival (PFS). These events are being reviewed and verified by an independent, blinded radiology organization to analyze all of the scans for the Phase 3 pivotal clinical trial from the 79 sites around the globe.

Top-line results are expected at the end of Q2 2016.

During internalization of drug protein conjugates, a large pH shift from 7.2–7.4 in the blood or extracellular spaces to 4.0–6.5 in the various intracellular compartments takes place during cellular uptake of the carrier-linked prodrugs. The significant drop in the pH-value is a unique physical property in living systems that can be exploited for intracellular drug delivery by coupling drugs to suitable carriers through acid-sensitive bonds. As a continuation of our development of aldoxorubicin, we set out to design a novel acid-sensitive drug release platform that is applicable to carriers such as serum proteins and antibodies. Key requirements for acid-sensitive bonds are high stability of the carrier-bound drug in the blood circulation and an effective or sustained release of the active drug in the acidic tumor interstitium and acidic endosomes/lysosomes of tumor cells. In addition, sufficient stability of the acid-sensitive bond facilitates galenic formulation and reconstitution.

For this reason, we explored novel aromatic hydrazone linkers and fine-tuned their pH-dependent release profile by substituting the aromatic moiety with a spectrum of electron-withdrawing groups. Resulting drug hydrazone derivatives conjugated to the cysteine-34 position of albumin demonstrated that drug release at pH 4.0 – 5.0 could be substantially modified in the range of 1-50 h. The new hydrazone linker technology was coined LADR™ (Linker Activated Drug Release) and has the additional advantage that the galenic formulation and reconstitution of LADRTM-based drugs is facilitated. The LADR™ technology has been applied to several anticancer drugs such as nemorubicin vinblastine and gemcitabine.

A drug derivative with gemcitabine is the most advanced example of an intelligently designed albumin-binding prodrug that makes use of the LADR™ technology. Gemcitabine (Gemzar®) at its recommended dose of ~1000 mg/m² has two major disadvantages: (1) approximately 90 % are deactivated by cytidine deaminase to the inactive uridine metabolite and excreted in the urine; (2) low expression level of the human equilibrative nucleoside transporter 1 (hENT1) on the cell surface of cancer cells lead to chemoresistance preventing cellular uptake of gemcitabine.

DK049 is an innovative prodrug that is capped at the amino group of the cytosine moiety with acetyl benzoic acid preventing deamination and in addition introducing a carbonyl group to which a proprietary maleimide-bearing LADR™ linker is bound forming a stable, but acid-sensitive hydrazone bond. Following intravenous administration, DK049 binds rapidly and selectively to circulating albumin and utilizes the advantages of albumin-mediated tumor uptake as well as cellular uptake by endocytosis [1].

DK049 is dosed at approximately one seventh of the dose of gemcitabine in nude mice. We could show that DK049 (8 x 18 mg/kg) is distinctly superior to gemcitabine (4 x 240 mg/kg) in four subcutaneous human patient-derived tumor xenografts (NSCLC: LXFE397, LXFE937, ovarian cancer: OVXF899, and pancreatic cancer: Panc11159) inducing long-term complete remissions for several weeks after end of therapy without any loss in body weight or bone marrow toxicity.

[1] Felix Kratz, Albumin as a drug carrier: design of prodrugs, drug conjugates and nanoparticles. J Control Release 8;132:171-83, 2008.

DIACHEMO – POC DEVICE FOR QUANTIFICATION OF CHEMOTHERAPEUTIC DRUGS

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The decision-making in chemotherapy nowadays depends on standard methods that are liquid chromatography followed by mass spectroscopy (LC-MS/MS) or capillary chromatography; both are labour- and cost-intensive and can be performed only in dedicated hospitals and laboratories. This lead to a minimal therapeutic drug monitoring in patients and hence that 30-60% of drugs are administered without clinical benefits.
We propose to develop a point-of-care device for quantification of chemotherapeutic drugs in small body fluid samples by highly selective nanoparticle extraction and liquid crystal detection incorporated in a microfluidic lab-on-a chip device (optofluidics based) allowing the real-time drug monitoring. This will improve the therapeutic outcome and reduced health care costs.

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THERANOSTICS: FROM TARGETING AND IMAGING TO TISSUE ENGINEERING

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Theranostics refers to the combination of diagnosis and therapy. In the nuclear medicine field, several theranostic constructs have already found their way into routine clinical practice. Theranostic concepts can also be employed in nanomedicine. By labeling polymers, liposomes and micelles with radionuclides, and by using them to preselect patients presenting with sufficiently high levels of EPR, nanomedicine treatments can be individualized and improved. In recent years, theranostics constructs and concepts have also emerged in the fields of regenerative medicine and tissue engineering. By labeling (stem) cells or scaffold materials with contrast agents, their localization and performance can be monitored non-invasively and longitudinally. In the present lecture, several of the abovementioned systems and strategies will be highlighted, together showing that theranostic constructs and concepts holds significant potential for improving disease treatment.

UNSOLVED PROBLEMS IN FUNGAL INFECTION

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Of all species of fungi on earth about 600 cause diseases and most infections are caused by the genera Trichophyton, Candida, Aspergillus and Cryptococcus. Fungi infect billions of people every year and the incidence of invasive fungal infections is rising as a result of modern medical interventions and immunosuppressive treatments. Hence, most infections occur as consequences of other health problems such as asthma, cancer, organ transplantation and corticosteroid therapies. Fungal infections are best divided into:

1. invasive infections, which are often fatal (i.e. cryptococcal meningitis, invasive aspergillosis, Candida bloodstream infection, Pneumocystis pneumonia) if not treated promptly,
2. chronic lung or deep tissue infections (i.e. chronic pulmonary aspergillosis),
3. allergic fungal diseases (i.e. allergic bronchopulmonary aspergillosis),
4. mucosal infections (i.e. Candida vaginitis), and
5. skin, hair and nail infections (i.e. onychomycosis).

Clinical presentations are not specifically, which results in missed or delayed diagnosis and compromised clinical care. Invasive fungal infections remain understudied and underdiagnosed when compared to other infectious diseases. Three unresolved problems require attention: We need a robust, rapid, simple, and cheap diagnostics for sensitive diagnosis to allow adequate antifungal treatment. Most diagnostics still suffer from long assay times and poor specificity and/or sensitivity. Appropriate diagnostics would immediately affect mortality and reduce morbidity. Safer and more effective antifungal drugs are also needed. Antifungal drugs suffer from restrictions in the route of administration, toxicity, a narrow spectrum of activity, detrimental drug interactions, the development of drug resistance, bioavailability in target tissues and high costs. There are currently no approved human vaccines for any fungal pathogen, despite the advances in our understanding of antifungal immunity. Accurate data on fungal disease burdens and their economic impact are needed to raise scientific interest and increase global investments. The population at risk for life-threatening fungal infections is growing worldwide, and tackling the challenges of these pathogens should become a much higher priority.

Reference: http://www.life-worldwide.org/fungal-diseases-

RADIONANOMEDICINE: EXOGENOUS AND ENDOGENOUS

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Radionanomedicine is the combined approach of nuclear medicine and nanomedicine. Use of radioisotopes such as Tc-99m (SPECT; single photon emission combined tomography) or Cu-64 (PET: positron emission tomography) are used as diagnostic-imaging help to nanomedicine and radioisotopes such as Lu-177 and Y-90 for therapy aide to nanomedicine. Radionanomedicine can be divided to exogenous or endogenous ones.
Exogenous radionanomedicine uses mostly inorganic materials such as iron oxide, gold, silica and doped nanoparticles (upconversion nanoparticle). It also can use organic materials of micelle or liposomes. We can use trace amount (nanomole) and avoid concerns of safety and also use tracer kinetic to elucidate the biodistribution with imaging and quantification. Using Ga-68-DOTA-labeled iron oxide, fluorescent-lableled SERS (surface enhanced Raman scattering) dots, and Cu-64 labeled UCNP (upconversion nanoparticles), we could characterize sentinel lymph nodes using mannose-macrophage targeting, FRES (fluorescence-Raman endoscopic system) imaging of HER2/EGFR1 receptors on the surface of the tumors and rapid hepatobiliary excretion of 40 nm-sized encapsulated UCNPs, respectively.
Endogenous radionanomedicine recently adopted endogenous organic nanovesicles which are collectively called extracellular vesicles (EVs) consisting of exosomes and microvesicles. Liposomes are void surrogate (of EVs) artificially made to mimic exosomes. EVS were proposed to replace cell therapy. Instead of fluorescent dye (Cy7), Tc-99m HMPAO or Cu-64 DOTA could label EVs and we now

began to understand biodistribution. Migration of EVs on microfluidic chips and their functional activity using luciferase-reporter or graphene-oxide-reporter could simulate intercellular *in vivo* EV action succefully *in vitro*. Thus, understanding of whereabouts and action at target sites (cells) of EVs were recently enabled by endogenous radionanomedicine and molecular imaging on a chip.

POLYMERIC NANOCARRIERS FOR IN-VIVO GENOME EDITING USING NUCLEASE ENCODING MRNA

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The discovery of sequence specific endonucleases (Zinc fingers, TALENs, CRISPR/Cas 9, etc.), allowing for targeted editing of disease-assoiated genes, has caused some shift of paradigm in the field of gene therapy. Rather than delivering these enzymes themselves, the encoding mRNA is delivered into the cytoplasm. This allows for minimizing off-target effects, provided that transient endonuclease expression can be achieved and integration into the genome be avoided. Besides an optimization of the nucleotides themselves, however, their controlled intracellular delivery still requires appropriated carrier technologies, preferably of non-viral nature. Poly-cationic nanoparticles, capable of condensation and polyplexes formation and thus mediating protection against nucleases, cellular uptake and endosomal escape, may provide a viable alternative. With respect to a safe translation into the clinic, however, such nanocarriers must be well tolerated, not immunogenic and capable to be eliminated from the body. Our approach is therefore to design nanocarriers with tunable physicochemical properties, such as size, charge density and surface energy, but always made of non-toxic and biodegradable materials.

STARCH-GRAFT-POLYMERS

A series of cationic starch derivatives was synthesized and characterized in molecular weight and degree of modification. Using a two-step synthesis with first oxidation and then a catalyst mediated coupling, diverse cationic side chains, 0.8kDa bPEI and various oligoamines, can be coupled to starch with controlled degree of modification. The resulting cationic starch derivatives were compared in cytotoxicity, pDNA transfection efficiency and enzymatic degradability.

Providing a sufficient charge density, all starch-graft polymers could bind pDNA. With a MW >100kDa stable nanoplexes were formed. Starch-derivatives with short, linear side chains had a lower cytotoxicity, but needed a higher degree of substitution for effective transfection. Starch-grafting of normally non-transfecting PEI 0.8kDa led to a transfection efficiency comparable to bPEI 25kDa, but by maintaining lower cytotoxicity and enzymatic degradation by α-amylase. (Yamada, 2014) These data point to an optimum of oligo-amine side chain length for balancing cytotoxicity, transfection efficacy and biodegradability

AMINO-FUNCTIONALIZED CALCIUM PHOSPHATE

Amino-functionalized, calcium phosphate (CaP) NPs were prepared by a novel one-step synthesis. Wet-precipitation in the presence of (N-(2-aminoethyl)-3-aminopropyl trimethoxysilane) as dispersant and surface modifier resulted in stable, cationic nanoparticles with a narrow size distribution (~140 nm) and positive zeta potential at physiological pH. Two crystal structures, hydroxyapatite and brushite, were obtained and studied for the impact of amino-group density on transfection efficacy, biodegradation, cytotoxicity and immune stimulation (Mostaghaci 2013, 2015).

The amino-modified CaP NPs enabled stable complexation of pDNA and more reproducible transfection for both obtained crystal structures compared to conventional CaP precipitates. Brushite NPs had a higher zeta potential, were more efficient in transfection and showed a faster biodegradation at physiological pH. The amino-modification decreased the release of pro-inflammatory cytokines from treated macrophages for both CaP NPs compared to the respective non-functionalized CaP NPs.

CATIONIC POLYMER COATED PLGA NPS

Poly(lactic-co-glycolid) PLGA) is very well established as a pharmaceutical polymer of proven safety. In order to use it for nucleotide delivery, however, cationic charges must be somehow introduced. Technologies to just coat PLGA NP's by cationic polymers allowed to reduce the necessary amounts of the latter, but still to form stable nucleotide nanoplexes. (Kumar 2004, Dong 2012) The effect of the nature of the cationic polymer for coating was studied by comparison of chitosan, protamine and cationic starch as coating material. The resulting particles were compared in their colloidal stability and effect in siRNA delivery *in vitro*.

NANOPARTICLE–ENABLED DELIVERY OF NUCLEASE ENCODING OF MRNA TO CORRECT LETHAL SURFACTANT PROTEIN B AS A PROOF OF CONCEPT FOR IN VIVO GENOME EDITING

A specific zinc finger nuclease encoding mRNA (Kormann 2011) was complexed with Chitosan-PLGA NPs and delivered via intratracheal instillation to transgenic mice with a doxycycline-dependent promoter for surfactant protein B expression. The hypothesis was that the nuclease would cleave the dox- promoter and insert via homology directed repair a dox-independent, constitutive promoter. The latter had been provided previously via adenoviral transfection. For comparison the zinc finger nuclease was also delivered by adenoviral transfection.

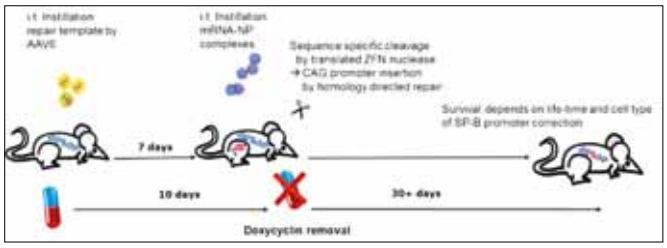

Figure 1: Treatment scheme of the genome editing study using chitosan coated PLGA NPs as carrier of mRNA in a mouse model with SP-B expression under a doxycyclin-dependent promoter.

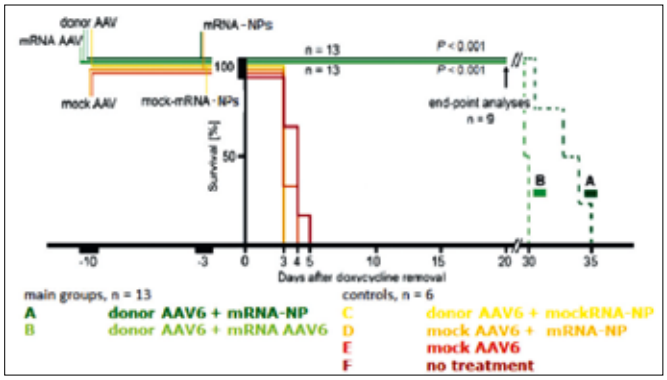

Figure 2: Kaplan-Meier survival curves for the various treatment groups in an SP-B deficient mouse model.

Complexation of mRNA with the Chitosan-PLGA NPs increased the expression compared to the level achieved by free mRNA administration. The treatment with Chitosan-PLGA NPs-mRNA was well tolerated, low immunogenic and resulted in a longer survival time compared with all control groups even including the AAV-mRNA transfection. (Mahiny 2015).

CONCLUSION

Optimized polymeric nanocarriers have outstanding potential for timely gene therapy applications, such as genome editing and may outperform viral carriers not only in safety, but also in efficacy.

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HOW CAN EU-NCL SUPPORT INDUSTRY?

CAROLINE LEMARCHAND, Onxeo, Preclinical and Pharmaceutical Development Director

There has been significant progress in the development and application of nanomedicines for drug delivery, diagnostic. Nevertheless, these specialized pharmaceutical dose forms remain very complex, qualified by expert group as Non Biological Complex Drugs (NBCD), between small chemical entities and large molecules. They are generally manufactured using a non-standard proprietary manufacturing processes and their characterization is always challenging.

Many of the manufacturing challenges applicable to conventional medicines eg stability, process impurities level, … also apply to nanomedicines. Although there are many "standard" validated characterization techniques used for conventional medicines (laser diffraction photo correlation spectroscopy, X ray diffraction that can be used for the qualification of the nanomedicines, there is as yet no "gold" standard or dedicated monograph for characterizing nanoparticles. There are few guidelines focused to nanoparticles. Available guidelines are based on the lessons learnt from existing and marketed nanoparticles (liposomes, iron oxide nanoparticles, …). In addition, regulatory agencies require as well a high level of characterization of nanomedicines in order to authorize their marketing. Therefore, in the development of nanomedicines, characterization of these nanoparticles represent one of the most difficult challenges and requires a lot of expertise.

Depending on the company developing nanomedicines (big pharma, generic, biotech, SME) characterization of the nanomedicines can be carried out internally/in house, through collaboration with academic laboratories, institutions, contract research organizations and/or platforms such as Nanotechnology Characterization Laboratory (US – NCL or very soon EU-NCL). As characterization of nanoparticles requires specific techniques and expertise focused on these materials, NCL appears a good scientific partner for developing nanomedicines. Although each nanoparticle is unique, NCL translates the lessons learnt from many products they have assessed, providing "standard" protocol developed and validated for these specific materials and covering the characterization from the physicochemical characterization to the *in vivo* assessment including the immune toxicity.

Onxeo is developing Livatag® a polymeric nanoparticle loaded with doxorubicin. Livatag is currently under clinical investigation in phase III compared to Best Standard of Care in patients with advanced HCC (Relive study). The company already initiated a collaboration with US-NCL for a better understanding and characterization of the drug product in order to support the industrialization of the process.

NANOATHERO – FIRST RESULTS OF THIS EU PROGRAMME NANOMEDICINE FOR TARGET-SPECIFIC IMAGING AND TREATMENT OF ATHEROTHROMBOSIS

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Atherothrombotic diseases remain the main cause of morbidity and mortality with clinical manifestations of angina, heart attack and stroke. There is a need for new approaches for early diagnosis and improved therapies. This is the focus of Nano-Athero, an European large scale project, started in February 2013. The aim is to demonstrate that nanotechnologies can be developed and clinically proven to be effective in tackling cardiovascular diseases (Chauvierre & Letourneur, 2015; Juenet et al., 2015). NanoAthero combines in-depth knowledge of nanocarrier bioengineering and production with state-of-the-art expertise in imaging and treatment of cardiovascular patients providing a full framework of 16 partners within one collabora-tive European consortium (16 partners from 10 countries - see http://www.nanoathero.eu/). NanoAthero project integrates several key elements: GMP production, the initiation of clinical investigations in patients at high cardiovascular risk, includ-ing the preparation of dossiers on regulatory issues, nanotoxicology, risk and ethical assessments, and the evaluation of the performance of optimized diagnostic and therapeutic compounds.

In NanoAthero, several systems (Matuszak et al., 2016) were studied and evaluated *in vitro* and *in vivo* (Almer et al, 2014; Suzuki et al., 2015). Using GMP liposomal nanoparticle (Lobatto et al., 2015), the clinical studies of liposomes encapsulating prednisolone in atherosclerosis were already performed (der Valk FM et al, 2015).

NanoAthero tackles critical current limitations in atherosclerotic disease management by using Nanomedicine, aiming to deliver nanosystems clinical validated by Phase-I Clinical Trials, and ready for future clinical development through Phase-II/III Clinical Trials and ultimate clinical and commercial/business translation in atherosclerosis. The discovery of new molec-ular targets, the better understanding of the pathophysiology of atherosclerotic disease, as well as the ongoing nonclinical and clinical trials for imaging and therapy, will undoubtedly improve the prevention, diagnosis and treatment, and finally the natural history and the prognosis of atherosclerosis.

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POLYPEPI612 PRECISION IMMUNOTHERAPY VACCINE FOR MELANOMA

JULIANNA LISZIEWICZ, eMMUNITY Inc.; Bethesda MD

eMMUNITY is meeting the need for a safe and effective vaccine-based immunotherapy that specifically attacks melanoma cells of patients identified as likely responders. Our research has addressed the main reasons for the clinical failures of cancer vaccines, namely (i) the inadequate identification of T cell targets, and (ii) the lack of biomarkers that can identify likely responders to the vaccines. First, we have discovered the mechanism by which T cells are activated to recognize HLA-presented targets on the tumor cells, enabling us to identify the T cell targets. Second, our PEPI Test is a retrospectively validated proprietary diagnostic device that predicts the antigen-specific T cell responses of HLA genotyped subjects, thereby enabling us to identify likely responders to a cancer vaccine.

This research led to our development of a precision vaccine-based immunotherapy for melanoma. Our product, PolyPEPI612, consists of 9 polypeptides that activate both helper and cytotoxic T cells against at least 3 melanoma specific antigens in 75% of the 228 melanoma patients for whom we have complete 4 digit HLA genotype data. Our PEPI Test can identify these likely responders based on their HLA genotype, and we predict that selected likely responders will experience clinical benefit after treatment with PolyPEPI612.

We are in the process to initiate clinical trial to demonstrate that PolyPEPI612 is a safe and effective treatment for melanoma in patients selected by our PEPI Test as likely responders. The secondary objective is to prospectively validate our computational vaccine development technology, enabling us to develop additional precision vaccine-based immunotherapies for other cancers for identified likely responders more quickly and more cheaply.

NANOMECHANICAL PROFILING OF BREAST CANCER: A NOVEL TOOL FOR CANCER DIAGNOSTICS AND PROGNOSTICS

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A crucial point in making treatment decisions for cancer patients is the assessment of tumor aggressiveness. Currently, for breast cancer, established prognostic markers exist that are routinely assessed by standard pathological examination. However, these parameters are often not sufficient to stratify patients, especially those with early stages of breast cancer and adjuvant therapy is frequently administered to patients who might have been cured by surgery and anti-hormonal treatment alone. The goal to avoid over- and under-treatment has led to an intensive search for prognostic and predictive markers in early breast cancer. Physical interactions between cancer cells and the extracellular matrix (ECM) that occur at the molecular (nanometer) scale are crucial for the metastatic process. Consequently, nanomechanical alterations of cells and ECM due to cancer progression can serves as potentially suitable markers of cancer aggressiveness that may help to optimize treatment strategies. This motivated us to develop an atomic force microscope (AFM)-based method for measuring nanomechanical (stiffness) profiles of unadulterated tissues in native physiological buffer conditions with an unprecedented stiffness sensitivity resolved at nanometer-scale spatial resolution. An AFM utilizes a ~10 nm-sharp stylus or tip that makes ~10'000 miniscule indentations across a living tissue specimen (Plodinec et. al; Nature Nanotech. 2012). In this clinical study, we have analyzed human 187 breast cancer samples from breast biopsies and tumor resections including primary breast cancers of various stage and grade, lymph node metastases, and non-neoplastic breast parenchyma. Post-AFM samples were fixed, paraffin embedded in an oriented manner and used for routine histology. Data showed for all human breast cancer samples, distinct stiffness phenotypes in comparison to the surrounding non-neoplastic and morphologically normal breast tissue and revealed specific nanomechanical profiles of phenotypes that lead to metastases. Interestingly, patients presenting nanomechanical changes in the adjacent tissue, which was histologically characterized as tumor free, typically exhibited local and/or distant metastases. Overall, our findings demonstrate the first application of nanomechanical profiling in a clinical setting that allows for fast, on-site assessment of specimen and does not suffer from inter-observer variability such as other markers. The relative size and distribution of nanomechanical profiles can provide an indicator of cancer aggressiveness, and therefore orientate therapy choice, and support patient follow-up.

THE MIRACLE OF PEGYLATION: SPECIFIC PROTEINS DETERMINE THE STEALTH EFFECT OF POLYETHYLENE GLYCOL

VOLKER MAILÄNDER

PEGylation is today's gold standard for drug delivery vehicles to reduce unspecific cell uptake, i.e. to establish a "stealth" effect. Nanocarriers with diameters of 100 nm were modified with PEG as the gold standard and for comparison with a hitherto less known polymer poly(ethylene ethyl phosphate)-modified (PEEP). PEEP is interesting as it is biodegradable, has additional chemical functionality, while for PEG recent studies have also pointed out medical problems with PEG, namely hypersensitivity or antibody formation. As it is thought that the reduction of protein absorption is the critical parameter for the stealth effect while still a detectable amount is present we chose to combine the critical techniques. We determined the exact number of polymer chains attached to the nanocarriers, which were then investigated with respect to amount of protein adsorption, cell uptake, and protein corona composition. Three intriguing findings are reported: First, both PEG- and – and also interestingly PEEP-modified nanocarriers - are not internalized by cells and exhibit a reduced protein adsorption after incubation with human plasma. Secondly and more intriguingly, all particles are strongly internalized by cells if not previously incubated with human plasma. Third and most importantly, we have shown by quantitative proteomic mass spectrometry of the protein corona on both stealth nanoparticles that clusterin – also known as apolipoprotein J (ApoJ) is the major component in both protein mixtures and that plasma as well as clusterin alone act as "cell-repellents". This established the necessity of distinct plasma proteins at the nanocarriers' surface to mediate the stealth effect.

HOSPHORUS DENDRIMERS-BASED ANTI-INFLAMMATORY PROPERTIES FOR DISEASES TREATMENT

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By virtue of their high degree of molecular uniformity, perfectly controlled size (1-15 nm), shape and surface chemistry, as well as their highly-branched three-dimensional architecture and versatile surface functionalization, dendrimers have been engineered for use in modern medicine as nanodevices, either in nanocarrier drug approaches or as drugs per se. They have been recently included in a new chemical/biological space named 'dendrimer space', developed by several of us[1,2], which represents a new vision for medicinal chemistry which should extend the possibilities to develop new nanodrugs to fight several diseases.
The intrinsic biological effect of terminal moieties carried by dendrimers as well as their number played a major role in the global efficiency decupling the overall therapeutic activity. Basically the main successes of dendrimers have been due to their appropriate, reproducible and optimized design parameters addressing the physicochemical limitations of classical drugs (e.g., solubility, specificity, stability, biodistribution and therapeutic efficiency) and overcoming biological obstacles to reaching the right target(s) (e.g. first-pass effect, immune clearance, cell penetration, off-target interactions, etc.). Recently we mentioned the recent progresses of dendrimers as nanoscale drug delivery systems for the delivery of drugs using enteral, parenteral and topical routes with a focus on the emerging and promising routes such as oral, transdermal, ocular and transmucosal routes [3]
Since several years, our objective is to develop first-in-class anticancer drugs based on cellular phenotypic screening and original biocompatible phosphorus dendrimers – developed at Toulouse, France - as novel biologically active and promising cohemotypes. Several applications of our phosphorus dendrimers were reported in the recent past: anti–prion, anti-inflammatory properties, NK cells multiplication, effects on Alzheimer and Parkinson diseases, use for diagnosis etc.

Three examples of the anti-inflammatory properties of phosphorus dendrimers will be mainly presented and discussed:

- Neutral phosphorus dendrimers bearing 48 trimannose or 96 dimannose caps were found powerful anti-inflammatory compounds that reduce lung accumulation of neutrophils in a mouse model of acute lung inflammation,
- A Polyanionic phosphorus dendrimer of generation 1 ended by 12 azabisphosphonate terminal groups displays original properties towards the human immune system: i) amplification of Natural Killer (NK) cells which play an important role to fight against viral and bacterial infections and again many different types of cancers, ii) activation of monocytes, inhibition of the proliferation of TCD4+ lymphocytes (pro inflammatory lymphocytes), iii) anti –osteoclastic activity on mouse and human cells. Preclinal assays suggest the potential use of this dendrimer as a nanotherapeutic for rheumatoid arthritis,
- Polycationic phosphorus dendrimers with surface protonated amines (pyrrolidinium or morpholinium) when complexed with siRNA targeting TNF- alpha showed high uptake and transfection efficiency in cells and high therapeutic efficacy in mice using a lung inflammation model. Therefore phosphorus-based dendrimers are suitable vectors for siRNA delivery and indicate that the performance of such dendrimers are highly dependent on the surface functional groups used.

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THE NANOMEDICINES ALLIANCE – HOW INDUSTRY BUILDS ITS NETWORK – FIRST ACHIEVEMENTS

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Nanotechnology has rapidly expanded into the realm of providing new and improved medicines, devices, and diagnostics. Biomedical and pharmaceutical companies translate nanomedicines research into the production and evaluation of needed products. Along with regulatory agencies and government policy makers, industry experts are critical to ensure that quality and large-scale production standards are met and that reviewers, policy makers, and the public are educated about the value, risks, and benefits of these new products. The Nanomedicines Alliance is a global organization of pharmaceutical and biotechnology companies that supports the global advancement of nanomedicines from research to commercialization that provides a focal point to define consensus positions among members and helps build relationships with regulators to establish clarity around the promise of nanomedicines through symposia, publications, policy initiatives, and other communications.

THE ROLE OF T-CELL/MACROPHAGE POLARIZATION FOR THE DESTABILIZATION OF ATHEROSCLEROTIC LESIONS

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Myocardial infarction and stroke are usually caused by a so called vulnerable atherosclerotic (AS) plaque lesions of the vascular wall. An improved early diagnosis and treatment of these lesions is essential to prevent fatal clinical endpoints. As vulnerable AS plaques are frequently non-stenotic, they remain preclinical undetectable by conventional imaging modalities. Levels of blood lipids (triglycerides , small LDLs, fatty acids), C-reactive protein, and interleukin-6 may be increased, but are insufficient for a useful preventive diagnostic assessment. Some biomarkers (e.g., troponin, natriuretic peptides, soluble ST2) indicate acute coronary syndrome or cardiac insufficiency, but not a critical destabilization of AS lesions in coronary or carotid arteries. Thus, valuable time that could be used to treat the patient is wasted. An improved understanding of the interactions between T-cells and monocytes/macrophages during the process of destabilization of AS plaques may help to find new strategies for the prevention of clinical endpoints.
Macrophage and T-cell polarization, innate- and adaptive immune responses (e.g., The Toll-like receptors 2, 4, 7), are critically involved in the process of destabilization. New biomarkers of interest are Pentraxin 3, Angiopoietin-like proteins, and the chemokines CCR7, CCL19 and CCL21. Nevertheless, the main challenge remains: which asymptomatic person should be screened? At which time? Furthermore, it is essential to act specific, effective, without side effects because the "patient" may yet feel healthy at the time of the successful prediagnosis.
We showed recently that globular adiponectin targeted pegylated "stealth" liposomes are useful tools for molecular imaging of AS lesions by means of NMRI. Under the scope of the EU project NanoAthero this approach is currently examined for a development to the human pipeline for a better detection of potentially instable AS lesions.

MICRO & MACRO ENVIRONMENT FEATURES TOWARDS DISEASE SIGNATURE IDENTIFICATION

MIRA MARKUS KALISH,
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The practice of medicine is nowadays in a moving stage from a passive coincidence mode to an active convergence model, including micro and macro environmental physical and mental effects, such as age, gender, clinical tests, biological markers, lifestyle, medical history, genetics, imaging, microbiome, etc.
The goal is to provide individually tailored treatment to patients in terms of efficacy and adverse events and actually to identify and define the term "disease signatures". A term that became a premise in the therapeutic targets, precision medicine and disease prediction processes. Eventually, disease outcome is a summation of the autonomous and microenvironment components combined with the macro-environment features of the individual patient, relying on mental, sociological and physical events.
Thus, the ability to define "disease signature" depends heavily on the comprising, analysis and translation of all relevant personal data. The remarkable achievements of science and technology are providing nowadays, a broader insight into the complex system of the individual physical and mental functioning in the surrounding - over times and modes of actions. The new sophisticated and advanced technologies are enabling the capturing of these parameters at various levels and degrees of sensitivity, providing "Big Data" initiated from various sources. These affordable technologies, such as high throughput diagnostic platforms, major gene and biomarkers discoveries and advanced imaging have led to targeted diagnosis and treatments.
The practical goal is to provide inspired translational research from basic science to preventive, predictive, precise and personalized medicine, while utilizing all knowledge and converging all available targeted technologies. This goal creates challenges in tackling the barriers such as replicability, reproducibility, overfitting, created by many parameters on few samples, database leakage, etc. For example, hospital databases, usually constructed upon specific purposes, suffer from leakage and reliability barriers, have limited availability of healthy records and include a big variety of diagnostic tests that the healthy and sick people pass through.
Furthermore, these issues of experimental methods arises debates in the public and scientific press with harsh criticism regarding the replicability and reproducibility of the published scientific results to the point that even the significance testing and the p-value is being debated. However, this last debate loses much of its sting when p-values are being adjusted to False Discovery Rate (FDR) control by using appropriate methods.
The 3C- Categorization, Clustering & Classification - strategy, developed as part of the Human Brain Flagship Project, the Medical Informatics sub-group, is our attempt to provide a broad and comprehensive insight by utilizing and converging medical expert knowledge, the disease manifestations and the potential biomarkers, including micro & macro features, towards reliable personalized prediction and treatment. The methods starts from the disease diagnosis, as assigned by the medical expert and recorded in the electronic health record (EHR). That is the knowledge we start with, even though some current diagnosis might be misleading and we want to refine it by identifying homogenous sub-groups.
The "3C" method consists of three major stages: Categorization of all the available information into clinical measurements and potential biological markers. Clustering- a feature selection process of the clinical measurements and an unsupervised learning creating sub-classes based on the disease manifestation differences while reducing dimensions. Classification - the third stage is a supervised learning feature selection of the potential biomarkers using the new subtypes as targets. The potential biomarkers includes genetics, imaging, micro and macro features, physical, psychological, behavioural and environmental aspects such as climate, culture, and community.
A preliminary feasibility study of the "3C strategy" was applied successfully to the Alzheimer's disease Neuroimaging Initiative (ADNI) cohort, bridging the barriers of missing values, etc. Thus, we were able to identify and suggest new sub-types, rather than the 5 assigned in the ADNI data set: the AD (Alzheimer disease), EMCI, LMCI (Early & Late Mild Cognitive Impairment), SMC (Significant Memory Concern) and CN (Normal). The new identified sub-types are currently in an evaluation and verification process, while embedding additional knowledge as well as clinical and biomarkers sources, towards "disease signature" identification, early detection and precise medicine.

DOXORUBICIN-FUNCTIONALIZED DEXTRAN-BASED SINGLE CHAIN POLYMERIC NANOPARTICLES AS NEW POTENTIAL THERAPEUTIC NANOSYSTEMS

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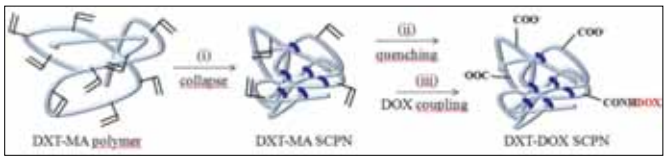
In route to obtain new types of biocompatible polymeric nanoparticles based on readily available and easily functionalized materials, our laboratory has developed a new synthetic strategy to generate well defined and water dispersible single chain polymeric nanoparticles (SCPNs). With respect to other polymeric nanoparticles, SCPNs are unimolecular soft nano-objects which can be obtained by a controlled collapse of single polymer chains [1, 2, 3]. However, most of the covalent strategies for the preparation of SCPNs suffer from the limitation of being performed at high temperatures, in the presence of metal catalysts and in organic solvents.
In this context, here we present a novel synthetic methodology based on the intramolecular cross-linking (collapse) of single polymer chains by means of bi-functional spacers (cross-linkers) in water and mild conditions (r.t. and absence of catalysts). This methodology allows the generation of SCPNs with different chemical compositions and a wide variety of functionalities.



Scheme 1. Schematic representation of the synthetic process developed to obtain dextran-based single chain polymeric nanoparticles (SCPNs) covalently functionalized with Doxorubicin (DXT-DOX SCPNs): (i) Dithiol linker-mediated intra-chain collapse of methacrylated dextran (DXT-MA); (ii) Mercatopropionic acid-mediated quenching of residual double bonds; (iii) Functionalization of DXT-MA SCPN with Doxorubicin (DOX) by peptide-like coupling.

As an example, biocompatible SCPNs based on a dextran polysaccharide (DXT) have been prepared by means of intramolecular reticulation of a single polysaccharide chain (Scheme 1). DXT is a water soluble and biocompatible natural analogue of polyethylene glycol, and has been widely used in biomedicine. DXT was first functionalized with methacrylate groups following a reported procedure [4]. The intra-chain collapse was then performed by controlled addition of a homobifunctional cross linker with dithiol terminal groups (Scheme 1). The formation of DXT-MA SCPNs was followed by different techniques, including nuclear magnetic resonance (NMR), transmission electron microscopy (TEM), dynamic light scattering (DLS), size exclusion chromatography (SEC) and Taylor Dispersion Analysis (Viscosizer). These SCPNs have a low rate of aggregation, a long shelf life, and are easily dispersible in water at relatively high concentrations.
Further reaction with mercaptopropionic acid (MPA) allowed the stabilization of the obtained SCPNs and the incorporation of car-

boxylic acid groups, suitable for further (bio)functionalization. As a proof of principle, the chemotherapeutic Doxorubicin (DOX) was conjugated to MPA-functionalized DXT SCPNs by covalent chemistry through peptide-like coupling (Scheme 1). Well defined nanoparticles with size < 100 nm were obtained (DXT-DOX SCPNs).
The cellular toxicity and uptake of DXT-DOX SCPNs were studied in HeLa cells. Cell viability analysis of free DOX showed an IC50 value of 0.38 µg/mL which was approximately 2-fold lower than that of DXT-DOX SCPNs (0.91 µg/mL, referred to the DOX) as showed in Figure 1A. Covalent conjugation reduced slightly the citotoxity of DOX. The nanocarrier without DOX did not affect cell viability, indicating that the obtained cytotoxicity was due to the DOX loading.

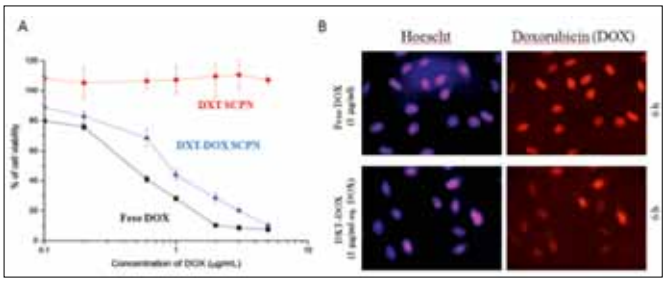


Figure 1. (A) In vitro cytotoxicity studies (MTS assay) with HeLa cells (48 hours; equivalent concentrations of doxorubicin ranging from 0.1 to 5 µg/mL); (B) Nuclear localization.

Further fluorescence studies (Figure 1B) indicated internalization of DOX into HeLa cells nuclei, suggesting that DXT-DOX SCPNs could be used as DOX delivery vehicles.
Biocompatible nanoparticles are attractive carriers for drug delivery and many efforts have been made in the last years for developing nanotechnology-based platforms against cancer [5]. Soft matter-based SCPNs seem to be promising nanocarriers for nanomedicine applications. Fine tuning of the size can be achieved by controlling the molecular weight of the precursor polymer chain and the quantity of intramolecular bonds generated in the collapse. An accurate selection of the starting materials guarantees biocompatibility/biodegradability. Incorporation of suitable functional groups opens up the opportunity to incorporate not only drugs, but also targeting moieties, dyes and/or chelating agents and convert SCPNs into interesting tools for nanomedicine. In the case of DXT-based SCPNs, the low cost of the starting material and the possibility to set up a large-scale production in GMP-like conditions make the SCPNs technology promising for translation.
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THERAPEUTIC EFFECTS OF TREHALOSE LIPOSOMES AGAINST LYMPHOBLASTIC LEUKEMIA ALONG WITH APOPTOSIS IN VITRO AND IN VIVO

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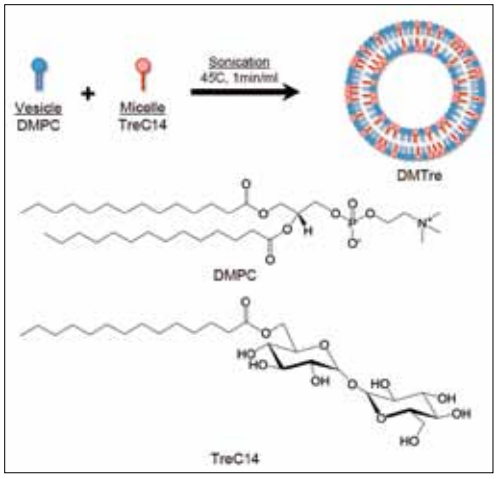
Saccharides play important roles in adhering to cells, transmitting information, and recognizing molecules on the cell membranes through receptors, including lectin. Hydration of saccharides with hydrogen bonds provides stability to the structure of water. Specific inhibitory effects of three-component liposomes composed of L-α-dimyristoylphosphatidylcholine (DMPC), Tween 20 and sugar surfactants on the growth of tumor cells *in vitro* has been reported.1-2 Trehalose stabilizes membranes and proteins in cells most likely by hydrogen bonding. Trehalose liposomes (DMTre) composed of DMPC and trehalose surfactants have been produced.3-4 The remarkable inhibitory effects of DMTre on the growth of human colon, gastric and hepatocellular carcinoma cells have been reported.3-4
In this study, we investigated therapeutic effects of DMTre (Scheme 1) without any drugs using human ALL (MOLT-4) cells *in vitro* and xenograft mice model of carcinoma after the inoculation of MOLT-4 cells *in vivo.*
Hydrodynamic diameter (d_{hy}) of DMTre composed of 30 mol% DMPC and 70 mol% TreC14 was 100 nm with single and narrow range of size distribution, which was preserved for a period remaining stable for more than one month. On th**E** other hand, DMPC liposomes were unstable and precipitated after 14 days.
DMTre inhibited the growth of MOLT-4 cells in a dose-dependent manner on the basis of WST-8 assey.5



Scheme 1

Apoptotic DNA increased after the treatment with DMTre as the dose of DMTre increased and reached a high apoptotic rate (95%), indicating that DMTre induced apoptosis of MOLT-4 cells. High activities of caspase 8, 9, and 3 were observed, indicating that DMTre induced apoptosis of MOLT-4 cells through the activation of those caspases (Figure 1(A)). The michondrial transmembrane potential of MOLT-4 cells decreased after the treatment with DMTre, indicating that DMTre induced apoptosis of MOLT-4 cells through mitochondria pathway (Figure 1(B)).
An increase of cell membrane fluidity has been reported in the induction of apoptosis for tumor cells. We evaluated membrane fluidity of MOLT-4 cells using fluorescence polarization (P) method. The P value decreased after the treatment with DMTre, indicating the increase in membrane fluidity of MOLT-4 cells. DMTre selectively fused and accumulated into hepatocarcinoma (Hep-G2 and HuH-7) cells but not into normal (WI-38) ones, suggesting that membrane of tumor and normal cells differently uptake DMTre.[4] The clustering of lipid rafts in plasma membranes of MOLT-4 cells was examined with a marker Cholera toxin subunit B conjugates Alexa Fluor (CTB), which binds to the pentasaccharide chains of ganglioside GM1 on

the cellular surfaces. The clustering of lipid rafts in plasma membranes of MOLT-4 cells was observed after the treatment with DMTre. These results suggest that DMTre could induce apoptosis for MOLT-4 cells after the increase in membrane fluidity and clustering of lipid rafts when DMTre fused to MOLT-4 cell membranes.

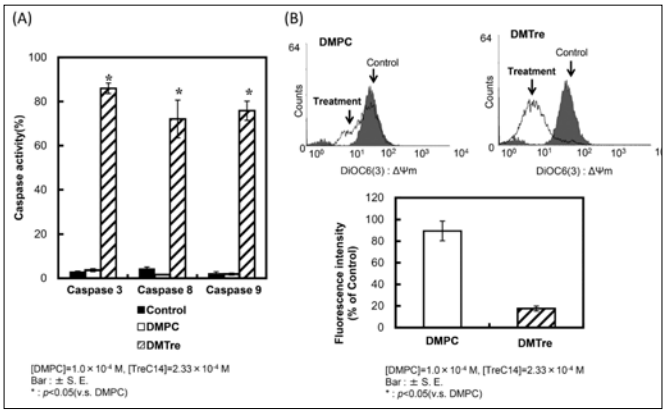


Figure 1: Activation of caspases (A) and decrease in mitochondrial transmembrane potential (B) for MOLT-4 cells after the treatment with DMTre.

We examined therapeutic effects of DMTre using xenograft mice models of carcinoma after the inoculation of MOLT-4 cells *in vivo*.[29] The results are shown in Figure 2(A) and (B). It is noteworthy that a remarkable reduction of tumor weight was obtained in xenograft mice models treated with DMTre after inoculating MOLT-4 cells. Tight bond of trehalose to water molecules and the phospholipids in bilayer interphase by specific interactions has been reported. Therefore, therapeutic effects of DMTre could be related to hydration of trehalose in tumor cells of xenograft mice models of ALL. The induction of apoptosis by DMTre for ALL was examined in xenograft mice using TUNEL method. The results are shown in Figure 2(C). Brown color indicating apoptotic cells was observed in many tumor cells of xenograft mice after the treatment with DMTre, although the apoptotic cells were slightly observed after adding DMPC liposomes. Furthermore, no cytotoxicity of DMTre on the growth of human normal fibroblast (WI-38) cells *in vitro* was obtained. These results suggest that DMTre could not have severe side-effects.
In conclusion, inhibitory effects of DMTre on the growth of MOLT-4 cells along with apoptosis through caspases and mitochondria *in vitro* were obtained. Furthermore, therapeutic effects of DMTre for xenograft mouse models of carcinoma were obtained *in vivo* along with apoptosis.

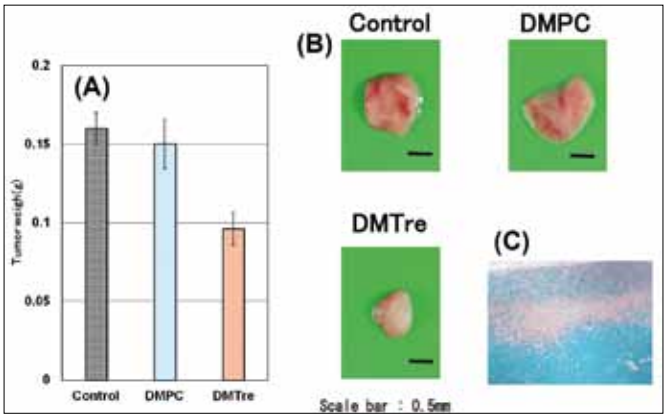


Figure 2 Decrease in tumor weight (A) and reduction of solid tumor (B) of xenograft mouse models of ALL treated with DMTre.Induction of apoptosis (C) for tissue section of xenograft mouse models of ALL treated with DMTre using TUNEL method.

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VIEW FROM THE NCL

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Assessing the equivalence of complex drug products such as nanomedicines and non-biological complex drugs (NBCDs), pose unique challenges compared to small molecules and biologics. There is a global interest in developing a rational, science-driven approach to evaluate these follow-on drug products, without compromising on safety or efficacy in comparison to the legacy formulation. As reflected in recent regulatory science initiatives by the FDA, there is increased need for development of novel nanomedicine fractionation methods to determine bioequivalence and facilitate development of the follow-on product. There is also increased expectation that new methods for physicochemical characterization, biopharmaceutics principles, and *in vitro* tools may be used to predict product performance and bioequivalence, and provide supplemental information to clinical endpoint studies. This presentation will provide NCL's perspective on the current challenges in assessing NBCD and nanomedicine equivalence. It will highlight recent advances in development of physicochemical and *in vitro* characterization methods to evaluate product equivalence, and revisit observed trends in how physicochemical parameters influence nanoparticle biocompatibility and toxicity. Funded by NCI Contract # HHSN261200800001E.

RATIONALLY DESIGNED GRAPHENE OXIDE FOR DRUG DELIVERY APPLICATIONS

KUO-CHING MEI, Pedro M. Costa, Vincenzo Abbate, N. Joan Abbott, Robert C. Hider, Khuloud T. Al-Jamal

Graphene oxide (GO), a 1 nm thin carbon sheet, has attracted attention of drug delivery scientists in the past few years. It is one of the thinnest 2D-material known offering many advantages such as huge surface area, good colloidal stability and ability to be taken up by cells. It is hypothesised that rational design of graphene oxide sheets, of controlled sizes and surface functionalities, is needed for their better utilisation in drug delivery.
We have developed, over the past few years, many approaches to surface functionalise GO using mechanochemistry, click chemistry, and click-mechanochemistry, with the vision of their utilisation for targeting delivery,1-3 or imaging purposes.4 The synthesised GO constructs were characterised using atomic force microscopy (AFM), transmission electron microscopy (TEM), thermogravimetric analysis (TGA), Raman spectroscopy, and attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR). The toxicity of the modified GOs was evaluated using a modified lactate dehydrogenase (mLDH) assay in a range of immortalised cancerous cell lines and primacy cell. Uptake in cells is monitored by TEM. Results so far have shown that double-clickable GO (bearing azide and protected alkyne group) offered more efficient uptake in cells than the starting GO material. The size plays an important role so methods to develop a relatively narrow size distribution are currently under development. Conjugation of imaging and targeting probes will be next performed to provide quantitative data on *in vitro* uptake and *in vivo* organ biodistribution. The field is still in its infancy and there are many questions to be answered regarding the optimal design required for safe and efficient delivery.

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NANOCORT PEGYLATED LIPOSOMAL PREDNISOLONE: CLINICAL EXPERIENCE IN INFLAMMATORY DISEASE

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Glucocorticoids (GC) are potent anti-inflammatory drugs but their systemic (parenteral/oral) use in inflammatory disorders such as rheumatoid arthritis and inflammatory bowel disease is limited by poor target localization and toxic effects in healthy organs. Targeted delivery of GCs to the site of inflammation with long-circulating liposomes can improve the therapeutic index. This approach has proven successful in our rat and murine arthritis studies and in several other preclinical inflammatory disease models. Furthermore, over the past few years we conducted small trials in patients with a variety of diseases of inflammatory origin. Doses of 37.5 up to 300 mg prednisolone in long-circulating liposomes (Nanocort) have been studied in patients with rheumatoid arthritis (RA), multiple sclerosis (MS), colitis ulcerosa (UC), and patients with inflamed and instable atherosclerotic plaques. In total 65 patients have been exposed to Nanocort. Besides obtaining a first view of its pronounced therapeutic efficacy profile in these indications, these studies allowed us to carefully assess the safety and pharmacokinetics of Nanocort as a prototype long-circulating liposomal GC product in humans. So far we can conclude that liposomal GC targeting is a safe and efficacious novel treatment strategy for several diseases with an inflammatory component.

MULTIFUNCTIONAL NANOPLATFORM FOR DRUG DELIVERY CARRYING CIS-PT, MAGNETIC NANOPARTICLES AND LUMINESCENCE THERMOMETRY

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One of the main advantages of nanoparticle systems in comparison with molecular systems in clinical applications is that they can carry multiple functionalities in a single object. There are several synthetic strategies to ensemble several tools in a single nanoparticle including encapsulation in empty shells (liposomes) or porous nanogeles, and covalent binding to the surface of nanoparticles or to the ends of side chains of branched polymers such as dendrimers. The strategy used in this case is based on the copolymerization of hydrophobic polymers and polyethylene polymer chains carrying diverse functionalities at one of the ends attached by covalent bonds. Moreover, inorganic nanoparticles (i.e. iron oxides) can also be encapsulated in the hydrophobic part of the copolymers. Several functionalities have been incorporated in a nanoparticle in this way, such as: imaging tags (fluorescence, magnetic resonance imaging contrast, radioactivity), targeting vectors (antibodies and polypeptides), magnetism, and therapeutical drugs such as cis-Pt. The performance of these multifunctional nanoplatforms in cell studies, MRI and SPECT biodistribution studies (Fig. 1), and magnetic heating[1] is shown. Furthermore, the nanoplatform shows blood compatibility, anticoagulant behavior, low toxicity in cell cultures, and no sign of toxicity in mice *in vivo* studies.

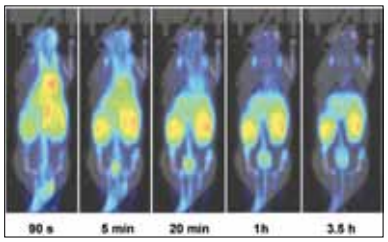

Figure 1. SPECT images of a mouse at different times after injection of ^{111}In doped iron oxide multicore nanoparticles coated with a poly(4vinylpyridine)-polyethylenglycol acrylate copolymer.

The nanoplatform has also been conjugated with cis-Pt complexes. Ligands in these complexes are members of the steroid acids family known as bile acids (Fig. 2) with the aim to avoid mostly of the cis-Pt drawbacks, because hepatic epithelial cells express a number of transport proteins that take up bile salts from the bloodstream.

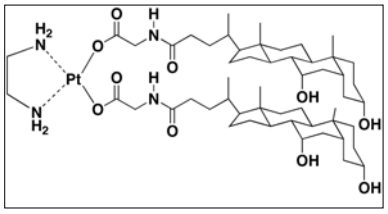

Figure 2. Ethylenediamine bisdeoxycholylglycinatepl atinum(II), Pt(en)(DCG)$_2$.

Studies on Jurkat, MG-63 and TPH1 cells showed a high internalization ratio on the last two, and deciphering the altered intracellular protein pathways has been performed by SILAC assays; showed the differential expression on RNA/DNA binding proteins in the cell treated with the cis-Pt nanoplatform in comparison with unconjugated nanoplatform.
The nanoplatform carries magnetic nanoparticles that can be used as a heat source by induction with an external ac magnetic field. The heating can be useful to potentiate the cis-Pt drug action and also as hyperthermia therapeutical agent. An alternative to hyperthermia treatments based on massive nanoparticles injection and overall tumor heating could be an intense local heating at points inside the cells to produce apoptosis. This strategy requires an adequate monitoring of the nanoheaters local temperature.
The nanoplatform can also be loaded with another element that can be useful to screen the local intracellular heat produced by magnetic induction or by internal cellular processes, which is a luminescence molecular thermometer[2]. The thermometer is based on lanthanide complexes that are encapsulated in the hydrophobic inner part of the nanoparticle copolymer coating. The most relevant features of the thermometer are a fast response (0.250s) a low uncertainty (0.5 K), and an appropriate working temperature range (295–315 K). We have used this thermometer to perform a proof of concept of temperature mapping on cells that were incubated with the nanoparticles[3]. The fluorescence microscopy in two different wavelengths simultaneously after beam splitting permits the mapping of the intracellular local temperature using the pixel-by-pixel ratio of the Eu^{3+}/Tb^{3+} intensities (Fig. 3).

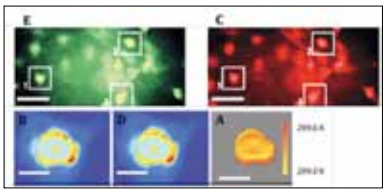

Figure 3. Imaging of Tb^{3+} (A) and Eu^{3+} (B) emissions from cell-internalized nanoparticles. Scale bars are 40 µm. Pseudocolour maps of spot 1 in Fig A&B illustrating the co-localization of the Tb^{3+} (C) and Eu^{3+} (D) emissions, temperature map (E) computed from this emissions at every pixel, and (D) histogram of the temperature distribution near the cell nucleus. Scale bars correspond to 10 µm.

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PROTEIN CORONA IN VITRO AS IN VIVO?

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The key issue in translational research of nanomaterials for biomedical applications is discrepancy between *in vivo* and *in vitro* studies. In the search for fundamental origin of this discrepancy, we started from the bases and that is that biological behavior of nanomaterials is determined by its surface. Moreover, the surface of nanomaterials gets modified in the interaction with biological environment, and become covered by biomolecules which form 'corona' around nanomaterial. The crucial part of this corona is long living part of tightly bound biomolecules called hard corona. The most studied type of hard corona is hard protein corona (HPC). There have been reported that HPC is influenced by the protein composition and concentration, but also numerous studies were done to correlate HPC and properties of nanomaterials, like for instance size of nanoparticles and with this associated surface curvature, the overall surface charges, as well as chemical compositions while keeping the surface charges the same [1-4]. Moreover, the dynamic of protein binding to the surface of nanoparitcles and the evolution of this dynamic with time were studied as well [5].

In fact the main aim behind all HPC studies is to correlate on one side physical and chemical properties of nanomaterials and on the other side their biological behavior which includes toxicity, biodistribution, therapeutic effects, side effects, etc. The ultimate goal is to be able to predict the biological behavior of nanomaterial by knowing its physical and chemical properties, like hydrodynamic radius, surface curvature and the overall surface charges. Unfortunately, this is still not possible, and thus, further studies and dipper understanding of HPC is needed.

If we again think of discrepancy found in *in vitro* versus *in vivo* studies and we know that HPC governs the nanomaterials fate, than it would be for expecting to find different HPC in *in vitro* and *in vivo* studies. Up today, there were few reported studies which compared HPC *in vitro* and *in vivo* and the expected difference was indeed found [6-9]. Therefore, we think that study of origin of this difference in HPC *in vitro/in vivo* and better understanding of it, can help us to understand and ultimately overcome the discrepancy found in *in vitro* and *in vivo* studies. This would be the key to enable successful translational nanomedicine which we are striving to.

In order to resolve this issue of this difference in HPC *in vitro/in vivo*, the crucial would be to have *in vitro* such conditions which would result in HPC as *in vivo*. Therefore, we studied *in vitro* influence of different parameters on HPC around neutral iron oxide nanoparticles. Interestingly, by including these novel study parameters, we obtained an *in vitro* HPC with the same class of most abundant proteins as found for *in vivo* conditions.

Loosely bound proteins were removed by centrifugation and washing, leaving only HPC which was afterwards remove from the iron oxide nanoparticles by fractional centrifugation and few stage of washing. The so obtained HPC proteins were loaded into SDS-Page gels and after digestion, analyzed by liquid chromatography-tandem mass spectrometry (LC-MS^2). The obtained data were analyzed by the Scaffold tool.

The detailed physical and chemical characterization was done, like FTIR, TEM and HRTEM of native and negatively stained iron oxide nanoparticles with HPC (see Figure 1).

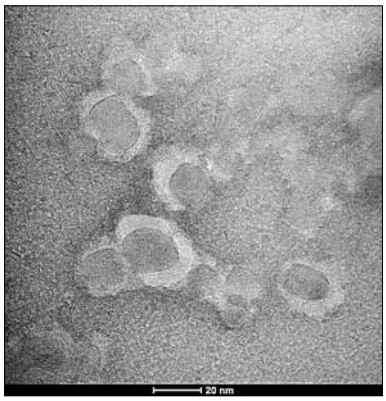

Figure 1: Transmission electron microscopy (TEM) micrographs showing the iron oxide nanoparticles with HPC which is negatively stained in order to increase the contrast of organic material on the surface of nanoparticles (proteins) and carbon background film on the TEM grid. The light parts around the surface of nanoparticles show proteins in HPC.

This research is bringing us closer to bridge the current gap in between *in vitro-in vivo* studies and by this to enable translational research in nanomedicine. This is especially important for the economic reasons because matching *in vitro-in vivo* studies would save billions (from industry side, but also from scientific foundations) typically spend in *in vivo* studies which at the end finish as toxic while previous *in vitro* studies showed the opposite.

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RELIABILITY OF IN VIVO MODELS IN PREDICTING NANOMEDICINE-MEDIATED INFUSION-RELATED REACTIONS IN HUMANS

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Pigs are often used as predictive models of nanomedicine-mediated cardiopulmonary distress reactions in humans. Unlike humans, pulmonary intravascular macrophages (PIM) are abundant in pig lungs. Robust phagocytosis of particles by PIM results in immediate release of large quantities of mediators that correlate with periods of peak cardiopulmonary disturbances. This raises questions on relevance of the pig model to human cases. However, there are suggestions of induction of pulmonary macrophages in certain human diseases (e.g., liver and inflammatory lung diseases). It is conceivable that highly responsive patients may have induced PIM, which could increase sensitivity to blood-borne particles, and the potential risk of pulmonary hemodynamic side effects. Accordingly, it would be necessary to search for constitutive or induced PIM in biopsied or autopsied human lungs, map their phenotype in liver and inflammatory lung diseases, and understand the pathologic implication of phagocyte residency in pulmonary capillaries. In this presentation, I will discuss the roles of PIM and the complement system activation on initiation of adverse cardiopulmonary distress on nanomedicine administration as well as simple strategies that could overcome these problems even in the pig model. Alternative animal models will be suggested for investigating the interplay between induced PIM and the complement system that could closely resemble the human cases and applicable for cardiopulmonary risk assessment in relation to biopharmaceuticals/nanomedicine administration.

BIOCOMPATIBLE PLATINUM NANOPARTICLES RESTORE PHYSIOLOGICAL ROS HOMEOSTASIS IN A REAL EXPERIMENTAL MODEL OF A HUMAN CEREBROVASCULAR DISEASE

MAURO MOGLIANETTI

Our recent findings show that pure, biocompatible platinum nanoparticles (Pt NPs) of different size and shape are able to counteract molecular dysfunctions that cause accumulation of intracellular reactive oxygen species (ROS) (Fig.1). We will also present our latest data on the importance of platinum nanoparticles shape (octahedral vs spherical) in the development of nano-carrier with selective enzymatic activity, uptake and drug-delivery properties with an outlook on topical dermal delivery.

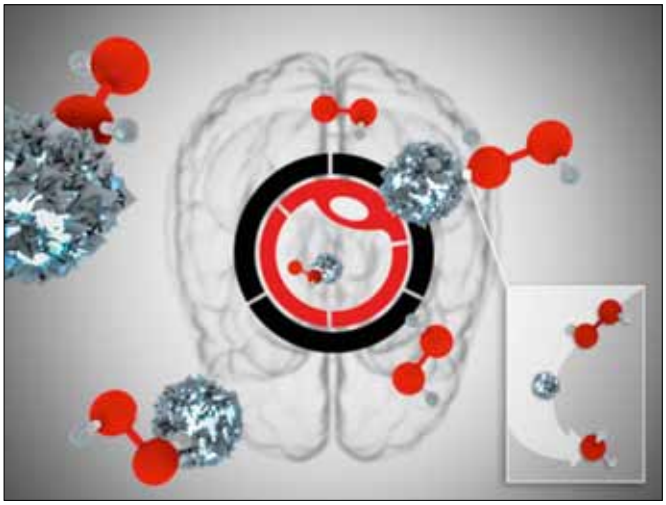


Fig.1 Graphical abstract

After performing a systematic characterization of Pt NPs as biocompatible and anti-oxidant materials (Fig. 2), we demonstrated, for the first time, that Pt nanozymes are capable to restore physiological ROS homeostasis in a real experimental model of a human cerebrovascular disease, namely Cerebral Cavernous Malformation (CCM). CCM is characterized by an increased level of intracellular ROS, and we found that Pt nanozymes can completely recover the cellular phenotype, similar to that of wild type cells.

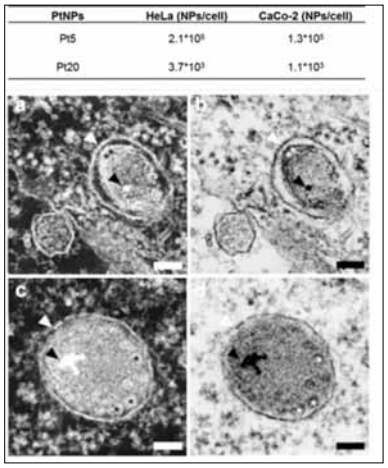
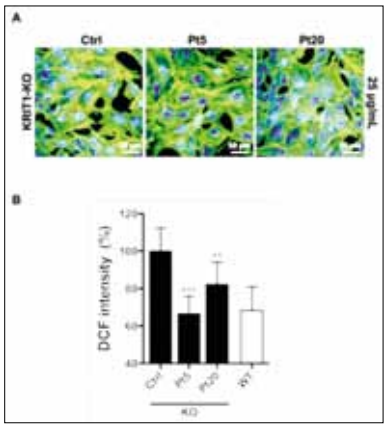


Fig.2. Cellular uptake and intracellular localization of Pt NPs. (Table) Pt NPs internalization in HeLa and Caco-2 cells 24 hours after incubation, at 50 µg/mL concentration. (a and c) STEM projection images of late endosomes/phagosomes (white arrowheads) containing, respectively, the 5 nm (a and b) and 20 nm (c and d) Pt NPs (black arrowheads).

This is possible because of the strong and broad anti-oxidant nanozyme activity of Pt NPs, which are simultaneously endowed with strong catalase-, peroxidase-, and superoxide dismutase-like activities, with superior performance than natural enzymes and higher adaptability/resistance to changes in environmental conditions (Moglianetti et al., Nanoscale, 2016, 8, 3739-3752) (Fig.3).

Fig.3. (A) Staining of Phalloidin (green) and Hoechst 33342 (blue) in untreated (Ctrl), Pt5 and Pt20 treated krit1-ko cells at 48 hours. (B) ROS levels in untreated MEF krit1-ko cells (Ctrl), krit1-ko cells exposed to Pt5 and Pt20 (25 µg/mL) for 48 hours, and untreated krit1-wt cells, evaluated by DCF assay. DCF intensity of Pt NPs-treated krit1-ko cells is expressed relative to untreated krit1-ko cells (Ctrl).



These findings are important and of broad interest, and open up novel perspectives in nanomedicine for the development of multifunctional active nanocarriers integrating the function of high-performance antioxidant drugs, with strong potential for therapies of complex oxidative stress-related diseases.

BREAST CANCER STEM CELLS: BIOLOGY AND APPROACHES FOR ELIMINATION

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The discovery of so-called cancer stem cells (CSCs) has changed paradigms for drug discovery and development, also impacting strategies in the nanomedicine sector. CSCs are considered as the tumorigenic founder population, possessing high metastatic potential and generally increased drug resistance. Thus, they are thought to be responsible for relapse of metastatic, drug resistant cancer, which commonly is incurable. Breast cancer stem cells (BCSCs) are among the most intensely investigated not at least due to high persistence and mortality rates of this cancer type. While

different novel drugs are presently in clinical testing, said to target the BCSCs, the molecular cues that play a role in the emergence and suppression of BCSCs are still ill-defined and not completely understood. Knowledge of these molecular cues, however, may offer novel starting points for nanomedicine to recover novel, more effective future cancer treatments.
Within the Lundbeckfonden Center of Excellence NanoCAN, we developed a pipeline that allows systematic testing of the functional effects of genes and mutations that have been recovered as changed from genome-wide next generation sequencing of cancers. We consecutively use this information to recover novel nanomedical strategies for therapeutic targeting of BCSCs by systematic high-throughput screening as well as by hypothesis-driven approaches. Here, we will present recent results, which identify a novel breast cancer stem cell suppressor from such systematic testing and discuss downstream strategies for the development of novel nanodrugs.

NANOPARTICLES AND THE ASSESSMENT OF ENDOCRINE DISRUPTING EFFECTS

REINHARD MÖLLER

Nanoparticles (NPs) are defined by the WHO (2002) as a nano-object with three dimensions in the size range of approximately 1-100 nm. Because of their very small size, NPs can enter the cells directly by penetrating the cell membrane and may interfere with important cell functions. The internalization of NPs can occur in a variety of ways and particle size largely influence their endocytotic processes and cellular uptake ability.
NPs have individual characteristics in regards to chemical composition, particle size, surface area, reactivity and functionalisation, charge, solubility, degree of agglomeration and used in different concentrations. Based on these different characteristics and the expanding use of NPs the effects of NPs on the endocrine system are of increasing interest.
Endocrine disruptors are chemicals that may interfere with the body's endocrine system and produce adverse developmental, reproductive, neurological, and immune effects in both humans and wildlife. A wide range of substances, both natural and man-made, are thought to cause endocrine disruption, including pharmaceuticals, dioxin and dioxin-like compounds, polychlorinated biphenyls, DDT and other pesticides, and plasticizers such as bisphenol A.
In vitro and in-vivo studies show endocrine disrupting effects of NPs in e.g. male and female reproductive systems, in the thyroid function, the insulin action and metabolism and neuroendocrine system. The disrupting effects are directed either against cellular and organ structures like ovarian cells or testicles or disrupt the normal levels of hormones.
In order to determine estrogenic active effects of compounds, exposition studies in fish are well suited as immature and male fish will respond to exogeneous estrogens with the synthesis of Vitellogenin, a female specific yolk-precursor lipoprotein, which is a well accepted endpoint determination for estrogenic active compounds. Several studies showed that NPs (AG-NPs; (CdS)/CdTe capped-QDs) can have estrogenic activities in male and premature fish determined by elevated Vitellogenin levels, whereas no effects of CdS-QDs in male stickleback was observed. These findings suggest that the endocrine disrupting effects might also be pending on species and sexual maturation.
A newly developed test system based on sensitive Vitellogenin determination in epidermal mucus of fish allows multiple non-invasive and non-destructive sampling in various fish species. Extensive studies showed that the Vitellogenin level in mucus correspond very well with the level of Vitellogenin in blood and homogenates. The in situ production of Vitellogenin in epidermis allows for the exposure of an estrogen-sensitive matrix directly to the NPs, independent from uptake, distribution and bioaccumulation in the test organisms. This new technology allows to detect NPs with estrogenic activities and to monitor these effects on the vitellogenin levels over time in exposition trials as well as in environmental monitoring programs.

MECHANISMS OF LOCAL DRUG DELIVERY WITH A COMBINATION OF ULTRASOUND AND MICROBUBBLES

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Recent studies have underlined the potential of ultrasound and microbubbles to enhance drug delivery. However, there is less consensus on the biophysical and biological mechanisms leading to this enhanced delivery. Sonoporation, i.e. the formation of temporary pores in the cell membrane, as well as enhanced endocytosis is reported. Because of the variety of ultrasound settings used - and corresponding microbubble behavior, a clear overview is missing.
We categorized the mechanisms contributing to sonoporation according to three ultrasound settings: i) low intensity ultrasound leading to stable cavitation of microbubbles, ii) high intensity ultrasound leading to inertial cavitation with microbubble collapse, and iii) ultrasound application in the absence of microbubbles.
Using low intensity ultrasound, the endocytotic uptake of several drugs could be stimulated, while short but intense ultrasound pulses can be applied to induce pore formation and the direct cytoplasmic uptake of drugs. Ultrasound intensities may be adapted to create pore sizes correlating with drug size. Small molecules are able to diffuse passively through small pores created by low intensity ultrasound treatment. However, delivery of larger drugs such as nanoparticles and gene complexes will require higher ultrasound intensities in order to allow direct cytoplasmic entry [(1)].
In addition, Fibered Confocal Fluorescence Microscopy (FCFM) was used to further evaluate in-vitro the involvement of clathrin- and caveolin-mediated endocytosis using specific inhibitors. C6 rat glioma cells were preincubated for 30 minutes with either chlorpromazine (CTZ), an inhibitor of clathrin-mediated endocytosis, or genistein (GEN), inhibiting the caveolin-mediated pathway. Inhibitor cytotoxicity was assessed, varying concentration and incubation time via XTT viability assay. Uptake of SYTOX Green fluorescent dye was triggered with 1.4 MHz US, (0.5 MPa p-p, Duty Cycle 10%, Pulse Repetition Frequency 1 kHz), in the presence of SonoVue at 20 microbubbles per cell. Uptake was monitored in real-time using FCFM. Results are expressed as median (interquartile range). Cells incubated with endocytosis inhibitors expressed a lower SYTOX Green uptake rate after sonoporation. GEN inhibition resulted in a shallow but significant ($p<0.05$) reduction of SYTOX Green uptake rate 1/k from 52.4s (20.1s, n=451) (no treatment) to 0'54" (0'22", n=378), 0'58" (0'21", n=455) and 0'58" (0'22", n=352) for 200µM, 250µM and 300µM of GEN, respectively. CTZ had a higher inhibitory effect than GEN. A significant ($p<0.05$) uptake rate reduction was observed from 0'52" (0'22", n=946) without treatment to 1'17" (0'36", n=227), 1'32" (0'46", n=448), 1'37" (0'52", n=215), and 2'11" (1'33", n=262), for 10 µM, 20 µM, 30 µM and 40 µM of CTZ, respectively. The data provide evidence for involvement of, in this case primarily clathrin-mediated, endocytosis [(2)].

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ASSESSING EQUIVALENCE OF COMPLEX DRUG PRODUCTS: EXPERIENCE WITH FOLLOW-ON NANOMEDICINES AND NON-BIOLOGICAL COMPLEX DRUGS (NBCDS)

STEFAN MÜHLEBACH, DAN CROMMELIN, on behalf of the NBCD Working Group at Ligature

Non-biological complex drugs (NBCDs), including many nanomedicines, are a class of medicinal products that cannot be fully quantitated and characterized by physico-chemical analytical means. They share that characteristic with other complex drugs belonging to the class of biologicals. Examples of NBCD products are glatiramoids, iron-carbohydrate complexes, polymeric micelles, complex ocular emulsions and liposomes. The complex nature of NBCD products means that minute variations in the manufacturing process can substantially change the composition of final products and their profile. Are the existing regulatory protocols indeed able to assess equivalence of these NBCD products or should a nano-similar approach (cf. biosimilars) be pursued? As patents of the first generation of "futuristic" drugs are expired and authorized follow-ons have demonstrated non-comparability in clinical studies, the importance of appropriate science-based approval standards is evident. They will be viewed and discussed in the symposium:

- View and Update from EMA
- View and Update from FDA
- View from the NCL
- Compendial Initiatives for NBCDs
- Ocular Emulsions
- A Comparative Study on Iron-Sucrose Formulations
- Questions and Debate

SINGLE-WALLED CARBON NANOTUBES: AS SENSORS OF ROS AND RNS AND ITS BIOLOGICAL INTERPHASE

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Key Words: SWCNT, Angiogenesis, Biosensors, ROS, RNS.

Reactive species, specifically nitric oxide (NO) and hydrogen peroxide (H_2O_2), activate signal transduction pathways during angiogenesis and other biological systems and therefore play important roles in physiological development as well as various pathophysiologies. Herein, we utilize a near-infrared fluorescent single-walled carbon nanotube (SWNT) sensor array to measure the single-molecule efflux of NO and H_2O_2 from human umbilical vein endothelial cells (HUVEC) in response to angiogenic stimulation. Two angiogenic agents were investigated: the pro-angiogenic cytokine, vascular endothelial growth factor A (VEGF-A) and the recently identified inorganic pro-angiogenic factor, europium (III) hydroxide in nanorod form. The nanosensor array consists of a SWNT embedded within a collagen matrix that exhibits high selectivity and sensitivity to single molecules of specific reactive species. We find that the production of H_2O_2 following VEGF stimulation is elevated outside of HUVEC, but not for stimulation via nanorods, while increased generation is observed in the cytoplasm for both cases, suggesting two distinct signaling pathways. In addition, we are able to detect the spatial resolution of NO in HUVEC cells in response to VEGF. Moreover, by employing transmission electron microscopy, confocal fluorescent microscopy, and UV-vis spectroscopic analysis, we have confirmed the internalization of DNA-SWCNT in HUVECs. Additionally, by using pharmacological inhibitors as well as genetic approaches, we have found that SWCNT is endocytosed through Rac1- GTPase mediated macropinocytosis in normal endothelial cells. Our work reveals a unique mode of entry of SWCNT in cells and might help to properly formulate SWCNT as nanovectors in biological systems.

THERANOSTICS NANOMEDICINE IN CANCER AND ATHEROSCLEROSIS

WILLEM MULDER,

A way to overcome a drug's side effects is by its more efficient delivery to diseased sites. This can be accomplished by nanoparticles, tiny carrier vehicles that can be loaded with drugs, known as nanomedicines[1,2]. The majority of nanomedicines are self-assembled of which the drug loading stability is strongly influenced by the *in vivo* environment. Therefore, thoroughly understanding *in vivo* drug-carrier association stability and dissociation kinetics should improve delivery efficiency and, as a result, therapeutic efficacy. Imaging techniques can monitor the drug-carrier association and help identify key parameters that determine drug-carrier compatibility. These findings can serve as drug delivery efficiency guidelines that can be applied to improve nanomedicines.

Despite nanomedicine's promise and the field's research activity, its potential is not being fully met and implementation in clinical care is falling behind. In part this is due to the technology's immaturity, but – more importantly – ways to stratify patients that may benefit from nanomedicine-based therapy are nonexistent.

The ability to non-invasively evaluate nanomedicine targeting would greatly improve patient care by allowing swift adjustments in dosage and/or treatment regimen. Strategies in which nanoparticle drug formulations are labeled for imaging-facilitated delivery are extensively studied[3,4]. Unfortunately, such theranostic approaches have little clinical relevance.

As has been shown for antibody therapy, an easy-to-prepare companion diagnostic for quantitative imaging of nanomedicines can overcome these issues. Practically, the companion diagnostic could be applied to screen for patient amenability, but could also be used as an agent that is co-injected with the actual nanotherapy to aid in treatment continuation decision. In this educational imaging-facilitated optimization of nanomedicine and the 'companion diagnostic' concept, the latest advances in these fields, and translational considerations will be discussed.

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NANOSCIENCE-INSPIRED MATERIALS RESEARCH IN DENTISTRY

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Caries is the commonest disease in the world [J. Dent. Res. 92 (2013) 592]. The disease destroys the mineral crystallites of the crowns. The crowns are made out of a unique biologically ordered material with internal interfaces. In healthy state, it remains stable for the entire human life and even beyond. Currently, no engineering process is known to bio-mimetically repair this unique material with a well-defined nanostructural organization. The economic impact of the necessary therapies is enormous. The World Health Organization estimated that the dental treatment costs accounted for 5 % to 10 % of healthcare budgets in industrialized countries. So far, therapy relies on the mechanical replacement of decayed tissue by isotropic polymers, ceramics, or composites. The analysis of the healthy and diseased crowns down to the nanometer scale has led to the necessary anatomical knowledge to develop nature-analogue dental fillings, which contain elongated nanostructures with the orientations present in dentin and enamel [Proc. SPIE 7401 (2009) 74010E]. Furthermore, the detailed analysis of the caries pathology

using spatially resolved X-ray scattering has shown that while bacterial processes dissolve the minerals in enamel and dentin, the dentinal collagen network remains unaffected, enabling the development of therapies to re-mineralize the dentin [Nanomedicine: NBM 7 (2011) 694; Biointerphases 7 (2012) 4]. As other research teams we struggle to reproduce the unique biological material having hierarchically ordered structures down to the nanometer scale. This is demanding, as for example, human enamel consists of ordered calcium phosphate crystallites organized in a fibrous continuum. Note, human enamel is about three times tougher than the geological counterpart and much less brittle than sintered material. In the oral cavity, enamel remains functional for many decades. The detailed understanding of the mineralization processes of enamel in the oral cavity is not only essential to further improve human health but will also generate the necessary basis for the bio-inspired fabrication of hierarchically organized materials with unique, locally varying, anisotropic properties.

OCULAR EMULSIONS: A CASE STUDY OF A COMPLEX DRUG PRODUCT DELIVERED TO A COMPLEX ORGAN TO TREAT A COMPLEX DISEASE

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Dry eye is a progressive and debilitating disease, if left untreated or undertreated, progressively damages the ocular surface and may lead to decreased visual acuity. It is also a complex disease to treat as the underlying pathophysiology is not fully understood as well as the disease is manifested in multiple target tissues in a complex organ (Eye). With the eye being a highly protected organ, treatments often involves local administration as systemic absorption is negligible. While this may present a perceived simplistic approach at some level; however, the absorption pathway is quite complex. The kinetics of absorption and distribution is such that differential rates are seen for different tissues, which poses a significant hurdle in order to deliver the drug for adequate effect. Further complicating absorption is that any eye drop application results in a very short residence time in the eye to enable bioavailability (< 30 seconds). In addition, the severity of a Dry-Eye patient's disease state can significantly affect drug release, partitioning and absorption, as ocular surface components such as tears are altered significantly. Emulsions are well known as thermodynamically unstable system consisting of at least two immiscible liquid phases; one phase that is dispersed as globules, in the other liquid phase, stabilized by the presence of an emulsifying agent. The product being discussed in this presentation, RESTASIS®, is a multi-phase system – contains oil phase, aqueous phase, interface consisting of surfactants and other stabilizing polymers as well as micellar structures. Drug is distributed in all these phases, which further complicates the delivery to the target tissues, as well as physico-chemical characterization of this product with traditional analytical tools. Being a complex dispersed product, manufacturing process plays a critical role on drug distribution to the various phases, as well as the drug delivery to various ocular tissues. Together, the complexity of the drug product along with the need to deliver to a complex organ with a goal to achieve therapeutic levels in multiple target tissues in the eye for treating a highly complex disease, renders this particular case study a perfect example of how traditional generic bioequivalence principles with *in vitro* characterization alone cannot be applied directly. Instead, consideration must be given to how a "totality of evidence" can be established to ensure that any generic copies provide the same benefit as the reference product in terms of safety and efficacy to patients. The data will also show that the "totality of evidence" in this case may need to take into account, a much more complex set of physico-chemical characterization, some of which may not have been established, and further research.. In addition, any assessment should keep in mind that it must be a predictor of product performance *in vivo*, not just *in vitro*. Hence a reasonable correlation between *in vitro* and *in vivo* behavior of each product should be mandatory.

DENDRIMERS IN NANOMEDICINE: IT IS TIME TO MEET THE EXPECTATIONS

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Nanomedicine is expected to solve some problems of modern medicine, and offers new hopes in several critical areas such as cancer, viral and bacterial infections, early diagnosis, artificial organs, tissue regeneration, and theranostics.[1] The icing on the cake of nanomedicine may be nanobots that travel through the human body to heal diseases without side effects; however they are not real yet.[2] Current efforts on interdisciplinary research at the interface of chemistry, physics, biology, engineering and medicine might achieve the creation and application of nanobots in the future. Many scientists are shifting their focus toward the search for new ways to deliver drugs using nanomaterials, rather than developing new drug candidates.[3] The driving force behind this paradigm shift is that drugs with promising performance *in vitro* frequently fail in clinical trials, because the candidate drug is not able to reach efficiently and selectively its intended location. Nanomaterials bring important benefits into the drug delivery field due to its nanoscale size and multivalency. Nanomaterial-based drug delivery systems generally cause higher drug accumulation in diseased tissues through enhanced permeation effect or through active targeting, reducing adverse effects of small molecule drugs. Other advantages of nanosystems include improvement on drug's solubility, stability, biodistribution and pharmokinetics, resulting in improved efficacy.[4] There has been significant progress in the application of a wide variety of nanomaterials in nanomedicine applications, however, extensive opportunities remain for the development of innovative nanotherapeutic systems. Multifunctional dendrimers have emerged as promising candidates for application in nanomedicine, especially in drug delivery, gene delivery, bactericides, MRI contrast agents, fluorescent imaging agents, radiotherapy, photodynamic therapy, neutron capture therapy.[5] Dendrimers are well-defined branched macromolecules that are built in a stepwise manner, which are accepted as being a special class of polymers with a monodisperse structure.5b, 6 In order to keep dendrimers monodisperse and obtain quasi-perfect structures, it is imperative to use efficient and selective reactions to avoid formation of species with branch defects, which are difficult to separate from the target dendrimer. Dendrimers' well-defined multivalent branched structure present several advantages when comparing with random-coiled polymers. Controlled multivalency on dendrimers can be used to attach drug molecules, imaging agents, stimuli-responsive moieties, targeting groups and/or solubilizing units at precise locations. Nanoscale size and branched structure of higher generation dendrimers yield materials with enhanced permeation effects and long *in vivo* circulation lifetime, while monodispersity enables reproducible pharmacokinetic behavior.[7] Therefore, the unique properties of dendrimers that prospect their use in nanomedicine applications are directly related to their multifunctional well-defined structure. Thousands of research articles forecasted exciting applications of dendrimers in nanomedicine; however, the number of dendrimer-based formulations that advance into clinical trials has been somewhat deceiving. This is partially due to the non-reproducible pharmokinetic behavior observed for multifunctional dendrimers synthesized through the random-statistical approach that leads to mixtures of products. To advance dendrimer-based materials into the clinic it is necessary to develop well-defined multifunctional dendrimers with controlled number and location of drugs, targeting groups, imaging agents, solubilizing agents and/or other relevant motifs. The demand for dendrimers' structural complexity brings new synthetic challenges that need to be overcome.5c, 8 While there are a wide variety of reports on the synthesis of well-defined bifunctional dendrimers[9] synthetic strategies to build well-defined dendrimers bearing three or more different moieties are still limited. *In vitro* and *in vivo* assays on well-defined multifunctional dendrimers are even more scarce.

In this talk I will present the research projects currently ongoing in my group. We have been developing innovative nanomaterials for drug delivery applications, including well-defined multifunctional dendrimers, biodegradable polymeric nanoparticles and supramolecular nanohydrogels. In particular, novel well-defined multifunctional

dendrimers are being synthesized taking advantages of orthogonality and click chemistry. Design of the new dendrimers has taken into consideration the trends that have been identified for the assembly of the "ideal" drug delivery carrier: (i) hydrodynamic diameter larger than 15 nm to increase blood circulation lifetime and enhance diffusion selectively into tumor tissues, (ii) branched structure to increase blood circulation lifetime, (iii) globular structure for higher tumor accumulation, (iv) engineered surface groups to increase water solubility and to minimize interaction with the immune system, (v) multifunctionality for attachment of drugs or apoptosis-inducing agents, imaging moieties and targeting groups, (vi) imaging moieties that absorb and emit in the NIR region, (vii) well-defined structure for reproducible pharmacokinetic behavior, (viii) biodegradable backbone to enable safe excretion and avoid long-term toxicity. The long term goal is to build a library of dendritic cores and dendrons bearing relevant moieties and orthogonal functional groups ready to be assembled according to the desired application. Recent developments on synthetic tools are defying the outdated perception that dendrimers' synthesis are complex, slow, tedious and cost-prohibitive. Indeed, the very low amount of dendrimers to be used in therapeutic applications will most likely dilute their cost, making their cost/benefit relationship interesting for further product development. Increasing complexity on dendrimers by locating precisely a wide variety of functionalities such as drugs, targeting groups, imaging agents, biosensors, stimuli-responsive linkages, interlocked moieties with controlled motion, catalysts, and propeller systems will result in versatile multifunctional nanomaterials, which might approach the conceptual nanobots in the future. The next steps in the dendrimer field will be very challenging and exciting, because it is time to meet the high expectations that were placed on dendrimers.

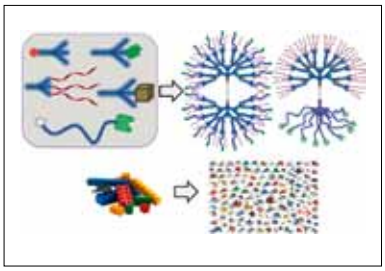

Fig. 1. Schematic representation of the long term goal: a library of dendritic cores and dendrons bearing relevant moieties and orthogonal functional groups ready to be assembled into well-defined multifunctional dendrimers, according to the desired application.

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PLASMA-INDUCED NANO-WRINKLES FOR ARTIFICIAL MUSCLES

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Dielectric elastomer actuators (DEA) are unique due to their large deformation capability of several ten percent. Many applications as actuators, sensors, and electric generators have been realized. They are considered as the most promising candidates for artificial muscles as they show strains and stresses comparable to human skeletal muscles. They show reaction times of some hundred milliseconds and are made of inexpensive materials. For planar DEAs, we have used polydimethylsiloxane (PDMS) elastomers to and transduce the electrical energy into mechanical work. The voltage required to deform the DEA increases with the elastomer thickness and is currently far too high for artificial muscles to be implanted in the human body. We aim to fabricate low-voltage DEA implants to treat incontinence.Low-voltage DEA are still limited by the fabrication of sub-micrometer elastomer membranes and the development of compliant and conductive electrodes. For this purpose, we have investigated the introduction of wrinkles to a flat PDMS surface by means of oxygen plasma. During the plasma treatment, a stiff silica-like layer some nanometer thick is formed. As the PDMS cooled down, wrinkles arose due to the mismatch of the elastic moduli and the thermal expansion coefficients of the thin film and the bulk PDMS. The periodicity of the wrinkles could be tuned between 0.4 and 2.0 µm.Furthermore, we have extracted the nanomechanical properties of the wrinkled PDMS structure using a spherical diamond-like carbon AFM tip. The stiffness increase during the plasma treatment was found to be moderate as shown by the stiffness maps of the wrinkled PDMS. Furthermore, we have deposited a gold electrode on the wrinkled PDMS and measured its mechanical impact on the overall DEA structure. An important and beneficial feature of this approach is a pre-stressing effect of the electrode. As the treatment of PDMS with oxygen plasma led to a spontaneous formation of wrinkles, a subsequent sputtering of the electrode leveled these non-oriented wrinkles. As expected, the electrodes showed fewer cracks and make this approach promising for the fabrication of artificial muscles.

Keywords: dielectric elastomer actuators, nano-wrinkles, oxygen plasma, PDMS, nanoindentation, atomic force microscopy, compliant electrode, MPTMS, adhesion layer

A CLINICAL PHARMACOLOGISTS VIEW OF NANO-MEDICINE REGULATION

ANDREW OWEN

Successful regulatory translation of any medicine requires a robust understanding of the clinical pharmacology, which is underpinned by knowledge of the pharmacokinetics, pharmacodynamics and the exposure-response relationship. There are a number of key differences between many nano-enabled therapy options and conventional small molecules, which present additional specific challenges when nanotechnologies are applied either to the reformulation of existing active pharmaceutical ingredients (APIs; including small molecules or biologics) or as enabling technologies for new chemical entities. Most of these challenges arise in a drug- and nanotechnology-specific manner and

appropriate regulation is therefore required on a case-by-case basis. This is particularly important in the context that nano-specific safety considerations (e.g. interference with the immune system) are apparent that should also be consider on the back-drop of immunological differences that occur in specific patient groups. Other important difference occur in specific sub-populations (e.g. difference in disposition between paediatric and adult populations) that need to be considered for certain applications. Nanomaterials often exhibit differences in biodistribution and routes of clearance from the body to conventional medicines, and a robust understanding of these processes is required to de-risk translation by improving prediction of the *in vivo* exposure-response relationship, needed to inform safe and efficacious preclinical and clinical dose selection. Classically, small molecules have been benchmarked according to their plasma pharmacokinetics, but altered biodistribution is a hallmark of certain nanocarrier systems, and this may mean that an understanding of tissue or cellular pharmacokinetics may be more informative for nanomedicines. Since many nanomedicines are presented as complex systems (potentially consisting of API, carrier, and targeting moiety), an understanding of the pharmacokinetics and pharmacodynamics of individual components as well as the consolidated medicine is required. Many recent nanomedicine strategies involve a change in route of administration or duration of exposure for an already clinically used drug, and this also requires consideration. Physiologically-based pharmacokinetic (PBPK) modelling is now extensively employed for conventional small molecule drugs, either in early dose selection or to probe the implications of specific clinical scenarios (e.g. a genetic susceptibility or drug-drug interaction), post licensing. A robust PBPK approach requires thorough understanding of the underpinning mechanisms for drug disposition and further nanomedicine-specific knowledge is required to strengthen its applications for nanomedicines. This presentation will provide a perspective on differences between nanomedicines and conventional therapeutics as they relate to pharmacokinetic and pharmacodynamic relationships.

THE APPLICATION OF NANOMEDICINE IN PAEDIATRIC INFECTIOUS DISEASES: FOCUS ON HIV

ANDREW OWEN

Nanotechnology-enabled drug delivery continues to deliver benefits across indications, with a focus on cancer still apparent within major global research efforts. However, benefits are now emerging in diseases beyond cancer and clear examples for treatment of infectious diseases are now emerging in terms of pre-clinical proof of concept as well as several nanomedicines in clinical development. Nanomedicines being explored for HIV are aimed at applications in simplifying and improving therapy options or for pre-exposure prophylaxis to prevent acquisition of HIV in high risk sub-populations[(1)]. Specifically, developments are aimed at either enabling lower dose alternative medicines for oral delivery [(2)], long-acting parenteral delivery[(3)], or novel materials for specific targeting of viral sanctuary sites that conventional medicines do not effectively penetrate[(1)]. Most applications are being directed towards improved adult therapy options but it is important to recognise and progress the opportunities for meeting the urgent need for additional paediatric therapy options. This is particularly important in the context that 2014 World Health Organisation statistics indicated that 2.6 million children <15 years are HIV positive, with only 32% of infected children currently on therapy. For some drugs, current paediatric formulations involve the ingestion of oral solutions containing high percentages (>40%) of organic solvents by very young children. Recent work at the University of Liverpool has developed a lopinavir nanosuspension aimed at delivering an entirely aqueous paediatric formulation of the drug, with the potential additional benefit for bioequivalence from a lower dose. Moreover, two long-acting HIV nanoformulations (rilpivine LA and cabotegravir LA originated by Janssen and ViiV Healthcare, respectively) have recently entered late stage development, and may provide therapy options with potential for monthly (or longer) administration formats while maintaining therapeutic plasma concentrations. This delivery strategy has a clear potential to simplify therapy, and may have a particular benefit in individuals that suffer pill fatigue or are non-adherent to therapy for other reasons. Adherence to medication is a major driver of therapy failure and a recent meta-analysis demonstrated the high incidence of non-adherence during adolescence. This presentation will outline recent developments in HIV nanomedicine, with a specific emphasis on benefits with a particular potential for improving paediatric drug delivery.

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VIEW AND UPDATE FROM EMA

MARISA PAPALUCA

"Nanotechnology is a most enabling technology for the development of innovative health products. I will present a review of key areas of interest and an outlook of regulatory science initiatives to support the contribution of nanotechnology to the success of personalised medicine".

WHAT HAPPENS TO NANOPARTICLES AFTER THEY HAVE BEEN INTERNALIZED BY CELLS?

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What happens to inorganic nanoparticles (NPs), such as plasmonic gold or silver, superparamagnetic iron oxide, or fluorescent quantum dot NPs, after they have been administrated to an animal or a human being? The review discusses the integrity, biodistribution, and fate of NPs after *in vivo* administration. First the hybrid nature of the NPs is described, by conceptually dividing them into the inorganic NP core, an engineered surface coating around the core which comprises the ligand shell and optionally also bioconjugation, and into the corona of adsorbed biological molecules. It is shown that *in vivo* all of these three compounds may degrade individually and that each of them can drastically modify the life-cycle and biodistribution of the whole hetero-structure. The NPs thus may be disintegrated into different parts, of which biodistribution and fate would need to be analyzed individually. Multiple labelling and quantification strategies for such purpose will be discussed. All reviewed data indicate that *in vivo* NPs no longer should be considered as homogeneous entity, but should be seen as inorganic/organic/biological nano-hybrids with complex and intricately linked degradation pathways.

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DISSECTING THE MECHANISMS OF LIQUID TO SOLID PHASE TRANSITION ASSOCIATED WITH NEURODEGENERATIVE DISEASES.

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FUS/TLS is a prion-like protein that contains intrinsically disordered domains and is associated with neurodegenerative disease. We recently showed that intracellular FUS/TLS compartments form under various cellular conditions and that these compartments exhibit liquid-like properties *in vivo* and *in vitro*. "Aging" experiments revealed that FUS/TLS liquid droplets undergo a phase transition to a solid-like state which is accelerated by disease mutations (Patel et al., 2015). We discovered that concentrating proteins by phase separation comes with the trade-off that can also promote protein aggregation. Solid-like aggregates of prion-like proteins are a hallmark of many aging-associated diseases. Aberrant phase transitions might be one trigger causing aging-associated diseases. However, the molecular mechanisms underlying this aberrant phase transition and the strategies cells have developed to sustain the function of these aggregation-prone proteins remain largely enigmatic. Here, we present recent advances we made in understanding the mechanisms cells might have developed to prevent the liquid-solid phase transitions, by using a wide range of biochemical, biophysical and cell biology techniques. We find that electrolytes, small compounds and protein interactors affect the liquid-liquid, as well as liquid-solid transitions. Insights gained from studying liquid-solid phase transition might help us developing drugs targeted to treat age-associated diseases.

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REGULATORY CONSIDERATIONS FOR NANOMEDICINES

ANIL K. PATRI, Ph.D., Chair, Nanotechnology Task Force & Director, Nanotechnology Core Facility, US Food and Drug Administration

The U.S. Food and Drug Administration (FDA) has reviewed and approved medical products containing nanoscale material for clinical use over the last 40 years. Product compositions that are approved include polymers, liposomes, micelles, emulsions, crystals, metals, and metal oxides with many other novel compositions in clinical trials. These products have evolved over the years with advances in innovation and research, leading to new complex products for personalized and precision medicines. More importantly, the evolution of new technologies and instrumentation enabled better characterization and understanding of the nanomaterial. In order to keep up with the emerging field and technologies, FDA formed the Nanotechnology Task Force (NTF) in 2006 to identify and address the knowledge gaps in our understanding of nanomaterial, coordinate collaborative regulatory research, staff training, interagency and international interactions to network with experts in the field. Over the last decade, FDA has produced guidance documents, participated in standards development activities, held public meetings, workshops, and engaged stakeholders to facilitate responsible development of nanotechnology.
Reproducible science is vital for regulatory review of any product. Nanomedicines brought new research opportunities and challenges due to the inherent complexity requiring development of appropriate methods, predictable assays, and models, for monitoring product quality, safety and efficacy. An in-depth understanding of the nuances in product manufacturing, scale up, and physicochemical characteristics that influence biological outcome is essential. Such knowledge provides for risk-benefit analysis as products are developed from proof-of-principle stage to clinical trials and beyond. This presentation will cover some of the important parameters to monitor for nanomedicines during product development, the challenges, and the need for standards to advance the field.

DISCLAIMER:

The views expressed are of the presenter and should not be considered as the official position or policy of the U.S.FDA.

IMMUNOMODULATION OF LEUKOCYTES VIA RNA BASED-NANOMEDICINES

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Hematological malignancies are group of diseases characterized by clonal proliferation of blood-forming cells. Malignant blood cells are classified to myeloid or lymphoid cells depending on their stem cell of origin. Lymphoid malignancies (leukemia, lymphoma and multiple myeloma) are characterized by lymphocytes accumulation in the blood stream, in the bone marrow or in lymphatic nodes and organs. Several of these diseases are associated with chromosomal translocation, which cause gene fusion and amplification of expression, while others are characterized with aberrant expression of oncogenic genes. Overall, these genes play a major role in development and maintenance of the malignant clone. The discoveries of RNA antisense oligonucleotides and RNA interference (RNAi) mechanisms offer new tools to manipulate gene expression of specific genes. Systemic delivery of inhibitory RNA molecules for manipulation of gene expression in lymphocytes holds a great potential for facilitating the development of an RNA-based therapy platform for lymphoid blood cancer. However, lymphocytes are among the most difficult targets for RNAi delivery, as they are resistant to conventional transfection reagents, and disperse in the body, making it difficult to successfully localize or deliver RNA payloads via systemic administration. In addition, systemic administration of RNAi molecules is usually accompanied with severe toxicity and the injected RNA molecules have a relatively short half-life in the blood stream as well as limited intracellular transition. Development of non-toxic, systemically applicable strategies for delivering RNA payloads *in vivo* to lymphocytes is a crucial step in applying RNA-based therapeutics in patients with lymphocytes-related malignancies.
Recent studies have addressed this specific problem by developing nano-sized particles that encapsulated inhibitory RNA molecules against specific targets known to be involved in the development of the tumorigenesis multistep process.
These nanoparticles are selectively directed to their target cells via surface-attached antibodies or ligands, which recognized abundant receptors on lymphocytes surface.
Herein, we will describe some of the challenges and opportunities in modulating cell response in malignant lymphocytes using inhibitory RNA –encapsulated nanoparticles and discuss adverse effects such as immuno-toxicity and specificity. Special emphasis will be made on novel lipid-based nanoparticles (LNPs) delivery platform that struggle with these challenges and design to selectively target and silence gene expression in malignant B-lymphocytes. We designed LNPs coated with anti-CD38 monoclonal antibodies that are specifically taken up by human mantle cell lymphoma (MCL) cells in the bone marrow of xenografted mice[(1)]. MCL is an aggressive lymphoid malignancy characterized by proliferation of mature B-lymphocytes in the mantle zone of lymph nodes. The lymphoma has often spread to the bone marrow, spleen and blood. In almost all cases of MCL, the lymphoma cells are associated with a characteristic chromosomal translocation, t(11;14)(q13;q32). This translocation resulting in an overexpression of cyclin D1 mRNA in >90% of MCL patients[(2,3)]. We demonstrated that CD38 targeted LNPs loaded with siRNAs against cyclin D1, induced gene silencing in MCL cells and prolonged survival of tumor-bearing mice. These results present a

novel RNA delivery system that opens new therapeutic opportunities for MCL as well as for other diseases characterized with high expression levels of CD38.

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EPITHELIAL TISSUES ENGINEERED IN VITRO THAT BEAR THE HALLMARKS OF LIVING TISSUE CAN RECAPITULATE CANCER INVASION

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The onset of metastasis occurs when cancer cells invade and breach the basement membrane (BM) that provides mechanical support to epithelial tissues. Yet, it remains unclear what triggers cancer cells to breach the BM, and how 'triggered' cells breach the BM. We have established an in vitro assay using native BM for culturing epithelial and stromal cells. Furthermore, we asked if carcinoma-associated fibroblasts (CAFs) isolated from cancer patients promote cancer cell invasion through a BM.

Using atomic orce microscopy (AFM) in combination with high-resolution- and life-microscopy, we have correlated the mechano-cellular attributes of the BM/epithelium interface to its biochemical and structural properties and followed cancer cell invasion through BM and BM remodeling. We demonstrated that the internal limiting membrane (ILM) isolated from human retinas acts as a native substrate for culturing epithelial cells in terms of BM composition, architecture and stiffness. Our findings contrast native BM and reconstituted BM (such as Matrigel), we find that Matrigel is mechanically 100-fold more compliant (i.e., softer) than native BMs and much less structured. We also show that activation of ß-1 and -4 integrins by the stiffness and architecture of the native alpha – 5 laminin chain plays a key role, not only as previously thought for maintenance of cell polarity but also for the establishment of a physiological mechanophenotype of the epithelium. Furthermore, we show that in the presence of CAFs, moderately invasive cancer cells invade in a matrix metalloproteinase-independent manner into mouse mesentery. CAFs actively pull, stretch and soften the BM, forming gaps making the BM permissive for cancer cell invasion. Hence, we propose that in addition to proteolysis, mechanical interactions between CAFs and BM represent an important additional mechanism of BM breaching. Given the mechano-biological relevance, native BMs allow us to further understand how mechanosignaling occurs between the epithelia and the surrounding stromal layers at the BM interface both in physiological and pathological states.

A COMPARATIVE STUDY ON IRON-GLUCONATE FORMULATIONS

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Patients with Chronic Kidney Disease experience severe anemia that must be treated with IV-iron preparations. There are currently 6 brand and 1 generic FDA approved iron products on the market in the US; all of which are colloidal nanoparticles composed of a polynuclear iron(III)-(oxy)hydroxide core stabilized by a carbohydrate ligand. Delivery of IV-iron preparations affects iron homeostasis in the cell. Under normal conditions, the iron is chelated by transferrin in the blood plasma. Transferrin then traffics the iron into the cell, via the transferrin receptor pathway, thereby providing iron as a protein co-factor. Under iron overload conditions, the transferrin receptor becomes saturated, and the remaining iron, which is collectively called 'non-transferrin bound iron' or NTBI, is taken up by the cell via non-iron specific pathways (e.g. DMT, ZIP). Once NTBI has entered the cell, it promotes production of reactive oxygen species (ROS) via the Haber-Weiss reaction damaging proteins, DNA and lipids. Thus it is critical that the IV iron products used to treat anemia deliver the optimal level of iron.

We are developing high-through-put bioanalytical assays to measure total iron (TI), transferrin bound iron (TBI), and non-tranferrin bound iron (NTBI) in plasma samples. This includes a high-throughput method to measure total iron (TI) concentrations in blood plasma that utilizes inductively coupled plasma mass spectrometry (ICP-MS), a liquid chromatography (LC) coupled ICP-MS protocol to measure the concentration of iron that is transferrin bound (TBI), and a chelation-ICP-MS strategy to measure Non-tranferrin bound iron (NTBI).

These assays will be applied to a clinical trial in which healthy subjects will be administered generic and reference sodium ferric gluconate. The primary outcome will be the assessment of non-inferiority of the generic colloid product against the reference colloid product with respect to non-transferrin bound iron (NTBI) exposure, after single-dose i.v. administration of brand and generic sodium ferric gluconate injections in n=44 healthy subjects. Secondary outcomes will contribute towards providing scientific evidence for consideration of any possible additional safety measures for IV iron.

PENETRATION ENHANCERS CAN ENHANCE EFFECTIVENESS OF INTRA-TUMORAL ANTI-CANCER DRUG DELIVERY SYSTEMS

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Anti-cancer drug delivery systems (DDSs) are designed to target chemotherapeutic drugs to their site of action in solid tumors, enhance their effectiveness and reduce the incidence of adverse events. Unfortunately, the currently available anti-cancer DDSs are characterized by very low tumor targeting efficiency and thereby possess limited pharmacological effect and poor safety profile. Even following intra-tumoral (I.T.) administration of DDS, only a thin layer of the tumor cells in immediate vicinity to the DDS are exposed to the therapeutic concentrations of anti-cancer drug.

We hypothesized that incorporation of penetration enhancers into the I.T. anti-cancer drug delivery systems can enhance their effectiveness and safety by affecting the intra-tumoral disposition of the released anti-cancer agent. Therefore, we investigated effects of several penetration enhancers on the anti-cancer effects induced by paclitaxel-loaded DDSs following their I.T. administration to the BALB/c mice bearing 4T1-Luc orthotropic breast cancer.

The experimental results demonstrate improved response to paclitaxel, decrease in tumor volume and potential reduction of lung metastases in mice treated with injectable implants containing 5%

paclitaxel and dexamethasone penetration enhancer incorporated in a polymeric carrier. Currently, we are investigating DDSs with different compositions (content of paclitaxel and of the permeation enhancers) to identify the optimized formulations with the best balance of anti-cancer vs. adverse effects.

INTRODUCTION

Solid tumors are characterized by complex and dynamic morphology that limits the uptake of chemotherapeutic agent, and its penetration to the deep parts of the tumor. New drug delivery systems (DDSs) are constantly being developed in order to improve the intratumoral permeability of the anti-cancer drugs and to enhance their therapeutic effectiveness. However, the targeting effectiveness of the currently available DDSs is still limited, and only a small fraction of the administered drug successfully accumulates in the tumor.[1]

Paclitaxel is a widely used chemotherapeutic agent. The clinically-used injectable formulation of paclitaxel leads to high incidence of adverse events and hypersensitivity reactions, raising the need for a new and improved DDSs to deliver paclitaxel safely and efficiently to the solid tumors.[2]

Intra-tumoral (I.T.) administration can be used to deliver efficiently the anti-cancer drug directly to the intended site of action in the solid tumor, and to limit its toxicity in the other tissues.[3,4] Over the last years, I.T. administration of various chemotherapeutic agents encapsulated into a semi-solid polymer [Poly(sebacic acid-co-ricinoleic acid ester anhydride)] was investigated in animal models of cancer disease. Administration of these formulations increased the survival rate, reduced the tumor rate growth, and prevented dissemination of metastases to the lungs and lymph nodes of the treated mice.[4-6] However, despite these promising findings, I.T. administration of various chemotherapeutic agents encapsulated into a semi-solid polymer rarely induced complete remission of the cancer disease. In part, this finding was due to the limited intratumoral distribution of the applied anti-cancer agent (e.g., the therapeutic permeability of paclitaxel in these studies was limited to ~3.5 mm from the implant surface).[6] We hypothesized that incorporation of penetration enhancers into the paclitaxel-loaded poly(sebacic acid-co-ricinoleic acid ester anhydride) I.T. implants will increase the permeability of paclitaxel to the to the cancer cells and will enhance the effectiveness of the anti-cancer effects. We investigated I.T. implants encapsulating paclitaxel with different types of promoter drugs, including those that affect the tumor microenvironment by modulation of inflammation (dexamethasone), reduction of stromal collagen (losartan), reduction of interstitial fluid pressure (IFP) and microvascular pressure (collagenase), lowering arterial blood pressure and tumor IFP (nicotinamide)[7] or topical penetration enhancers (azone, oleic acid).8 Following the initial screening experiments, we focused on the most promising penetration enhancers: dexamethasone, azone and oleic acid.

OBJECTIVE

To investigate the effect of penetration enhancers on the efficiency of paclitaxel-loaded intra-tumorally injected drug delivery systems, and to identify the content of the permeation enhancers that leads to the most effective anti-cancer effects.

Table 1: Control and treatment groups

I.T. Saline (negative control)
I.T. Polymer (negative control)
I.T. Polymer + I.P. Paclitaxel 10 mg/kg
I.T. Polymer – Paclitaxel 5%
I.T. Polymer – Paclitaxel 5% - Dexamethasone 10%
I.T. Polymer – Paclitaxel 5% - Azone 10%
I.T. Polymer – Paclitaxel 5% – Oleic acid 10%

EXPERIMENTAL METHODS

We used an orthotropic breast cancer model, based on injection of 4T1-Luc cells to experimental mice. The tumor cells were injected into the mammary fat pad to 8 weeks old female BALB/c mice (2.10^5 cells, SC). When the tumors reached ~50 mm^3 volume (day 16 after the tumor inoculation), mice were randomly assigned to treatment and control groups (n=5-7, see Table 1). For the I.T. treatments, 50 µl of the studied formulations were injected into the individual mice' tumor at room temperature.

The tumor size, animal body weight and behavior were closely monitored over a 10-day period. After that, the mice were anesthetized, injected with 150 mg/kg luciferin and imaged using IVIS® system. Subsequently, the mice were sacrificed, tumors and other organs were collected, weighed, checked for presence of metastases (by visual inspection and re-imaging using IVIS® system), and processed for further histological analysis (evaluation of the necrosis and inflammation in the tumors using different staining techniques, including hematoxylin & eosin staining, TUNEL assay, blood vessels staining, etc.).

RESULTS AND DISCUSSION

Animal survival: All the animals in the treatment groups survived during the follow-up period, but substantial part of animals in the control groups (I.T. polymer, I.T. polymer + I.P. paclitaxel) suffered from progressing cancer disease (tumor size > 10 mm, or weight loss > 10%) and had to be sacrificed.

Animal weight: Reduced body weight was observed in the group receiving I.P paclitaxel, which indicates loss of appetite and distress (see Figure 1). The dexamethasone group maintained steady body weight and the other groups demonstrated increase of up to 15% in body weight.

Tumor volume: The tumor volume increased during the follow-up period in all the groups, except of the mice that received I.T. injection of dexamethasone-containing formulation. The tumor volume was significantly lower in the dexamethasone group, as compared to the saline, polymer-paclitaxel, azone and oleic acid groups (see Figure 2). The tumor volume in the oleic acid group was significantly higher than in the polymer and dexamethasone groups.

Lung mass: The lung mass was used for assessment of tumor metastatic behavior in the individual groups. Treatment with the dexamethasone-containing formulation reduced the average lung mass, as compared to the control groups (data not shown). On the other hand, animals treated with oleic acid-containing formulation had significantly higher average lung mass than in the control groups, suggesting higher rate of lung metastasis.

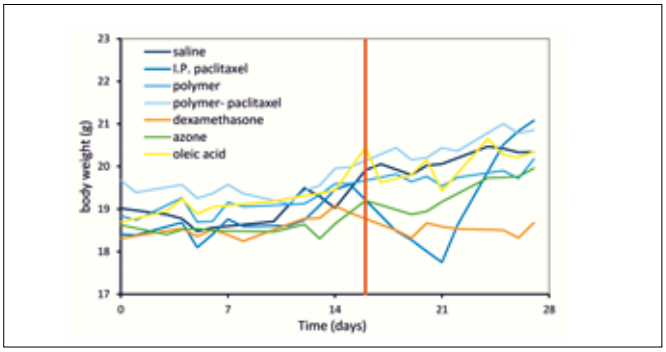

Figure 1. The effect of the studied treatments on the body weight of mice with 4T1-Luc tumors.

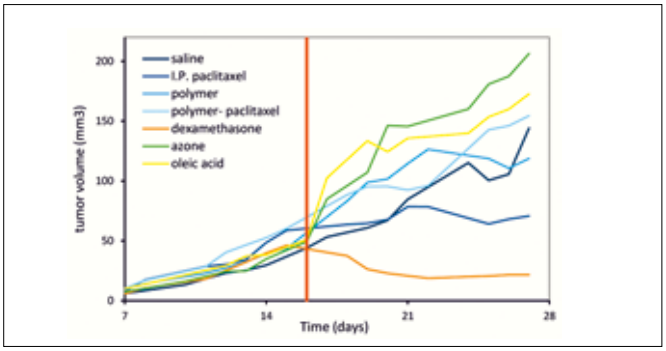

Figure 2. The effect of the studied treatments on the volume of 4T1-Luc tumors.

CONCLUSION

Addition of dexamethasone to the I.T. paclitaxel-containing drug delivery system enhanced the effectiveness of its anti-cancer effects and apparently inhibited spread of metastases. Additional experiments are required to reveal the optimal content of dexamethasone in the implantable formulations, its effects on the tumor microenvironment and on the intratumoral distribution of paclitaxel released from the implant.

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DIFFERENTIATING BETWEEN CANCER AND INFLAMMATION: GOLD NANOPARTICLES OFFER CT IMAGING OF METABOLIC ACTIVITY

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One of the main limitations of the highly used cancer imaging technique, PET-CT, is its inability to distinguish between cancerous lesions and post treatment inflammatory conditions. The reason for this lack of specificity is that [^{18}F]FDG-PET is based on increased glucose metabolic activity, which characterizes both cancerous tissues and inflammatory cells. To overcome this limitation, we developed a novel nanoparticle-based approach, utilizing Glucose-Functionalized Gold Nanoparticles (GF-GNPs) as a metabolically targeted CT contrast agent. Our approach demonstrates specific tumor targeting and has successfully distinguished between cancer and inflammatory processes in a combined tumor-inflammation mouse model, due to dissimilarities in angiogenesis occurring under different pathologic conditions. This study provides a new set of capabilities in cancer detection, staging and follow-up, paving the way for improved specificity.

INTRODUCTION: Cancer detection is based on both structural and functional imaging techniques. Structural techniques (e.g., US, MRI and CT) identify anatomic details and provide information on tumor location, size and spread, based on endogenous tissue contrast. However, they are not sufficiently sensitive for detecting critically small tumors or metastases since they lack structural manifestation. The development of the main clinically applicable functional imaging technique, positron emission tomography (PET) using the glucose analog ^{18}F-2-fluoro-2-deoxy-d-glucose ([^{18}F]FDG) has eventually revolutionized the field of medical oncology. [^{18}F]FDG-PET is based on the increased metabolic profile of malignant cells, and provides the ability to discern molecular and cellular alterations associated with pathological conditions, even before structural modifications occur. However, [^{18}F]FDG-PET lacks anatomical information, and thus necessitates the incorporation of an additional structural imaging modality such as CT or MRI in order to obtain an accurate anatomic localization of the foci of increased metabolic activity. The combination of PET with CT (PET-CT) enables both functional and anatomical information in a single setting. However, in view of the relatively high cost of PET scans, the dependence on the short-lived [^{18}F]FDG ($T_{1/2}$<2h) and its non-specificity for cancer which leads to high rate of false positives[1,2] (glucose uptake is not cancer-specific), the development of a single modality which will overcome these drawbacks is highly desirable. In the present work we demonstrate the development of a novel metabolic-based CT imaging technique using GF-GNPs, which provides simultaneous functional (metabolic) and structural imaging capabilities. In addition, as nanoparticles have unique biodistribution properties and tumor targeting profile, this technique allows distinction between cancers and inflammatory processes.

First, we have synthesized GF-GNPs and studied their interaction with cancer cells, investigating whether the glucose molecule (~1 nm) retains some of its activity and can be recognized as glucose by cells, when conjugated to a 'large' GNP (20 nm). To this end, four types of GF-GNPs were studied, wherein the GNP was attached selectively to one of four possible intra-molecular glucosamine sites. The hydroxyl groups (-OH) of glucose can be substituted by amine groups (-NH_2) at different and specific molecular sites, denoted 1, 2, 3 or 6 (C-1, C-2, C-3 and C-6, respectively). Identical, 20 nm GNPs were linked selectively to each of the 4 glucosamine sites, one at the time, resulting in four distinct GF-GNPs of the same shape and size, differing only in the intra-molecular glucose site being functionalized (Fig 1A).

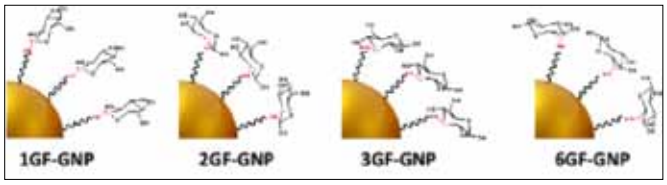

Figure 1: Selective uptake of 2GF- GNPs. A) Schematic diagram of the four distinct GF-GNPs, with the same shape and size, differing only in the intra-molecular glucose conjugation site (C_1, C_2, C_3, and C_6).

In order to examine the interaction between these GF-GNPs and cancer cells, we first performed an *in vitro* study. The four types of GF-GNPs were incubated with squamous cell carcinoma (SCC) human epidermoid A431 cancer cells (n=3 per group) for 30 minutes, and atomic absorption spectroscopy was used to quantitatively determine the amount of internalized Au. Interestingly, despite their identical shapes and sizes, a significantly higher uptake was observed for the GNPs that were conjugated to glucose through its 2' carbon position (denoted as 2GF-GNP). The uptake was about 3 times greater than that of the other three GNP types. The same trend was also observed *in vivo*. GF-GNPs were intravenously (IV) injected into mice bearing human A431 tumors (n=5 for each group), and gold concentration in the tumor was quantitatively measured by atomic absorption spectroscopy. In addition, CT scans of the mice were performed pre-injection and at 3.5 h post-injection of the GF-GNPs. Both atomic absorption spectroscopy and CT results clearly demonstrated that the uptake of 2GF-GNP was significantly higher than that of the other three GF-GNP conjugates (Fig. 2). Given that the four nanoparticle types are of the same material (gold), coated by the same molecule (glucosamine) and have the same physicochemical characteristics, while differing only in the intra-molecular glucose conjugation site, one would expect to obtain a similar tumor uptake values of the four GNP types. Unexpectedly though, both *in vitro* and *in vivo* experiments showed a remarkably selective accumulation of one of the four isomer-conjugates.

This difference in uptake provides unequivocal evidence that the 2GF-GNPs are recognized and preferred by cancer cells, probably because of the specificity of the glucose coating. Interestingly, the 2' carbon position is also the one to which the ^{18}F is connected in [^{18}F]FDG, supporting our result that chemical modification of the 2' carbon position does not prevent glucose recognition by cells. In addition, the differential uptake *in vivo* underscores the distinction between passive targeting of the 1GF-GNP, 3GF-GNP and 6GF-GNP, which is due to the enhanced permeability and retention (EPR) effect, and metabolically active targeting of the 2GF-GNP. Most importantly, the results markedly show that small tumors (approximately 4-5 mm in diameter), which are undetectable by CT without the use of GNP contrast agents, become clearly visible and detectable following administration of 2GF-GNP, which like [^{18}F]FDG can detect glucose metabolic activity while inducing distinct contrast in CT imaging.

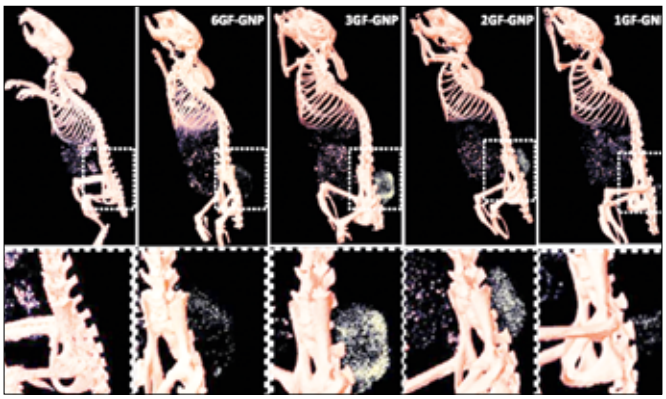

Figure 2: CT volume-rendered images of five mice; one without injection of nanoparticles (left), and four mice at 3.5 h post IV injection of the four types of GF-GNPs. Upper images: whole body volume-rendered images. The tumor area is marked with a white dashed rectangle. It is demonstrated that the tumor cannot be identified without injection of GNP (upper left image), while a significant accumulation of GNP can be observed following injection of 2GF-GNP. Some accumulation of GNP can be observed in the tumor area following injection of 1GF-GNP, 3GF-GNP and 6GF-GNP, which can be attributed to the passive targeting mechanism. For all mice, CT contrast is observed also in the digestive system due to food. In addition, in mice which were injected with GNPs, nanoparticles can be identified in the abdomen, as they accumulate in the kidneys, liver, and spleen according to their well-described clearance mechanism. Bottom images are enlarged images of the white marked tumor area.

We have further compared the abilities of 2GF-GNPs and of [^{18}F] FDG to differentiate A431 tumors from turpentine-induced inflammation in a combined tumor-inflammation mouse model. Inflammation was established in mice bearing A431 tumors (n=14) by a subcutaneous injection of turpentine oil, and four days post turpentine injection, 2GF-GNP or [^{18}F]FDG were IV injected. It has been previously demonstrated that maximum uptake of [^{18}F]FDG occurs at 4 days post injection in this inflammation model, and therefore, this time point was selected for PET imaging. Subsequently, CT scans were performed at 3.5 h after 2GF-GNP injection, and after sacrifice, gold concentration in the tumor and in the inflammatory lesion were quantitatively measured by atomic absorption spectroscopy.

Interestingly, both CT and atomic absorption spectroscopy results showed high-density accumulation of gold in the tumor, while practically no gold was detected in the inflammation region. For comparison, [^{18}F]FDG PET-CT scans were performed on four mice at 40 - 60 minutes after [^{18}F]FDG injection, showing no differentiation between cancer and inflammation, which exhibited equal accumulation of the radioactive tracer (Fig. 3).

In summary, we demonstrate a novel nanoparticle-based CT imaging methodology that overcomes the main drawbacks of the currently used [^{18}F]FDG-PET: (1) 2GF-GNP is cancer-specific and allows the distinction between cancer and inflammatory processes, (2) it offers cancer detection and imaging with no dependence on short-lived radio-tracers, and (3) provides simultaneous anatomical and functional information using CT. In addition, unlike specific immune-targeting approaches, this imaging modality does not target the expression of one molecule, but provides unique data about the functional state of the tumor tissue. We further showed that despite the conjugation to the GNP, the glucose molecule preserves some of its activity, allowing glucose recognition and cellular internalization by receptor mediated endocytosis. In addition, we showed that due to the unique characteristics of tumor vasculatures and dissimilarities between cancer and inflammatory processes, accumulation of GNPs occurs in the tumor and not in the inflammatory lesion, thus preventing false-positive results. Therefore, our new concept of functional CT imaging provides a new set of capabilities in cancer detection, staging and follow-up, and can be applicable to a wide range of cancers which exhibit high metabolic profile.

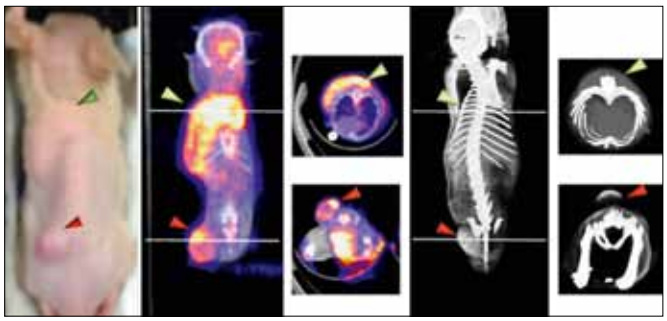

Figure 3: Differentiation between cancer and inflammation: Green arrowheads indicate location of inflammation; red arrowheads indicate location of A431 tumors; A) Representative image of a combined tumor & inflammation mouse model, before 2GF-GNP injection. B) [^{18}F]FDG-PET/ CT slice images of a representative mouse at 40 – 60 min post injection. [^{18}F]FDG accumulates equally in both tumor and inflammation, and does not distinct between them. C) CT surface-rendered images of the same mouse at 3.5 h post IV injection of 2GF-GNP. Gold accumulation is observed in the tumor, yet not in the inflammation, allowing a clear distinction between the two.

TUMOR STROMA AS A BARRIER AND TARGET IN CANCER: NOVEL MODELS AND TARGETING STRATEGIES

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BACKGROUND

Within the tumor microenvironment, malignant tumor cells and nonmalignant stromal cells actively interact with each other and thereby make the microenvironment supportive for the tumor growth. The tumor stroma is comprised of extracellular matrix and several cell types such as cancer-associated fibroblasts (CAFs), endothelial cells, tumor-associated macrophages (TAM) and other immune cells. On one hand, tumor stroma acts as a barrier to drug delivery, nanoparticle penetration and on the other hand, it becomes an important target to design new therapeutic strategies[(1)]. In this study, we developed *in vitro* 3D models to study nanoparticles penetration and also designed new strategies to target miRNA to tumor stroma. miRNA represent a important class of therapeutics for modulating cellular processes[(2)].

METHODS

In vitro 3D model: Tumor cells and fibroblasts were cultured in microwell-stamped petri dishes for generating 3D-spheroids array. The spheroids were characterized for their size and cellular reorganization using a confocal microscope and for the presence of CAF biomarkers using immunostaining and qRT-PCR. Then, we studied the

penetration of Cy5-conjugated PLGA nanoparticles in 3D-spheroid systems containing different ratios of fibroblasts and cancer cells (1:1 and 5:1) for up to 48 hours.
Stroma targeting: miRNAs (miR-199a) was identified in CAFs isolated from pancreatic tumors. We then designed a targeting system to deliver anti-miR-199a into CAFs based on cell penetrating peptide (CPP). Nanocomplexes were formed with anti-miR and CPP which were used to deliver anti-miR-199a to CAFs and the effect was determined on CAF activation using qPCR and immunocytochemistry.

RESULTS

Nanoparticle penetration study: Tumor stroma mimicking 3D-spheroids formed spontaneously within 48h. Confocal live imaging of spheroids showed that fibroblasts distributed throughout the spheroids and reorganized after 48 hours. Furthermore, spheroids showed a significant induction of collagen-1a1 and FSP-1, markers for the activated fibroblasts. Furthermore, 3D-spheroids were used for studying nanoparticles' penetration. Confocal live imaging showed that Cy5-conjugated PLGA NPs had faster and deeper penetration in tumor cell spheroids compared to spheroids containing both tumor cells and fibroblasts. The quantitative analyses of the fluorescence show that the nanoparticle's penetration decreased proportionally with increasing amount of stroma. These data demonstrate that tumor stroma acts as a strong barrier for the nanoparticle penetration.
Stroma targeting: To target tumor stroma for therapeutic application, we identified miRNA-199a in patient-derived stromal cells. The nanocomplexes formed with CPP led to the delivery of anti-miR into CAFs, as shown with fluorescent microscopy. In the effect studies, we found that nanocomplexes of anti-miR-199a strongly inhibited the activation of CAFs, as shown with qPCR and immunocytochemistry. *In vivo* studies are currently going on to investigate the stromal distribution and effects of these nanocomplexes.

CONCLUSION

In conclusion, tumor stroma poses a strong barrier for nanoparticle penetration which we can study our 3D heterospheroidal *in vitro* system. Furthermore, we have identified specific miRNA targets in tumor stroma which could be applied for inhibiting the tumorigenic effects of tumor stroma using novel stroma-targeting systems.

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CRISPR-CAS: FROM BACTERIAL IMMUNITY SYSTEM TO A VERSATILE AND UNIVERSAL TOOL FOR GENOME EDITING AND REGULATION"

ÜMIT PUL

CRISPR-based technologies are novel and easy-to-use genome engineering/editing tools that have been successfully applied in many eukaryotic organisms. The different methods are based on the modulation of the sequence-specificity of DNA endonucleases (e.g. Cas9) by a non-coding RNA of ~100 nucleotides (nt) in length, known as single-guide RNA (sgRNA). Loaded with the sgRNA, CRISPR nucleases screen the genome for the presence of a DNA sequence that is complementary to the first 20 nt of the sgRNA. Thus, CRISPR nucleases, e.g. Cas9, can be easily directed to nearly any desired DNA sequence within a genome simply by modulation of the 20 nt guide sequence of the sgRNA. Once the target region is identified through Watson-Crick base pairing of the sgRNA and the complementary DNA strand, Cas9 introduces double-strand DNA break (DSB) precisely within the target site. The DSB is repaired by the cell´s-own endogenous non-homologous end-joining
(NHEJ) pathway, which is error-prone and leaves short indel (insertion - deletion) mutations. In that way, it is possible to introduce inheritable mutations within genes- of-interest. In addition to knock-out studies, CRISPR technology can also be used for regulation of gene expression.
B.R.A.I.N AG has established a versatile CRISPR-based genome engineering toolbox allowing gene knock-out, chromosomal deletion or activation of gene expression in different human cells in order to develop novel screening model cell lines. The toolbox consists of a variety of different CRISPR-based technologies that allow to introduce mutations in any gene of interest (loss-of-function) but also to co- activate the expression of silent genes (gain-of-function). In this talk the biological function of CRISPR systems in bacteria will be introduced, followed by the methodical descriptions of the applications of CRISPR-nucleases and their variants in eukaryotic cells.

THE PHARMA INDUSTRY APPROACH IN THERANOSTICS

RAUSCH MARTIN

The need for predictive pharmaco-dynamic readouts and diagnostic tools is starting well before compounds are entering clinical testing. At present the way of how compounds are selected and profiled in pre-clinical research is changing significantly because of recent refinements of 3D cell culture models, co-cultures or organ explants. These enable scientists to mimic the human disease conditions much better than traditional approaches would do. This presentation will exemplify different approaches of functional tissue analysis at microscopic and macroscopic resolution.

HEPATOCELLULAR NUCLEAR FACTOR 4α (HNF-4α) ACTIVATION BY SARNA RESCUES DYSLIPIDEMIA AND PROMOTES FAVORABLE METABOLIC PROFILE IN A HIGH FAT DIET (HFD) FED RAT MODEL.

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Non-alcoholic fatty liver disease (NAFLD) culminates in insulin resistance and metabolic syndrome. As yet there is no approved single agent that targets steatosis or its pathological progression to hepatitis. Hepatocyte-nuclear-factor 4-alpha (HNF-4α) is at the centre of a complex transcriptional network where its disruption is directly linked to diabetes and steatosis. Resetting HNF-4α expression in NAFLD is therefore crucial for re-establishing normal liver function. Here, small activating RNA (saRNA) specific for upregulating HNF-4α was injected in rats exposed to a high fat diet for 16 weeks. Intravenous delivery was carried out using 5-(G5)-triethanolamine-core PAMAM dendrimers.We observed a significant reduction in liver triglyceride; increased HDL/LDL ratio and decreased white adipose tissue/body weight ratio. This suggested that HNF4A-saRNA- treatment induced a favorable metabolic profile. Proteomic analysis showed significant regulation of genes involved in sphingolipid metabolism, fatty acid β-oxidation, ketogenesis, detoxification of reactive oxygen species and lipid transport. We demonstrate that

HNF-4α activation by oligonucleotide therapy may represent a new paradigm as a single agent for the treatment of NAFLD and insulin resistance.

„UNSOLVED FIELDS IN EYE DISEASE"

HERBERT A REITSAMER

Ophthalmologists are concerned with one of the smallest organs in the body – the eye. Nevertheless, of all sensory systems, the eye transmits the largest amount of information to the human brain and vision became the most important of all senses in our species. The most common diseases in Ophthalmology are age dependent or associated the metabolic syndrome and diabetes. The prevalence of Glaucoma and age related macular degeneration is strongly increasing with age. The demographic pyramid has change into a demographic mushroom and the strongest growing part of the population are the elderly people beyond 60 years of age – one of the reasons, why Ophthalmology is the subject in medicine with the highest projected demand of doctors within the next 15 years.

Treatment of ocular diseases requires special attention in many respects as the eye is part of the central nervous system and an extension of the brain but also a precise optical apparatus with delicate optical organs inside. The nervous part - the retina with more than 50 different types of neurons is built similar to the Neocortex and its circuitry is matter of scientific research for more than 150 years. The retina is nourished by two independent vascular beds, one being innervated by the autonomous nervous system – similar to peripheral vascular beds of the body – and the other one follows the rules of blood flow regulation within the brain. These completely different vascular beds meet within the 200 µm thick layer of retinal tissue. Very little is known about the interaction between these two largely independent systems, but changes in their functionality are among the most important reasons for neurodegenerative diseases of the retina.

A major challenge is the fact, that neuronal tissue of the retina is irreplaceable. It cannot be transplanted like the cornea, which is the reason, why whole eyes cannot be transplanted either. Its regenerative capacity is extremely low and damage to the optic nerve, which connects the retina to the rest of the brain is inherently detrimental to vision. Consequently, retinal diseases and diseases of the optic nerve are subjects of intensive research in vision sciences and first gene therapies or cell replacement therapies are investigated in clinical trials (e.g. Leber's hereditary optic neuropathy, Stargardt disease). Currently no such treatment approach is available for glaucomatous optic neuropathy. In addition, and as a consequence of the inability of tissue replacement in the retina, early diagnostics and precise longitudinal measurements of retinal function are essential for preventing unnecessary loss of neuronal tissue. Major advances have been made in the field of optic coherence tomography and further improvements of diagnostics based on this technology are expected within the next years.

Drug application to the eye is an additional challenge in ophthalmology. Since the retina is protected by the blood retina barrier and diffusion through ocular tissue is largely limited to small molecules application of large molecules or long term application of drugs is not sufficiently possible. Injections of drugs into the eye was established during the last decade, however, this being a non-satisfying way of applications, drug delivery systems are developed and some of them are used in clinical trials for the delivery of e.g. glaucoma drugs.

Despite major advances in treatment options for diseases of the optical part of the eye, therapies for ocular neuronal and vascular tissue is very limited. Reduction of intraocular pressure was shown to be protective against progression of Glaucoma, but not all patients respond to this treatment, lost retinal cells are not replaced by it and when stopped, the disease continues its course. The situation in retinal disease like age related macular degeneration or diabetic retinopathy is similar. Hence, early diagnostics, the treatment of neurodegenerative diseases and the replacement of already damaged tissue are currently among the major unsolved challenges in therapy of eye diseases.

CRIPEC® NANOMEDICINES: PRINCIPLES, APPLICATIONS, PREPARATION, PRECLINICAL EVALUATION AND EARLY CLINICAL TRANSLATION

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INTRODUCTION

Cristal Therapeutics is a pharmaceutical company developing innovative nanomedicines for an enhanced therapeutic performance. The lead product, CriPec® docetaxel, is currently in clinical development for the treatment of solid tumours while other CriPec® products are in (late-stage) preclinical development. CriPec® is a pioneering approach to transform a broad range of therapeutic compounds into nanoparticles. The use of custom-made polymers with biodegradable drug linkers allows for the rational design of nanomedicines to assure optimal treatment of various diseases[1-4]. A generic, cGMP-grade manufacturing process has already been successfully developed.

RESULTS AND DISCUSSION: CRIPEC® DOCETAXEL

A significantly enhanced anti-tumour efficacy of 65 nm sized CriPec® docetaxel has been demonstrated in preclinical breast, prostate and gastric cancer mice xenograft models. The superior efficacy is attributed to an improved pharmacokinetic profile, confirmed higher tumour uptake and improved tolerability of CriPec® docetaxel as compared to Taxotere. The first-in-human trial of CriPec® docetaxel, started in H2 2015, follows a traditional dose-escalating approach. Initial results demonstrate a significantly longer systemic circulation of CriPec® docetaxel nanoparticles as compared to Taxotere. Comparison of plasma concentration levels at different CriPec® docetaxel doses indicated a clear PK-dose linearity. Safety evaluation is still ongoing.

CRIPEC® ACTIVELY TARGETED NANOPARTICLES

The conjugation of targeting ligands (e.g. peptides, nanobody or antibody fragments) to the surface of CriPec® nanoparticles (CriPec® ATN) increases target cell interactions, thereby further enhancing therapeutic effects whilst reducing off-target toxicity. The improved efficacy was proven with anti-EGFR-nanobody targeted CriPec® doxorubicin in a head & neck cancer mouse xenograft model[5]. Ligand conjugation is straightforward with full control over the number of ligands per nanoparticle. Moreover, smaller CriPec® nanoparticles (tuneable between 30 and 60 nm) have been generated while assuring a low polydispersity, high drug entrapment efficiency and tuneable drug release.

CRIPEC® OLIGONUCLEOTIDES

The therapeutic potential of oligonucleotides is still hindered by effective tissue and cellular targeting. Recently, CriPec® is successfully combined with AHA1 (ds-siRNA) and yielded ~ 55 nm nanoparticles (PDI < 0.2). Incubation of CriPec® AHA1 under physiological conditions demonstrated the release of native AHA1 over time, so opening opportunities for enhanced tumour targeting.

CONCLUSION

CriPec® allows for the rational design of nanomedicines for a superior therapeutic performance in several indications. Initial results of the clinical phase I evaluation of CriPec® docetaxel are promising. Moreover, additional CriPec®-based products carrying different types of payloads are being developed, partially in co-development with external parties.

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3D TISSUE MODELS - NEW PERSPECTIVES FOR MEDICINE

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Three-dimensional (3D) cell culture models are well established. Different technologies are available to grow cells in 3D. Among the scaffold-based 3D cell culture approaches the promising additive manufacturing technology called bioprinting is gaining more and more interest. Bioprinting allows the spatial control of cells, bioactive materials and molecules in 3D and is therefore being expected to produce 3D tissues that reflect the inherent complexity of native tissues to a high degree.
In industry-driven projects we are developing bioprinting solutions. The current bioprinting setup includes: i) inkjet- and extruder-based printheads with temperature control for cell jetting and contact printing into well plates, ii) different ECM-like printable matrices (bioinks), iii) a photopolymerization unit to crosslink bioinks with UV-LED (365 nm) and iv) a cell mixing unit to avoid cell sedimentation in the print cartridge while printing. For tissue generation alternating layers of bioink and cells are printed to produce a multi-layered 3D tissue construct. In a proof-of-concept study we established robust protocols to print full-thickness skin equivalents for future use in the cosmetic industry.
In a current industry research project we focus on an *in vitro* tool for drug assessment to find new treatments for muscle-related diseases. The final goal is an all-in-one solution to produce and analyse printed *in vitro* muscle/tendon tissues in a customized well plate. Each of the well in the plate contains to posts to build muscle/tendon tissues around and in between them. With bioprinting the respective precursor cells are printed in co-culture and differentiated in the muscle/tendon tissues. First, monocultures of primary human myoblasts and primary rat tenocytes were printed separately in a dumbbell-shape around the posts. After cell differentiation the myoblasts were stained positive for myosin heavy chain (MHC) and myotubes developed and for tendon the characteristic collagen I-distribution around the cell nuclei was detected. The printed muscle tissue is contracting on electrical stimulation and shows physiological functionality. The integration of electrodes in the well plates will allow electrical stimulation and subsequent read-out in the well plate.
In a precompetitive research project we are elaborating the possibility to rebuild parts of the kidney, the so called proximal tubulus, because nephrotoxicity is a major cause for effective drugs not being marketed. In preliminary experiments we printed the proximal tubule of the kidney seeded with proximal tubule epithelial cells to cultivate them under physiological flow conditions.
The development of standardized 3D *in vitro* tissue models combined with read-outs is a prerequisite for the future success of 3D tissues in substance testing and medicine in general.
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REVERSIBLE ESTERIFICATION-BASED LIPOSOMAL LOADING FOR ENHANCED RETENTION OF HYDROPHOBIC DRUGS

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Liposomes are a proven technology to encapsulate drugs at high concentrations. However, numerous anticancer drugs are hydrophobic which results in the design of challenging strategies to prevent drug leakage. We hypothesized that glucuronide derivatives of hydrophobic drugs might enhance liposomal retention by increased membrane impermeability. However, active loading of glucuronides in liposomes was severely limited by their high water-to-lipid partitioning. To overcome this issue, we transiently increased the lipophilicity of glucuronides by facile generation of glucuronide methylesters in acidic methanol (Fig. 1a). Glucuronide methylesters rapidly accumulate inside liposomes and spontaneously saponify back to glucuronides at basic pH (Fig. 1b). Reversible esterification helped encapsulate anticancer drugs 9-aminocamptothecin-β-D-glucuronide (9AC-G), 5,6-dihydro-4H-benzo[de]quinoline-camptothecin-β-D-glucuronide (BQC-G) and 4-methylumbelliferyl-β-D-glucuronide (4MU-G) at 260, 10.5, and 42.3 fold higher amounts as compared to a standard weak acid drug loading method. In addition, liposomal 9AC-G and BQC-G are significantly more stable in biological environments for prolonged periods compared to the liposomal formulation of hydrophobic parental drugs 9AC and BQC. Moreover, liposomal 9AC-G retained toxicity and performed better than liposomal doxorubicin (Doxisome®) against numerous human cancer cells. *In vivo*, liposomal 9AC-G was able to cure all treated mice bearing subcutaneous MDA-Mb-468 human breast cancer tumors without significant body weight loss. This approach may help stably retain a wide variety of lipophilic drugs inside liposomes to help reduce premature "burst" release *in vivo*.

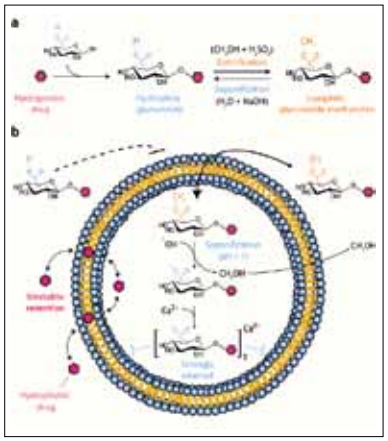

Figure 1. General loading strategy and mechanism. (a) Hydrophobic drugs are conjugated to a water-solubilizing agent such as glucuronic acids to become hydrophilic glucuronides. The methyl ester form (glucuronide-mE) is simply synthesized from glucuronides in methanol in the presence of a strong acid. This reaction is reversible in water in the presence of a strong base (b) Glucuronide-mE freely crosses lipid bilayers while an internal high pH saponifies the methylester and generate intraliposomal glucuronide. At high pH, glucuronides are present in a negative carboxylate form, which stably precipitates inside the liposome with free calcium cations.

SURVIVING NEBULIZATION-INDUCED STRESS: DEXAMETHASONE IN PH SENSITIVE ARCHAEOSOMES

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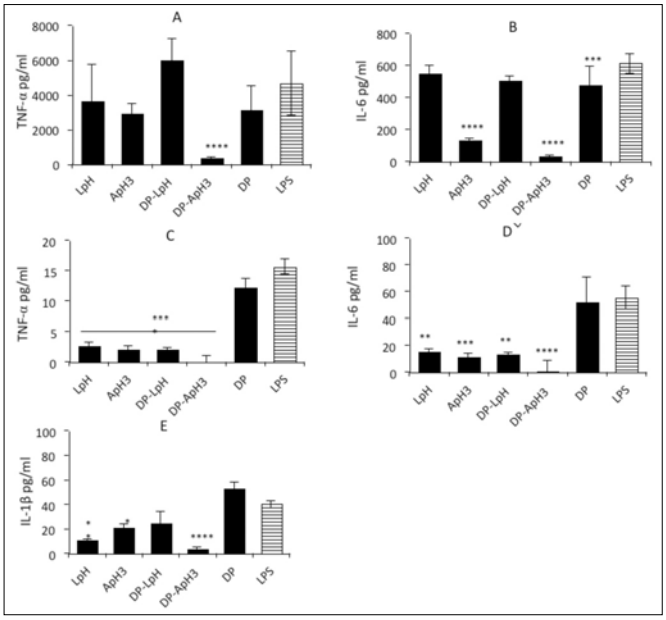
The inhalatory route gives the most direct access to the gas exchange surface of the lungs. This route is used for systemic drug delivery, where the drugs enter the blood by crossing the thin interface of the alveolar epithelial-vascular endothelia, or for site specific drug delivery, to the alveoli in the lower airways.
Despite of the meaningful advantages associated to the advent of inhalable corticosteroids, (IC), the inhaled therapies may evolve towards more refined products. The inhaled particulate carriers for instance, are proposed to maximize the residence time in the lungs

and protect the chemical structure of drugs. Today, two inhaled liposomal formulations for amikacin (Arikayce®) and ciprofloxacin (Pulmaquin) are in late stage clinical trials. These carriers for site specific drug delivery, however, may yet evolve towards more sophisticated nanoliposomes displaying new functionalities, compatible with their industrial manufacture. It would be desirable for instance, to count on carriers enabling the drugs to be delivered to a specific subcellular site (tertiary targeting). Once endocytosed, classical nanocarriers remain trapped within endo/phagocytic vesicles of growing acidity, where end up destroyed. Because of that, unless the site of drug action is in the endo-lisosomes, escaping the endocytic machinery is required for drugs to target cytoplasmic or nuclear sites. That is the case of corticosteroids or antibiotics, having cytoplasmic receptor. Ordinarily, to access the cytoplasmic receptor the drugs have to diffuse across the cell membrane. Endocytosed within properly engineered carriers, the drugs would enter the cytoplasm even in the absence of a huge concentration gradient. The endocytic machinery thus, may account for therapeutic subcellular concentrations of drugs given at low doses

We hypothesize that the performance of nebulizable drugs could be improved by designing pH sensitive nanoliposomes (LpH) including total polar archaeolipids (TPA). The inclusion of TPA may result in LpH exhibiting new pharmacodynamic properties with increased mechanical bilayer stability (responsible for the loss of the liposomal drug) during nebulization. In this work we describe the design and test the performance of mixed nanoliposomes DOPE:TPA:CHEMS for nebulization of the model corticosteroid dexamathasone phosphate (DP) to alveolar macrophages and alveolar epithelial cells involved in asthma inflammation. DP was used instead of classical IC, because the hydrophobic drugs partitioned in the bilayer of a LpH are not efficiently released in front of the acidity.

The anti-inflammatory effect of 0.18 mgDP/mg total lipid, 100-150 nm DP-containing ApH made of DOPE: TPA: CHEMS 6.5:0.5:3 w:w (ApH1), 5.6:1.4:3: w:w (ApH2) and 4.2:2.8:3 w:w (ApH3) was tested on different cell lines. Size and HPTS retention of ApH and LpH before and after nebulization were determined.

We found that DP-ApH3 completely suppressed il6, TNFalpha and il1beta, on phagocytic cells (figures AB in J774A1 cells; C,D,E in HTP1 cells). Nebulized after 6-month storage, LpH increased size and completely lost its HPTS while ApH3 conserved size and polydispersity, fully retaining its original HPTS content. These results suggested that the physical stability of TPA- containing nanoliposomes to nebulisation may well manage without including cholesterol or expensive hydrogenated lipids of high phase transition temperature in the nanoliposome bilayer.



The high cost/benefit of the targeted approaches difficult their industrial implementation. In order to reduce their high attrition rates, it is essential thus, to count on methods to prepare targeted nanoliposomes as simple and cheap as possible. Our results show for the first time that by simple mixing archaeolipids and ordinary phospholipids, nanoliposomes of higher colloidal endurance than liposomes, exposing specific ligands, were obtained. The inhaled targeted liposomes are still an emergent technology, and in such scenario, our results suggest that *in vivo* the inhaled pH-sensitive archaeosomes may enhance the activity of drugs having cytoplasmic receptors.

QUANTIFICATION OF NANOPARTICLES AT THE SINGLE CELL LEVEL USING MICROSCOPIC AND ANALYTICAL TECHNIQUES

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Engineered nanoparticles (NPs) are increasingly produced and their promising potential for diagnostic and therapeutic applications requires a thorough understanding on how these particles interact with single cells. There is convincing evidence from the literature that the physico-chemical properties of NPs, e.g. size, shape, material, and surface functionalisation, have a strong impact on NP uptake, intracellular fate and induction of cell response [1-3].

In order to understand how NPs interact with cellular systems, potentially causing adverse effects, their detection, localisation and quantification within cells is of central importance to understand how physico-chemical parameters might influence the possible interaction with a specific cell type. Once intracellular NPs are identified, their distribution in different cellular compartments, such as endosomes, lysosomes, mitochondria, the nucleus or cytosol may also provide some indications as to their potential biological impact, as well as how to specifically design nanocarriers for cell targeting and drug delivery.

The method of choice for the intracellular detection of NPs depends on the characteristics of the particles (chemical composition, fluorescence, size, and structure or electron density) and on the cellular structure of interest. In addition, due to the small size of Nps, their identification and localisation within single cells is extremely challenging. Therefore, various cutting-edge techniques are required to detect and to quantify metals, metal oxides, magnetic, and fluorescent NPs [4]. Several techniques will be discussed in detail such as inductively coupled plasma atomic emission spectroscopy, flow cytometry, laser scanning microscopy combined with digital image restoration, and quantitative analysis by means of stereology on transmission electron microscopy images. An overview will be given regarding the advantages of those visualisation/quantification techniques including a thorough discussion about limitations and pitfalls.

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ENTRY OF NANOPARTICLES INTO CELLS: MECHANISMS AND CONSEQUENCES

KIRSTEN SANDVIG

Nanoparticles can be used to deliver drugs or other substances both *in vivo* and *in vitro*[1-3], and are commonly used to study basic cell biology. To enter cells the particles exploit the endocytic machinery, and they have been demonstrated to induce changes in cellular uptake and intracellular transport[4,5]. Crosslinking of cell surface molecules may cause signaling in cells[6], and nanoparticles have been found to induce macropinocytosis that facilitates uptake of particles. In several instances this process has been shown to be dependent on the large GTP-binding protein dynamin. To optimize nanoparticle delivery into cells one needs to understand the cellular mechanisms involved in their uptake. Such information may help in deciding the type of particle to use, the size of the particle as well as which components to include at particle surface. Today we know that cells have different types of endocytic mechanisms[7], some giving rise to small vesicles (60-200 nm diameter), whereas other mechanisms such as macropinocytosis are required for uptake of larger particles. One should be aware of that cells growing in a polarized manner are likely to have different endocytic mechanisms which are under differential influence of signaling substances at the two poles[7], and studies of nanoparticle uptake in nonpolarized cells may not give the same results as if uptake in polarized cells is investigated. Furthermore, increased cell density may induce changes in membrane lipids and intracellular transport[8], and modification of membrane lipids may change the mechanisms of uptake. Clearly, well controlled conditions for the cell experiments performed and correct interpretation of the results obtained from cellular studies are essential. For instance, cholesterol is often mistaken for only being important for caveolar uptake, but is involved in several endocytic processes including macropinocytosis[7]. Also, robust methods to determine whether a particle is internalized or only at the cell surface are important to provide the investigator with correct data about uptake efficiency.

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BLOCKING TOLL LIKE RECEPTOR 4 IN NON-HUMAN PRIMATES WITH SEVERE INFECTIOUS DIARRHEA

PERE SANTAMARIA

The complexity of autoimmune diseases is a barrier to the design of strategies that can blunt autoimmunity without impairing general immunity. We have shown that systemic delivery of nanoparticles (NPs) coated with autoimmune disease-relevant peptide-major-histocompatibility-complex (pMHC) class-II molecules triggers the formation and profound expansion of antigen-specific T-regulatory-type-1 (TR1)-like CD4+ T-cells in different mouse models, including mice humanized with lymphocytes from patients, leading to resolution of established autoimmune phenomena. Eleven pMHC-class II-based nanomedicines show similar biological effects, regardless of genetic background, autoimmune disease type, prevalence of the cognate T-cell population or MHC restriction. Specifically, these compounds can: (1) restore normoglycemia in type 1 diabetic (T1D) mice; (2) restore motor function in paralyzed mice with experimental autoimmune encephalomyelitis (EAE, a model of Multiple Sclerosis, (MS)); (3) promote active re-myelination of the CNS in a model of lysolecithin-induced demyelination in the context of EAE; (4) resolve joint inflammation in arthritic mice; and (5) blunt liver inflammation in mice with spontaneous primary biliary cirrhosis (PBC). pMHC class II-NP therapy functions by expanding, in an epitope-specific manner, cognate T-regulatorytype- 1 (TR1) CD4+ T-cells that are virtually identical (phenotypically, transcriptionally and functionally) to TR1 cells cloned from patients. These nanomedicines promote the differentiation of disease-primed autoreactive T-cells into TR1-like cells, which in turn suppress autoantigen-loaded antigen-presenting cells and drive the differentiation of cognate B-lymphocytes into disease-suppressing B-regulatory cells, without compromising systemic immunity. pMHC class II-based nanomedicines thus represent a new class of drugs useful for treating a broad spectrum of autoimmune conditions in a disease-specific manner.

NANOMEDICINES FOR ENHANCING AGRICULTURE AND AQUA-CULTURE YIELD

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Keywords: nanotechnology, food, hunger, RNAi, pesticides, agriculture, aquaculture

As the world population grows, there is a need for efficient and ecologically-friendly agricultural technologies to deliver food requirements. Here, we describe a plant-derived nanomedicine system used to deliver nutrients and crop-protection-agents to plants and RNAi to shrimp.
In plants, we show that 100-nm nanoparticles, loaded with Mg and Fe, penetrate the leaf cuticle and travel in a bidirectional manner, distributing to the other leaves and down the roots. The particles remain intact until they are internalized by the plant cells, where they release their payload.
In aqua-culture, shrimp treated with nanoparticles loaded with siRNA, exhibited reduced gene expression, protecting shrimp from disease. We find that nanomedicine technologies developed for humans, can be adapted for the benefit of animals and plants. Cross pollination of these disciplines may help defeat hunger and create healthier food.

NEW ANTIBODY DERIVATIVES FOR TARGETED PAYLOAD DELIVERY

FELIX SCHUMACHER, Senior Scientist, Roche Diagnostics GmbH, pRED, LMR

The idea of using antibodies as ‘carriers’ for chemically attached or genetically fused payloads has been realized in a large variety of combinations and constructs over the past few decades. While the basic concept is simple, its transfer into clinical reality has proven to be notoriously difficult as one of the major players in this field, the ADC (antibody-drug conjugate) industry, has learned over the past years from a long list of failed clinical trials. The main focus of current efforts to solve this problem has been the nature of the payload (moving towards more potent or overall more payloads) and the conjugation method (moving from random to site-specific tech-

nologies). While the first results look promising these approaches are overlooking a major component of the system, which is readily amenable to modifications: the antibody itself. Applying the wealth of knowledge of antibody engineering to the carrier-molecule will enable the design of derivatives with favorable PK, penetration and binding profiles. Combining these with innovative payloads and elegant conjugation methods has the potential to form the basis of the next generation of targeted therapies.

INCREASING SENSITIVITY OF TUMOR CELLS TO TREATMENT WITH NANOMEDICINES

SIMO SCHWARTZ, Jr MD, PhD

Cancer Stem Cells are highly resistant to chemotherapy and are responsible of the metastatic spread of the disease reducing the overall survival of cancer patients. New treatments should target this resistance and sensitize CSC to improve cancer outcomes. There are several options that might possibly overcome this resistance and fight against metastatic spread. Targeted inhibitors against specific CSC active signalling pathways or use of targeted nanomedicines against CSC able to deliver higher amounts of antitumor drugs or siRNAs inside CSC, are two of these options. We will discuss about them using specific bioluminiscent CSC models and how targeting specific pathways CSC reversión is hampered, increasing the sensitivity of CSC to chemotherapeutic treatment.

ACTIVE TARGETING AS MODULATORS OF NANOMEDICINES PHARMACOKINETIC PROFILES

SIMO SCHWARTZ, Jr MD, PhD

Pharmacokinetic profiles of currently used drugs can be modulated by using nanomedicines as delivery vehicles. The existence of enhanced vascular permeability (EPR) within tumors and macrometastasis and in inflammed areas helps to accumulate drugs if desired. However, it does not substantially modify the pharmacokinetic profile of a nanoconjugate because this mainly depends on other parameters such as it size and 3D structure or its biological behaviour *in vivo*. Here we report with some examples how the use of specific targeting moities might modify the pharmacokinetic profile of a nanomedicine, independently of the EPR effect and their cellular internalization capability.

A NEW NANO WORKBENCH TO STUDY AND CONTROL THE FORMATION OF OLIGO-FIBRILS MADE BY ALFA-SYNUCLEIN IN PARKINSON'S DISEASE

GIACINTO SCOLES

Nanografted patches of alfa-Synuclein (alfa-S; the protein contained in the Lewy 's bodies that are responsible for cell death in the brain of PARKINSON patients) of varying densities (coverage) can be prepared on a flat piece of (111) gold by an application of the method of DNA Directed Immobilization of Protein (DDI). These patches result from the hybridization between ss-DNA nanografted on the (111) face of gold and the complementary ss-DNA conjugated at one of the two terminals of alfa-S. Placing the patches in contact with a solution containing the free protein and polymers containing positive groups fibrils are formed and can be studied in the presence of polydentate nanobodies to see if it is possible to bring the alfa-S back in solution.
The implications of a possible solution of this problem for the cure and or the diagnostics of Parkinson's disease are clear and important.

HEALTH CANADA'S APPROACH TO NANO–TECHNOLOGY BASED HEALTH PRODUCTS

HRIPSIME SHAHBAZIAN

Health Canada uses existing legislation and regulations to mitigate potential health risks of nanomaterials and to help realize their benefits. Consistent with other major regulatory bodies around the world, the Department takes a case-by-case approach to assessing the safety of products and substances that may either be or contain nanomaterials.
To support the regulation of nanomaterials, Health Canada developed a working definition for Nanomaterial. The Policy Statement on Health Canada's Working Definition of Nanomaterials was adopted on October 6, 2011.[1] The working definition provides Health Canada with a consistent approach across its diverse regulatory program areas to identify regulated products and substances that may be or may contain nanomaterials. The working definition is relevant for all products and substances regulated by Health Canada.
Health Canada's Health Products and Food Branch (HPFB) is the national authority that regulates, evaluates and monitors the safety, efficacy, and quality of therapeutic and diagnostic products available to Canadians. The HPFB created a nanotechnology webpage[2] informing
stakeholders regarding Health Canada's Working Definition of Nanomaterials and providing general guidance. It advises sponsors and other stakeholders to communicate with responsible regulatory areas early in the development process if their products contain or make use of nanomaterial and provides examples of the type of information that may be required for a nanotechnology-based product's safety assessment. To address unique physical, chemical and biological properties of nanomaterials each product is assessed on a case-by-case basis.
The first step to assuring adequate risk assessment and risk management is to identify potential nanomaterials using the Working Definition as a tool.
To facilitate identification and tracking of nanomaterial containing drug submissions Health Canada revised Drug Submission Application Form for Human, Veterinary, Disinfectant Drugs and Clinical Trial Application/Attestation (HC/SC 3011).[3] Section 56 of the revised form asks the sponsor to self-identify when their application concerns a nanomaterial or 'nano-product'.
Natural health products, such as vitamin and mineral supplements and herbal products for which therapeutic claims are made are regulated under the Natural Health Products Regulations and not as drugs under the Food and Drug Regulations. For Natural Health Products, the Natural and Non-prescription Health Products Directorate allows sponsors to identify nanomaterials on Eelectronic Product Licence Application form, if there are any.[4]
To facilitate identification and tracking of nanomaterial containing device submissions Health Canada issued a revised Medical Device License Application form to ask the sponsor to self- identify when their application concerns a nanomaterial and provide size range of nano-scale material particles.[5]
Health Canada believes that, in general, its current risk assessment methodologies are applicable for nanomaterials as they allow for sufficient flexibility. Maintaining a flexible approach is important to integrate new knowledge about risks and benefits related to nanomaterials into regulatory decision-making processes.
Joint efforts are needed to accelerate the achievements promised by Nanotechnology. Health Canada continues to work closely with domestic and international partners toward consistency with relevant international norms. The Department has developed strong partnerships with other levels of government, academia and stakeholders and ensures that their perspectives are included in its assessment of the health benefits and risks of every therapeutic product it reviews. Health Canada works with international organizations, including regulatory authorities in other countries, to harmonize regulatory standards and processes for therapeutic products.

As part of its international collaborative work on nanotechnology and nanomaterials, Health Canada is engaged, with Environment Canada, in the Canada-United States Regulatory Cooperation Council (RCC) Nanotechnology Initiative. Its goal is to share information and develop joint approaches on regulatory aspects of nanomaterials, including terminology and nomenclature, as well as risk assessment and management.[6]
Health Canada is a member of the International Regulators on Nanotechnology Working Group that was established in summer of 2009 to discuss nanotechnology related issues relevant to regulated products that may contain nanoscale materials. The WG established product specific Subgroups in various regulated product areas (including pharmaceuticals, devices and food). The main purpose of this WG was to facilitate more detailed discussions in key regulated product areas that would enable more detailed communication and collaboration among interested parties.
The International Pharmaceutical Regulators Forum (IPRF) was established in June 2008 as a regulators-only platform, who met in the margins of the ICH Conferences. One of the objectives of the IPRF is to identify the need for harmonization or regulatory convergence, as well as for regulatory cooperation, including work-sharing, in specific areas. As an emerging product category, nanotechnology-based therapies became a topic for the Regulators Forum in 2014. In 2015 agreement was reached to establish a Nanomedicines Working Group under IPRF to share non-confidential information.[7] Health Canada is a member of this new Working Group.

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4 Electronic Product Licence Application form: http://www.hc-sc.gc.ca/dhp-mps/pubs/natur/eplaguide-eng.php#fig29
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BLOCKING TOLL LIKE RECEPTOR 4 IN NON-HUMAN PRIMATES WITH SEVERE INFECTIOUS DIARRHEA

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Shigella causes the most severe of all infectious diarrhoeas and colitis. We infected rhesus macaques and treated them orally with a small and non-absorbable polypropyletherimine dendrimer glucosamine that is a Toll-like receptor-4 (TLR4) antagonist. Antibiotics were not given for this life-threatening infection. Six days later, the clinical score for diarrhoea, mucus and blood was 54% lower, colon interleukin-8 (IL-8) and interleukin-6 (IL-6) were both 77% lower, and colon neutrophil infiltration was 75% less. Strikingly, vasculitis did not occur and tissue fibrin thrombi were reduced by 67%. There was no clinical toxicity or adverse effect of dendrimer glucosamine on systemic immunity.
This is the first report in non-human primates of the therapeutic efficacy of a small and orally bioavailable TLR antagonist in severe infection. Our results show that an oral TLR4 antagonist can enable controlled resolution of the infection-related-inflammatory response and can also prevent neutrophil-mediated gut wall necrosis in severe infectious diarrhoeas.
The dissection of complex biological pathways by biologists is opening up fundamentally new opportunities for the therapeutic evaluation of novel polyvalent drugs that are based upon dendrimers. Our results highlight their clear potential as new medicines. Results in several animal models strongly suggest that small dendrimers with only 16-32 peripheral groups will make for the best infection and inflammation related medicines. Public-private partnerships are now needed to drive these small dendrimer drugs into proof-of-concept clinical trials.

RNAI AND TUMOR MICROENVIRONMENT: PRE-CLINICAL AND CLINICAL EVIDENCE

AMOTZ SHEMI, CEO Silenseed LTD Israel

The distribution of drugs within solid tumors presents a long-standing barrier for efficient cancer therapies. Tumors are highly resistant to diffusion, and the lack of blood and lymphatic flows suppresses convection. Moreover, efficacy in targeting oncogenes by current monoclonal antibodies or small-drug inhibitors is limited. RNAi-based medicine enables effective targeting of 'undrugable' targets' such as KRAS, and, when is based on prolonged and continuous intratumoral drug delivery from a miniature drug source, offers an alternative to both systemic delivery and intratumoral injection. At delivery onset the drug mainly affects the closest surroundings. Such 'priming' enables drug penetration to successive cell layers. Tumor 'void volume' (volume not occupied by cells) increases, facilitating lymphatic perfusion. The drug is then transported by hydraulic convection downstream along interstitial fluid pressure (IFP) gradients, away from the tumor core. After a week tumor cell death occurs throughout the entire tumor and IFP gradients are flattened. Then, the drug is transported mainly by 'mixing', powered by physiological bulk body movements. Steady state is achieved and the drug covers the entire tumor over several months. We present measurements of the LODER™ system, releasing the siG12D (siRNA against mutated KRAS) drug over months in pancreatic cancer in-vivo models. siG12D-LODER was successfully employed in a Phase 1/2a clinical trial with pancreatic cancer patients, and is planned to enter Phase 2 multinational trial in 2016 (Golan et al., 2015, Oncotarget; Shemi et al., 2015, Oncotarget).

NANOBODIES IN MEDICAL DIAGNOSTICS: NEW TOOLS FOR REVIEWING OLD CONCEPTS

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INTRODUCTION

The early 1990s discovery of naturally occurring heavy-chain-only antibodies (HCAbs) in Camelidae[1] and their further development into small recombinant nanobodies2 provided a new tool in the well-established and quite lucrative antibody-based diagnostics portfolio. Easily expressed in microorganisms and amenable to engineering, nanobody derivatives are soluble, stable, versatile, with unique refolding capacities, reduced aggregation tendency and high target binding capabilities 3. A ready-to-use potential refers to diagnostic imaging, *in vivo* monitoring, immunotherapy and targeted drug delivery. Notwithstanding, the applicability domain could broaden to the benefit of other technology fields, as well.
The harsh engineering that nanobodies can sustain make them perfect candidates as biorecognition moieties in biosensor platforms. Biosensors, using natural recognition processes with a view to detecting targets with extremely low detection limits, have enjoyed low marketability since they have an operational versatility and stability that is, by large, dictated by the biological moiety immobilized on top of the transducer. Nanobodies can confer their extended shelf-life and ruggedness to biosensors, allowing for real

time and in situ detection in biological fluid, an area that, so far, only enzymes can serve adequately at the expense of selectivity, sensitivity and specificity.

SCOPE OF RESEARCH

The development of immunosensors using conventional antibodies or fragments has been proven challenging due to the low affinities expressed in the presence of binders. A thin lipid film electrochemical platform has been used to evaluate the potential of nanobodies in biosensing. Conventionally developed immunosensors have been reviewed using the new material. The interaction of nanobodies with artificial lipid membranes have been investigated using the simpler format of metal supported lipid films; nanobodies have been immobilised on the lipid bilayer using physisorption. Sensor optimization using polymerised membranes incorporated with nanobodies and modified electrodes with graphene nanosheets and zink oxide nanowalls is presented.

RESULTS

Lipid membrane immunosensors built on antibodies relied on the steric hindrance of the proteinaceous moiety to adsorb on the surface of the bilayer in a self-regulated orientation 4. The interactions of nanobodies with artificial lipid bilayers did not reproduce conventional antibody behaviour; their small size allowed some permeation of the bilayer resulting in both, increased noise levels and low reproducibility of response. In effect, nanobodies may not be suited for random coupling to solid surfaces unless advanced engineering produces formats that would permit directional immobilization. Published works on surface plasmon resonance biosensor platforms have come to similar conclusions[5].

Mixing nanobodies with the lipid mixture before polymerisation enabled the optimization of membrane loading with analyte binding sites. The polymerization could take place either by using UV irradiation or thermal polymerization[6]; since most biorecognition elements exhibit thermal instability, UV polymerisation is preferred at the expense however of sensor shelf- and operational stability 3. The thermal stability of nanobodies allowed polymerisation at 80 oC for 10 hours resulting in more rugged biosensing interfaces. When coupled to nano-modified electrodes two basic conclusion were drawn: (i) graphene nanosheets provide some degree of lipid anchoring that appeared to aid a self-regulated directional immobilisation of the nanobodies resulting in highly reproducible responses and satisfactory reliability of measurements; (ii) zink oxide nanowalls provide alternating layers of positive and negative ions along the nonpolar plane that create an inherent signal amplification mechanism and a capability for high sensitivity; detectability was greatly enhanced offering a ten times lower detection limit than conventional antibodies and a four times lower detactability than graphene-modified sensors; yet, sensor reliability was greatly compromised from increased noise levels.

CONCLUSIONS

Preliminary results are very promising as per the feasibility of preparing sensors that could simulate better natural chemoreception. Yet, the kinetics and binding properties of nanobodies should be further investigated before their participation in sensor architectures.

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DEVELOPMENT OF NANOPARTICLES FOR CLINICAL USE: IMPORTANCE OF DEGRADATION AND EXCRETION

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There are huge expectations for the use of nanoparticles (NPs) to deliver therapeutics and for imaging of different diseases, such as cancer. Carefully designed experiments, both *in vitro* and *in vivo*, are essential in order to fully explore this technology. Despite many promising NPs being made during recent years, the biological studies performed with such NPs very often do not have the quality needed to support the conclusions drawn[(1,2,3)]. More interdisciplinary collaboration to improve the quality of such studies is required. With a long experience from pharmaceutical R&D, I will discuss improvements that should be made in biological studies with NPs. The design of animal studies, including which time points to take samples and which parameters to analyze, is critical when aiming at developing drugs for clinical use[(1)]. Biodistribution, metabolism and excretion studies are extremely important not only to generate such data (e.g. for an imaging agent), but also to evaluate safety and to predict whether it is likely that the NPs studied ever can receive market approval for clinical use[(3)].

It is of utmost importance that NPs made of non-endogenous substances are degraded and excreted. The impact of having biodegradable versus non-degradable NPs on toxicity studies, cost of development and the risk/benefit analyses one can expect pharmaceutical companies to perform will be discussed[(3)].

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PAEDIATRIC NANOMEDICINE: CHALLENGES TO CLOSE THE ADULT-CHILD GAP BEFORE IT EMERGES

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Nanotechnology has become a key tool to overcome the main (bio) pharmaceutical drawbacks of drugs and to enable their passive or active targeting to specific cells and tissues. Traditionally the development of paediatric treatments relies on previous clinical experience in adults, which is relatively scarce for most nanotechnology platforms[(1)]. Children present biological and/or metabolic differences with respect to adults due to the gradual development and maturation of the different organs and systems after birth. In the case of diseases that hit both adults and children, nanomedicines need to be primarily adjusted to fit the paediatric use, a process that might demand the development of different pharmaceutical products and their later clinical trial. The perspectives are more

uncertain for diseases that are children-specific, that have a different pathophysiology or that show substantially greater morbidity in children, where nanomedicines need to be especially developed. On one hand, it is clear that the treatment of disease in children cannot be simplified to the direct adjustment of the dose to the body weight/surface. On the other, the need for innovative paediatric medicines enters into conflict with the complexities of the fragmented market and the challenging clinical trials that discourage researchers in both academia and industry to investigate pediatric nano-drug delivery systems. This is leading to the generation of a new adult-child gap, this time around the use of nanomedicines that if not addressed timeously will probably become unbreachable. In this presentation, the main challenges faced for the implementation of nanomedicines in children will be briefly overviewed with emphasis in two groups of diseases, paediatric cancer and infections.

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REGULATORY SCIENCE CORE COMPETENCIES TO GUIDE EDUCATION AND TRAINING PATHWAYS

SCOTT STEELE

Regulatory Science is defined by the U.S. Food and Drug Administration (FDA) as "the science of developing new tools, standards, and approaches to assess the safety, efficacy, quality and performance of FDA regulated products." One goal of Regulatory Science is to enhance the overall translational research process and improve the development of safe and effective medical interventions. This is particularly relevant for anticipating rapidly advancing technology areas such as nanotechnology.

There is increasing recognition of the critical need to provide trainees and current researchers with educational opportunities in Regulatory Science. The future workforce addressing Regulatory Science needs will come from diverse fields impacting new medical product development (e.g., nanotechnology, toxicology, genetics, pharmacology, biostatistics, etc.) with roles as researchers/ scientists, physicians, engineers, reviewers, policy analysts, or other professionals in academia, industry, government or other organizations.

As Regulatory Science is a broad area, it requires a multidisciplinary team science approach bringing together diverse topics in drug discovery, clinical trial design, ethics, data analytics, just to name a few. Since Regulatory Science is so diverse and no single institution is likely to have complete expertise in all of these areas, this field is ideally suited for a collaborative approach. One challenge to address this educational need has also been the lack of a roadmap to guide Regulatory Science training. This presentation outlines the development of a set of Regulatory Science core competencies to inform curriculum development and diverse training pathways. This extensive effort involves collaborations with academic institutions (particularly from the Clinical and Translational Science Award Network), the pharmaceutical and medical device industry, FDA, the U.S. National Institutes for Health and scientific organizations. The competencies serve as a guide for networks of organizations to catalog existing educational resources, develop new resources to address educational gaps and establish experiential training opportunities in academia, industry and regulatory agencies.

NANOMEDICINES: IN VITRO-IN VIVO CORRELATIONS

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Controlling the pathways of drug disposition using specialized drug delivery systems (DDSs) can be an efficient way to increase the drug concentrations and prolong the retention time of the drug at the target site, and to decrease the drug concentrations at the sites where toxicity may occur. This will lead to enhanced magnitude of the desired effects (i.e., higher effectiveness of drug treatment), reduced magnitude of adverse effects (i.e., less toxicity), and higher efficiency of drug administration. In the past few decades, significant progress has been made in development of nano-DDSs intended for targeted delivery of the drug to its site of action. Several dozens of systemically or focally-administered DDSs have been approved for clinical use.

Despite these achievements, the biofate of the drug/DDS following their administration is complex and is controllable only to a low extent. As a result, only low amounts of the drugs reach the target tissue, and the currently available DDSs are characterized by low clinical effectiveness and/or high magnitude of adverse effects.

For instance, the currently available anticancer drugs/DDSs possess limited clinical effectiveness and safety. It appears that only the small fraction of the administered drug is available to exert the pharmacological effect at the intended site of action in the tumor, and the overall targeting efficiency of the drugs/DDSs to the tumors (i.e., the ratio of drug concentrations in the tumor tissue vs. other tissues) is low. In addition to limited tumor accumulation of the anticancer drugs/DDSs, it emerges that their inefficient intratumoral distribution and low permeability to the 'deep' parts of the solid tumor is a major factor that limits the treatment efficiency, increases the risk of adaptation of the tumor cells to drug effects and contributes to evolvement of drug resistance (treatment relapse).

In my talk, I will focus on the anticancer nano-DDSs for treatment of solid tumors, will summarize the major pharmacokinetic and pharmacodynamic parameters that govern the clinical effectiveness of these nanoformulations, and will discuss the ways to control the drug/DDSs disposition and to enhance the efficiency of the anticancer pharmacological responses.

NANOMEDICINE: BALANCING RISK OF TRIAL PARTICIPATION AND RELEVANCE OF THE NEW TREATMENT FOR PATIENTS – AN ETHICIST'S VOICE

NICOLA STINGELIN

"The proposal is made that an optimal analysis of benefit and risk issues in clinical research should be based on a thorough appreciation that such research is a complex multi-step staircase. A main task of each of the multiple iterative steps is to reduce uncertainty, and improve the basis available for an assessment of risk; movement up the stairs must also be driven by the existence of a justified hope of bringing new benefits for future patients by filling a gap in our knowledge. Many parties and institutions have onerous responsibilities that must be fulfilled in a step-wise manner before thought can be given to approaching potential trial participants for consent.

Applying such an approach will not reduce the complexity of nanomedicine research ethics deliberations, but supports a sound and just decision making process."

LIPOSOMAL CORTICOSTEROIDS IN A RABBIT MODEL OF NONINFECTIOUS ANTERIOR UVEITIS

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Noninfectious anterior uveitis (AU) are a group of immune-related, sight-threatening inflammatory conditions that account for 60% of all cases of uveitis seen in eye centers. These patients contribute significantly to the clinical load. The incidence of uveitis varies from 14 to 52.4/100,000 globally, with an annual prevalence of 69.0 to 114.5 per 100 000 persons. Uveitis is the cause of up to 10% of legal blindness in the United States, or approximately 30 000 new cases of blindness per year. AU may run a relapsing and remitting clinical course. Sight threatening eye complications can occur with prolonged uncontrolled inflammation, such as cataract, glaucoma, and swelling of the central retina. These complications lead to blindness in up to 25% of patients. The aim of this presentation is to show the positive effects of corticosteroid-containing liposomes in a well established model of AU in rabbit eyes. The results suggest that liposomal corticosteroids may revolutionize the way we treat patients with AU and avoid visual loss in these patients.

INFLAMMATION AS A TARGET TO REDUCE RESIDUAL CARDIOVASCULAR RISK. SYSTEMIC OR NANO?

ERIK STROES

Atherosclerosis is a lipid-driven inflammatory disease. Targeting inflammation to attenuate cardiovascular disease holds promises, yet also harbours challenges. Both systemic and nanomedicinal interventions are under evaluation. Systemic anti-inflammatory strategies such as interleukin 1-beta inhibition, which is currently under evaluation in cardiovascular endpoint trials, inhibit inflammation non-specifically. In view of the risk of systemic 'adverse' effects, local delivery strategies carry a theoretical advantage. We previously showed that liposomal nanoparticles loaded with prednisolone (LN-PLP) accumulated in plaque macrophages, however, induced proatherogenic effects in patients.
Here, we substantiate in low-density lipoprotein receptor knockout (LDLr $^{-/-}$) mice that LN-PLP accumulates in plaque macrophages. LN-PLP infusions at 10mg/kg for 2 weeks enhance monocyte recruitment to plaques. After 6 weeks of LN-PLP exposure we observe (i) increased macrophage content, (ii) more advanced plaque stages, and (iii) larger necrotic core sizes. In subsequent *in vitro* studies we demonstrate that macrophages become lipotoxic after LN-PLP exposure, exemplified by enhanced lipid loading, ER stress and apoptosis. These findings indicate that liposomal prednisolone may paradoxically accelerate atherosclerosis by promoting macrophage lipotoxicity. Future (nanomedicinal) drug development studies should anticipate the multifactorial nature of atherosclerotic inflammation when selecting best candidates for attenuation of atherosclerotic inflammation.

Keywords: Atherosclerosis, macrophages, lipotoxicity, prednisolone, liposomal nanoparticles

Abbreviations: CVD, cardiovascular disease; DPPC, dipalmitoylphosphatidylcholine; DSPE, distearoylphosphatidylethanola-mine; GC, glucocorticoids; LDLr-/-, low-density lipoprotein receptor knockout; LN, empty liposomal nanoparticles; LN-PLP, liposomal nanoparticle encapsulating prednisolone phosphate; PBS, phosphate-buffered saline; PEG, polyethylene glycol; PLP, free prednisolone phosphate.

BIPHASIC IMMUNE REACTIVITY AND IMMUNOGENICITY OF PEGYLATED LIPOSOMES WITH AND WITHOUT ENCAPSULATED DOXORUBICIN: APPROACHES OF INHIBITION IN A PORCINE MODEL

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Background: PEGylated liposomes and other nanoparticles are widely used as drug carrier nanosystems, however, they can be recognized by the immune system as foreign pathogens leading to more or less severe adverse events. One of the adverse immune effects is complement (C) activation that may entail a potentially severe, or even lethal anaphylactoid reaction, called C activation-related pseudoallergy (CARPA). Yet another related immune side effect is immunogenicity, i.e., the formation of specific IgM and IgG antibodies against the nanosystems (so called anti-drug antibodies, ADAs), which may lead to acceleration of the blood clearance of repetitively administered nanomedicines (ABC phenomenon) by induced antibodies, and, hence, decrease of therapeutic efficacy.
Novel findings: The experiments presented in the lecture suggest that CARPA is mediated, at least in part, by natural IgM and IgG antibodies against PEG and other lipid components of PEGylated liposomes (anti-LIP antibodies), regardless of the presence of doxorubicin inside the vesicles. Accordingly, scavenging these natural antibodies by Doxil lookalike drug-free (placebo) Doxil (Doxebo), or by slowly and/or repetitively administered Doxil, attenuates or prevents CARPA. The binding of natural antibodies occurs within minutes and keeps the anti-PEG and anti-LIP levels on baseline until about 2-3 days, after which time massive formation of anti-PEG and anti-LIP IgM and IgG takes place peaking at 7-9 days and returning to baseline over ≥ 6 weeks. During the time of elevated anti-liposome antibodies the anaphylactoid reactions to liposomes turn into anaphylactic shock, the phenomenon serving as a highly sensitive functional endpoint of immunogenicity. Importantly, both antibody induction and the anaphylactic reactivity of liposomes were abolished by co-administration with Doxebo of the human equivalent dose of Doxil, suggesting that an immune suppressive effect of Doxil prevents its own immunogenicity, which effect is critical for its clinical efficacy.
Conclusions: Based on other studies in murine models, the formation of anti-PEG and anti-LIP antibodies in our experiments represents splenic marginal B cell-mediated, so-called "T cell-independent" immunogenicity, a time-limited subacute immune phenomenon typically observed with pathogen-mimicking synthetic nanoparticles that contain homologous arrays of non-protein surface antigens (carbohydrates, nucleic acids). Our data suggest that it can lead to, or accelerate CARPA into potentially lethal anaphylactic shock, upon repeated administration of reactogenic nanomedicines.
Outlook: In addition to showing different approaches for the prevention of a safety problem with nanomedicines, the present results provide direct evidence for a beneficial immunosuppressive effect of Doxil, which explains the lack of immunogenicity of this drug in clinical practice and the inhibition of the dose-limiting allergic reactogenicity of co-administered other drugs. Our experiments also highlight the utility of pigs to serve as a new model of nanoparticle induced T-cell independent immunogenicity, a model that enables quantitation of not only the specific antibodies formed but also their potential to cause anaphylactic shock, and, hence, sudden death in an occasional hypersensitive patient.

ACKNOWLEDGEMENTS
This research was supported by the Technology Innovation Found of Hungarian Government (TÉT 13 IL-2-2014-0001).

ASSOCIATION OF CARDIOVASCULAR STRESS WITH INCREASED ANTIOXIDANT DEFENSE DURING FERROUS NANOPARTICLE-INDUCED ANAPHYLACTOID REACTION IN PIGS

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BACKGROUND AND GOALS:

Nanoparticles, including some iron containing imaging agents (Fe-NPs), are known to carry increased risk for complement activation-related pseudoallergy (CARPA), a potentially serious, occasionally lethal acute hypersensitivity (allergic) reaction which involves anaphylatoxin-induced activation of granulocytes with oxidative burst and free radical production. Iron-compounds, on the other hand, are known to undergo or catalyze oxido-reduction processes in the body, potentially modulating free radical production. Nevertheless, the complex interplay between Fe-NP-induced oxido-reductive processes, anaphylatoxin production, granulocyte activation, oxidative burst and free radical production has never been scrutinized *in vivo*, to give hints about potential adverse effects in man. Our goal in the present study was therefore to initiate such queries, by evaluating the effect of a CARPAgenic FeNP on the redox status of pigs following Fe-NP-induced CARPA.

METHODS:

The oxidation–reduction potential (sORP) and antioxidant capacity (cORP) of heparinized pig blood was determined by the RedoxSYS Diagnostic System (Aytu Bioscience, www.RedoxSYS.com). CARPA was induced by a proprietary Fe-containing nanoparticle system under development as imaging agent. The method of CARPA induction in pigs and the use of zymosan as positive control has been described earlier [(1)]. Here we used the pulmonary arterial pressure (PAP) as the most quantitative and reproducible measure of CARPA.

RESULTS:

Our data to-date suggest that it is cORP, and not sORP, that changes during CARPA in good correlation with the changes of PAP, regardless of the trigger of CARPA. Moreover, we have established that sampling arterial vs. venous blood makes no significant difference in cORP readings, and that storing blood samples at -80C leads to slow gradual decline of absolute cORP values on a time scale of weeks, but the relative changes (expressed as % of baseline) remain consistent over time. Figure 1 shows a typical experiment wherein the Fe-NP (ferrous substance, FS) was injected i.v. in a pig and the changes in sORP (A) and cORP (B) in fresh and old stored (rep) samples were correlated with the pulmonary hypertensive effect of FS and zymosan. The major FS-triggered rise of cOPR proceeded in close parallelism with the rise of PAP, while the sORP did not show any change, and zymosan consumed, rather than further increased cORP.

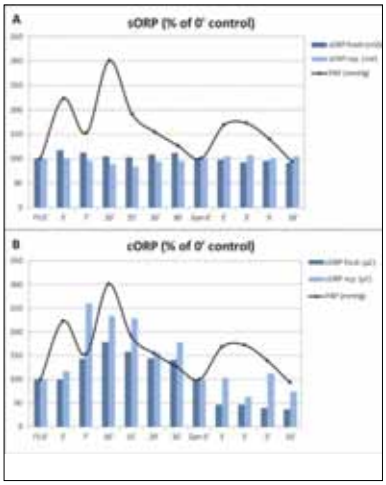

Figure 1. Pulmonary arterial pressure (PAP – mmHg, % of 0' control), actual redox state (sORP – mV, % of 0' control) and antioxidant capacity (cORP – mV, % of 0' control) in response to ferrous nanomaterial (FS) i.v. bolus injection (1 mg/kg-bw) and Zymosan A (Zym) i.v. bolus injection (0.1 mg/kg-bw).

CONCLUSIONS:

The presented pilot study can most easily be rationalized by FS-induced and ROS-triggered compensatory increase of endogenous antioxidants (e.g., metallothioneins) or reductive enzymes (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase) in blood, which may lead to "reductive stress", a phenomenon that by itself might have deleterious health effects via mitochondrial dysfunction[(2)]. Zymosan caused no reductive stress, perhaps because it was administered at a late stage, or because the presence of iron in the NPs is critical in the phenomenon. The consumption of cORP by zymosan, which was consistently seen in other experiments as well, remains to be explained. Our data raise the possibility that ROS could contribute to the cardiovascular stress and other symptoms at least in the case of FeNP-induced CARPA, and point to cORP as a potentially useful endpoint for laboratory assessment of this phenomenon. The study indirectly suggests the possible benefit of antioxidants in the prevention or treatment of Fe-NP-induced CARPA.

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ACKNOWLEDGEMENTS

This research was supported by TECO Medical and the Research and Technology Innovation Found of Hungarian Government (TÉT 13 IL-2-2014-0001). Sincere thanks are due to Marieluise Wippermann for providing the RedoxSYS system with sensors and Maria Velkei for her excellent laboratory assistance.

MODELING BREAST CANCER WITH TRIDIMENSIONAL BIOMIMETIC SCAFFOLDS: EFFECT OF THE MICROENVIRONMENT ON HYPOXIA RESPONSE, MIGRATION, PROLIFERATION, AND DRUG RESISTANCE

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The biology of tumors cannot be fully investigated considering solely the cellular and molecular features of cancer cells. The essential role that the microenvironment plays in several tumor processes, as progression, metastasis initiation and therapy response, is often underestimated because the study of cancer biology is based on culturing cells on bidimensional plastic substrates that fail to reproduce tumor native microenvironment. To address this limitation we established an ex vivo breast cancer model based on a three dimensional collagen scaffold mimicking the biological and physical structure of native breast tissue. Our hypothesis is that in this context, cancer cells show features closest to those of *in vivo* growing tumors, increasing the predictive potential of *in vitro* studies. We characterized proliferation rate, viability, senescence, migration and expression of biomarkers involved in aggressiveness, cell-ECM signaling and response to hypoxia. We compared the data with those obtained for standard monolayer cultures and with breast tumors implanted *in vivo* and demonstrated that cancer cells in

the 3D scaffolds showed the same behviour of cells in the native tumor environment. We showed that tumor cells had an altered growth profile, extended proliferation phase, migrated toward non hypoxic areas overexpressing the cytoskeleton contractility mediator RHO GTPase and the EMT marker Vimentin, or underwent phenotypical changes related to low oxygen tension, mimicking cells in the hypoxic regions of *in vivo* tumors (tumor necrotic core). The 3D-cells showed the activation of HIF-1 alpha signaling pathway, and enhanced the expression of the angiogenesis mediator VEGF and the glycolytic enzyme GAPDH, the same metabolic switch described in the Warburg effect. Finally, cells in this 3D model proved to be less sensitive to chemotherapeutic agents than the monolayer cultures, allowing for the onset of drug resistance in a subset of the cell population. We believe that our bio-inspired system will allow for a more physiologic characterization of cancer cell behavior as a function of microenvironmental conditions.

LEUKOLIKE TECHNOLOGY TO AVOID NANOPARTICLE RECOGNITION BY THE IMMUNE SYSTEM

ENNIO TASCIOTTI

The development of targeted cancer treatments with increased therapeutic efficacy is still a major challenge in drug delivery. A multitude of micro- and nano-particles has been developed to control the transport of systemically administered pharmaceuticals. Regardless of their chemical features, they are all subjected to a number of biological barriers that limit their optimal biodistribution. Bio-inspired approaches emerged as an alternative solution to address the complexity of biological barriers by offering a one-step solution to simultaneously confer the ability to overcome multiple biological barriers.

Inflammation plays a key role in several traumatic and pathological conditions and it has been associated with the onset of cancer, cardiovascular, autoimmune and metabolic diseases as well as with the failure of implants and the delay of tissue healing after injury. The inflammatory signaling cascade occurs both locally and systemically through the interaction of native cells with the components of the immune system, and offers ideal molecular and cellular targets to identify the areas of cell/tissue/organ malfunction.

In response to these observations, we developed nanomaterials with unique biomimetic features able to recognize and target tissue inflammation, to imitate the composition of immune cells and to recapitulate some of the function of these cells. In particular, the physiology of circulating immune cells and their involvement in the inflammatory process inspired us to develop injectable carriers with biomimetic functions.

Leukocytes freely circulate in the bloodstream and accumulate in the diseased tissue through the selective interaction with the inflamed vasculature. We demonstrated how leukocyte membranes could be manipulated to obtain a proteolipid material to formulate a biomimetic drug delivery system, the Leukosome, that combines the leukocyte's ability to escape immune surveillance and selectively target the inflamed vasculature, with the liposome's physical features, and the capacity to load, retain and release a cadre of different payloads. Compared to unmodified liposomes, Leukosomes showed 10 fold increase in circulation time, 50 fold reduction of liver accumulation and up to 100 fold accumulation of the payload in breast and melanoma tumor models in mouse.

The high versatility of this approach suggests that the leukosomes might be an effective delivery platform also for the treatment of a broad range of disorders that have low therapeutic alternatives (e.g. rheumatoid arthritis, cancer, inflamed bowel diseases) but share the same inflammatory background, thus offering the opportunity to develop novel forms of therapeutic intervention.

SELIGO™, A NEW CLASS OF CUSTOMIZABLE AFFINITY REAGENTS FOR DIAGNOSTICS AND THERAPEUTICS

JOHN THORNBACK, Managing Director, Apta Biosciences Pte Ltd, Singapore (SGP)

Apta Biosciences is developing novel wholly synthetic DNA-amino acid hybrid molecules called Seligo that bind to proteins by a protein to protein interaction.

These next generation affinity reagents have potential applications in the fields of research, diagnostics and biotherapeutics.

Seligo are

- Small (10-25kDa) DNA-amino acid hybrid molecule
- Engineered to display a peptide binding sequence
- High affinity and specificity to a specific protein target
- Fully synthetic, GMP manufactured
- Create libraries with diversity >1013
- Site specific modification and of dyes, radioactivity and cytotoxics

In this presentation the concept of Seligo will be discussed along with the process of selection from the random libraries to nanomolar affinity specific reagents.

Examples of the application of Seligo to the field of infectious disease detection and treatment will be given.

SYSTEMATIC ENGINEERING OF DENDRIMER CNDPs PRODUCES NEW EMERGING PROPERTIES CRITICAL TO NANOMEDICINE

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Systematically engineering the critical design parameters (CNDPs) for dendrimers (i.e., size, shape, surface chemistry, flexibility/rigidity, architecture and elemental composition) has led to a multitude of unprecedented dendrimer properties 1 (Figure 1).

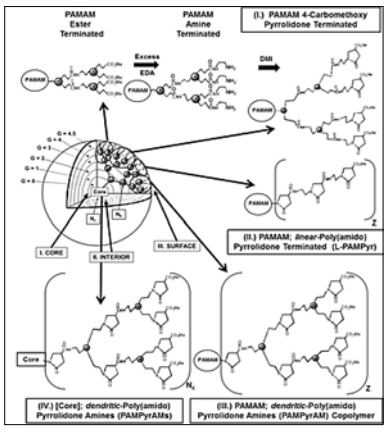

Figure 1. Engineering PAMAM dendrimer CNDPs such as: surface chemistry, architecture and interior composition.[1]

These new emerging properties are now providing many new therapy options,1 strategies 2 and applications in nanomedicine.3-5 Furthermore, it is now widely recognized that CNDPs for essentially all well-defined, Hard/Soft nanoparticles will broadly influence and literally define highly predictive nanoperiodic property patterns.6, 7 These new emerging properties and highly predictive nanoperiodic property patterns may now be used to develop new nanotherapy strategies, as well as to guide more effective nanoparticle optimization toward higher clinical success results.

This satellite entitled: Advanced Dendrimer Based Results in Medicine will overview many important new advances/applications in nanomedicine that have resulted as a consequence of systematically engineering these six - CNDPs associated with dendrimers.

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GENOME-WIDE TARGET-ID & VALIDATION TECHNIQUES FOR NOVEL DRUG DISCOVERY

CHRIS TORRANCE

In several disease areas, the rate of increase in our understanding of disease causation is impressive and is not now a bottle-neck to developing new and more effective therapeutic options. In theory, such key information on what a good biological target is, should start to accelerate the development of novel 'targeted' or 'personalised' medicines, especially now the human genome can be edited almost at will, at least *in vitro*.

In practice, however, technical barriers still significantly limit our ability to: 1) Design conventional small-molecule drugs to the majority of disease-driving targets; and 2) Deliver therapeutic levels of biological drugs, such as antibodies and genome editing vectors, to disease targets that invariably reside inside of a cell, *in vivo*.

Faced with these challenges for emerging biologics, there is still an important need to find new 'druggable' target space for conventional small-molecule drugs. This talk will explore how genome-editing and proteomic techniques, which work well *in vitro*, can also aid the design of small chemical agents that typically work well *in vivo*.

PRINTABLE BIO-ELECTRONICS FOR FUTURE POINT- OF CARE APPLICATIONS

LUISA TORSI, 1Dipartimento di Chimica, Università degli Studi di Bari "Aldo Moro", Bari, Italy, E-mail: luisa.torsi@uniba.it

Point-of-care (POC) biosensors are integrated diagnostic systems employed for the detection of clinically relevant analytes in biological fluids such as blood, urine and saliva. These devices offer the advantage to provide rapid results directly where the information is needed (e.g. patient's home, doctor's office or emergency room), thus facilitating an earlier diagnosis and a prompt patient's treatment. Various technologies have been proposed for the realization of POC biosensors including label-free techniques based on optical, mechanical and electrochemical transducers. However, reliable, quantitative and ultrasensitive devices have been not yet commercialized. Electronic biosensors based on organic thin-film transistors (OTFTs) are a promising choice for the development of the next generation of POC devices. These biosensors can be combined with integrated electrical circuits, microfluidic systems and wireless technologies. Furthermore, they offer high sensitivity, biocompatibility and possibility to produce all-printed low-cost biosensors in flexible and disposable formats. Among them, electrolyte-gated (EG)-OTFTs have been identified as ideal candidates for biosensors development as they operate at low voltages directly in aqueous buffer solutions. Using these configurations ultrasensitive label-free immunosensors for the detection of C-reactive protein (CRP), a specific biomarker of inflammatory and infection diseases, at the femtomolar concentration level have been developed. The devices are also able to perform chiral differential detection of odorant molecules. The specific features of the proposed EGOTFT biosensors as well as their analytical performances will be discussed.

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COSMOPHOS INITIATIVE

PANAGIOTIS (PANOS) N. TROHOPOULOS
E-mail: panagiotis.trohopoulos@cosmophos.com

The CosmoPHOS-nano Project (GA 310337) is a Large-scale EU FP7 NMP Funded Translational Nanomedicine R&D Project in Cardiovascular Diseases, and more specifically in Atherosclerotic Heart Disease. The Project co-funded by the European Union under the FP7 Programme/NMP Theme (Nanosciences, Nanotechnologies, Materials and New Production Technologies) with 8,5 Million Euros, and additionally co-funded by All Project Beneficiaries with 4,5 Million Euros, having a total project budget of 13 Million Euros.

Dr med Panagiotis (Panos) N. Trohopoulos, a Distinction of Excellence Greek (Ellin) Medical Doctor – Cardiologist, is the Founder (12 years ago, since 2004) and the Scientific/Exploitation/Strategic Coordinator of the EU FP7 NMP Funded Large-Scale CosmoPHOS-nano Project (GA 310337), which is a Multidisciplinary Five-year R&D Project started on March 1, 2013 and will be concluded on February 28, 2018, and consists of 19 World-Class Participants, including 13 Universities and Research Foundations and 6 Companies, from 11 European Countries, Japan, and USA, with a wide variety of complementary and cutting-edge scientific, technological and manufacturing expertise and know-how. The EU FP7 NMP Funded Large-Scale CosmoPHOS-nano Project (GA 310337) is the World's Largest R&D Project of Nanomedicine in Cardiology aiming to develop a Radical Innovative Theranostic (Diagnostic and Therapeutic) "Smart" Nanomedicine Product, the CosmoPHOS System, which enables:

- A. Near-Infrared Fluorescence-based Molecular In Vivo Imaging (NIRF-based Molecular In Vivo Imaging);
- B. Targeted Near-Infrared nanoPhotodynamic Therapy (Targeted NIR nanoPDT); and
- C. Real-time and Follow-up Therapy Monitoring;

of Atherosclerotic Coronary Artery Disease (CAD) of the Heart which causes the myocardial infarctions (heart attacks) and is the number one cause of human death and morbidity in Europe and worldwide. The CosmoPHOS System is anticipated to significantly reduce the number of deaths and the morbidity caused by CAD. This is forecast to result in a significant decrease of the European and Global Healthcare Costs caused by CAD, increase the income of the European Healthcare Industry from CAD market which is the global largest, and alleviate the European and Global Society.

The CosmoPHOS-nano Project (GA 310337) is the First EU FP7 NMP Funded Large-scale R&D Project planning to apply Nanomedicine for Cardiac Patients. It foresees conducting during the final Project-year, a First-in-man Phase-I Clinical Trial in CAD Patients, to evaluate the safety and feasibility of the novel CosmoPHOS System for human use.

The CosmoPHOS System comprises of three interacting components:

- 1) Theranostic Nanoparticles;
- 2) Therapeutic Medical Devices; and
- 3) Imaging Medical Devices.

The CosmoPHOS System addresses mainly, but not only, the Vulnerable Atherosclerotic Plaques with main focus in Atherosclerotic Coronary Artery Disease (CAD) of the Heart and Atherosclerosis in general, but also addresses other Diseases as well.

The CosmoPHOS Ltd, an Innovative European SME for Translational Nanomedicine based in Thessaloniki, Greece (Ellas), is the Commercialization Cornerstone of the EU FP7 NMP Funded Large-Scale CosmoPHOS-nano Project (GA 310337) with the Strategic Mission to commercialize the CosmoPHOS System worldwide.

POLYMERIC MICRO-CANTILEVER ARRAYS FOR DETECTING DNA FRAGMENTS AND BIOLOGICALLY RELEVANT METAL IONS

PRABITHA URWYLER

The invention of atomic force microscopy spurred the development of micro-cantilever-based sensors. Their applications in biomedicine require disposable, low-cost cantilevers for single usage. Polymeric micro-cantilever arrays might be a beneficial alternative to the established silicon-based microstructures. This study demonstrates that injection-molded polymeric micro-cantilever arrays have characteristics, which compare reasonably well to silicon ones and permit the quantification of medically relevant species.
Using variothermal polymer micro-injection molding, disposable arrays of eight polymer micro-cantilevers each 500 µm long, 100 µm wide and 25 µm thick were fabricated. The present study took advantage of an easy flow grade polypropylene. After gold coating for optical read-out and asymmetrical sensitization, the arrays were introduced into the Cantisens® Research system to perform mechanical and functional testing. We demonstrate that polypropylene cantilevers can be used as biosensors for medical purposes in the same manner as the established silicon ones to detect single-stranded DNA sequences and metal ions in real-time. A differential signal of 7 nm was detected for the hybridization of 1 µM complementary DNA sequences. For 100 nM copper ions the differential signal was found to be (36 ± 5) nm.
Nano-mechanical sensing of medically relevant, nanometer-size species is essential for fast and efficient diagnosis. Rather simple further adaptations to the fabrication process will allow an easy tailoring for their application in other systems. It may result in dedicated bedside systems for the benefit of patients.

TRANSLATIONAL OPTICAL MOLECULAR IMAGING: FROM EXPERIMENTAL TO CLINICAL PHASE III EVALUATION IN ONCOLOGY

GOOITZEN M. VAN DAM, MD, PhD, Professor of Surgery, Nuclear Medicine and Molecular Imaging and intensive Care
University Medical Center Groningen

The main focus of the talk will be an overview of translational optical molecular imaging in various solid tumors: breast cancer, colorectal cancer, esophageal and pancreatic cancer. In particular based on the initial clinical study using a folate-receptor alpha targeted imaging approach in ovarian cancer, more recent developments using therapeutic antibodies, nanobodies, small peptides and smart-activatable probes for intraoperative imaging and drug development will be presented for each tumor type in patients. Newly developed Standard Operating Procedures from the operating theatre into the pathology department will be showcased in order to validate and cross-correlate data from multicenter studies and per tumor type. Besides tumor detection, new developments for treatment of (microscopic) residual disease will be presented as based on targeted photodynamic therapy. In particular the aspect of fluorescence imaging into the workflow of the pathologist will be outlined and showcased with clinical studies.

RECENT DEVELOPMENTS IN MEASUREMENT FOR NANOMEDICINE

HANS VAN DER VOORN

In nanomedicine size matters, the number of particles matters and the particle surface properties matter. As nanomedicine products evolve out of university research groups to detailed development, clinical testing and clinical use, the quality and discipline around measurement and analysis becomes increasingly critical. Measurement technology has developed to meet this need and extremely accurate measurements can now be done quickly and easily.
TRPS in particular offers the level of detail and certainty that the medical world requires. Recent developments include the measurement of very small differences in DNA binding structures by the analysis of individual particle electrophoretic mobility through a pore. Zeta potential is typically derived from the measurement of bulk electrophoretic mobility but there are other factors at work which mean that the traditional assumptions need to be re-evaluated.
The extracellular vesicle research community is beginning to make advances in a new kind of nanotherapeutics using extracellular vesicles, mainly exosomes in a number of different configurations including gene therapy, drug delivery and stem cell derived exosomes as the therapeutic agent. These are biological particles, typically more heterogeneous than synthetic particles and typically more adapted for survival in the body than synthetics such as liposomes. The need for precise isolation and measurement of these nano-bio structures provides some additional challenges which TRPS is being developed to meet.

TRANSPORT, DEGRADATION AND DRUG RELEASE MECHANISMS OF NANOMEDICINES

PETER VAN HOOGEVEST, Lipoid GmbH, Ludwigshafen, Germany

The fate of liposomes, as model and reference nano-systems, and their drug release characteristics after subcutaneous (s.c.) and intramuscular (i.m.) administration is discussed. The subcutaneous or intramuscular injection of nanomedicines is, within the field of parenteral administration, compared to intravenous administration less explored. The s.c. and i.m. routes of administration may, however, be of particular interest for targeting of nanomedicines to lymph nodes. The lymphatic system plays a crucial role in the immune system's recognition and response to diseases, and most solid cancers initially spread from the primary site via the tumor's surrounding lymphatics before hematological dissemination1. Hence, the lymphatic system is an important target for developing new vaccines, cancer treatments, and diagnostic agents. In addition, the s.c. and i.m. routes of administration are of importance for depot injectables with a slow release profile of a drug with short biological half-life.
For lymph node targeting especially smaller liposomes (< 300 nm diameter) are suitable, since it is demonstrated that smaller liposomes are partially drained from the subcutaneous injection site into the lymphatic circulation in a size dependent manner2. Such liposomes, loaded with antigens and/or immune-modulators can be used for vaccination purposes (probably by targeting to macrophages located in the lymph node) as adjuvant and after loading with a suitable marker they can be used for lymph node imaging3. The liposome products presently clinically tested are reviewed.
In order to achieve a depot effect, it is necessary that the liposomes are not immediately removed from the subcutaneous or intramuscular injection site. In particular large liposomes (> 20 µm diameter) or viscous liposome formulations are suitable for this purpose, able to release the encapsulated water soluble drug over a period of several days. At present, the DepoFoam® technology using multivesicular liposomes is an established depot formulation approach as underscored by a few marketed products.

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NANOMEDICINE FOR OPHTHALMIC DISEASE- 2 CASE STUDIES FOR TRANSLATION

SUBBU VENKATRAMAN, Director, Ocular Therapeutic Engineering Centre, Nanyang Technological University, Singapore
Director, NTU-Northwestern Institute for Nanomedicine

Very few sustained-release Nanomedicine products have been approved yet, primarily because cancer Nanomedicine does not require sustained release. It is difficult to achieve sustained delivery of more than a few days using diffusion control from nanoparticles. In this talk we outline translational efforts in sustained Nanomedicine for the eye, using two entirely different carrier systems. Nanomedicine translation appears to be slower than other forms of translational therapeutics, as shown in this 2013 summary:

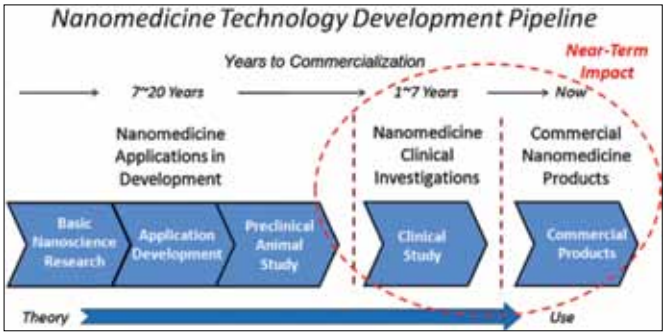

Fig 1: From concept to commercialization, the average timeline for a Nanomediciine product[1]

One case study from our own academic experience will be presented on the development and therapeutic evaluation of a liposomal nanocarrier for sustained release of latanoprost, for treatment of glaucoma[2]. This experience yields some lessons on how we can accelerate the speed of pre-clinical studies, and bring nanomedicine products useful for patients to human studies in a relatively short period. The pre-clinical phase can be greatly accelerated by judicious inter-disciplinary interactions and feedback. Additionally, feedback from potential patients is essential for design iteration. Early human trials are critical to obtaining funding for larger clinical trials; subsequently, a spin-off company Peregrine Ophthalmic) was founded in Singapore[3].

Fig 2: Picture of a latanoprost-loaded Nanoliposome that sustains efficacy of action over 3 months with a single sub-conjuctival injection

In another example, we highlight the use of layer-by-layer particles for sustained action of an siRNA against fibrosis in the eye, often a problem that limits the success of glaucoma filtration surgery. Compared to nanoliposomes, it is even more difficult to use polyelectrolyte layers to control release of drug from coated particles. We have shown that only when the core particle sizes are in the nanometer range that a layer-by-layer coating (with at least 3 layers) can control the release of drug from the core. In using LbLNPs to deliver siRNA we have made use of two factors: first, the siRNA itself is charged and can form one or more of the polyelectrolyte layers, and thus increase loading; and secondly, the outermost layer can be positively charged to facilitate cellular entry. Using this approach, we show that the combination of cellular penetration, intra-cellular survival and slow release of siRNA exhibit sustained anti-scarring action for 2 weeks.

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CELLULAR RESPONSES TO GRAPHENE OXIDE SHEETS: EFFECT OF LATERAL DIMENSION AND THE OXIDATIVE STRESS PARADIGM

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The adverse responses to nanomaterials could be correlated with their structural characteristics and explained by the oxidative stress paradigm. Increased levels of ROS, due to the exposure to nanomaterials, activate cellular anti-oxidative defence and pro-inflammatory pathways, eventually leading to cellular death. In the present study we determined whether the lateral dimensions of endotoxin-free graphene oxide (small GO <1 μm compared to the large GO >1 μm) influenced cellular responses of human lung epithelial cells (Beas-2B). Our second aim was to determine whether the observed response was in agreement with oxidative stress paradigm.
ROS production was assessed using two techniques: hydroethidine oxidation (HE) by flow cytometry and dichloro-dihydro-fluorescein diacetate (DCF-DA) oxidation measured by fluorescence plate reader. We found a dose-dependent increase of ROS levels in correlation with the lateral size of the material. Furthermore, we show the activation of pro-inflammatory pathways involving increased expression of IL-6 and IL-8 genes, analysed by PCR, after treatment with the large GO material.
Cellular death was analysed using modified lactate dehydrogenase (LDH) assay and Propidium iodide/Annexin V staining by flow cytometry. A dose-dependent toxicity was observed, with toxic response being more pronounced for the large material. Agglomeration of the GO, modulated by dispersing the material in the presence or absence of serum, was also shown to increase the ROS production, induce pro-inflammatory response and cellular death. In conclusion, we show that cellular responses to exposure of cells with GO is dependent on the size of the 2-dimensional sheets. Small and well-dispersed GO sheets had no adverse effects on cellular activity. On the other hand, more agglomerated and large GO sheets could induce the production of ROS and subsequently impair cellular activity significantly. The obtained data should assist in “safe-by-design” fabrication of 2D materials in relation to their lateral dimension.

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FILLED AND FUNCTIONALISED RADIOACTIVE NANOCAPSULES TOWARDS TARGETED RADIO-THERAPY IN VIVO

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INTRODUCTION

Carbon nanotubes (CNTs) have been exploited as delivery systems for theranostic applications[1]. Their unique needle-like structure enables cell penetration capacity[2, 3]. Moreover, the payload can be increased either externally by chemical modifications on the largely available surface, or internally by filling into the interior space[4]. We have previously developed targeted and functionalised radioemitting carbon nanocapsules for *in vivo* imaging[5]. This pioneer work demonstrated the use of high-density iodine-125 (^{125}I)-filled and glycosylated CNTs for ultrasensitive imaging. The current study employed a modified approach to construct therapeutically active and functionalised nanocapsules. Both single-walled and multi-walled CNTs (SWNTs and MWNTs) were filled with non-radioactive samarium-152 (^{152}Sm) in the form of $^{152}Sm_2Cl_3$[6]. Following neutron irradiation, chemical functionalisation was carried out to covalently conjugate EGFR-targeting antibody onto CNT surface[7], yielding tumour-targeting therapeutic ^{153}Sm-filled CNT nanocapsules (^{153}Sm-CNT-Ab). The tissue biodistribution profiles were assessed by 3D-single photon emission computed tomography/computed tomography (SPECT/CT) imaging and quantitative γ-scintigraphy, following intravenous (i.v.) injection. The therapeutic efficacy was examined using a syngeneic murine B16F10-Luc-EGFR melanoma lung metastasis model.

RESULTS

SPECT/CT imaging and quantitative γ-scintigraphy showed both ^{153}Sm-CNTs accumulated mostly in spleen, lung and liver up to 24 h post i.v. injection (Fig. 1). Taking advantage of the prominent *in vivo* uptake in lung, the radio-therapeutic efficacy of ^{153}Sm-CNT-Ab was challenged using a B16F10-Luc melanoma lung metastasis model. The luciferase-expressing cells enabled tumour growth monitoring by whole body bioluminescence imaging. The results demonstrate that single i.v. administration of ^{153}Sm-MWNT-Ab containing ~20 MBq of radioactivity (200 µg of CNTs per mouse) significantly inhibited the tumour growth in lung (Fig. 2). Significant tumour growth reduction was measured from Day 10 (6 days after CNT injection) until the experimental endpoint compared to untreated mice (**p<0.01). Representative bio-luminescence images of untreated and ^{153}Sm-MWNT-Ab treated mice on Day 13 and Day 17 post-tumour inoculation showed lower signals detected from ^{153}Sm-MWNT-Ab.

CONCLUSIONS

This work presented a novel approach to produce radiotherapeutical ^{153}Sm-filled CNTs following neutron irradiation. Further surface functionalisation with antibodies yielded a single nanocapsule, capable of *in vivo* targeting, imaging and therapy.

FIGURES

Fig. 1: Whole body SPECT/CT imaging and tissue biodistribution of ^{153}Sm-SWNT and ^{153}Sm-MWNT in mice up to 24 h. (A) SPECT/CT imaging (B) Tissue biodistribution profiles. Normal C57BL/6 mice were i.v. injected with 200 µg of ^{153}Sm-SWNT or ^{153}Sm -MWNT containing 10 MBq or ~1 MBq for SPECT/CT imaging or γ-scintigraphy analysis respectively. The radioactivity of blood and major organs sampled at specified time points were measured by γ-scintigraphy. The results are expressed as %ID/g of organ and presented as mean ± S.D. (n=3-4).

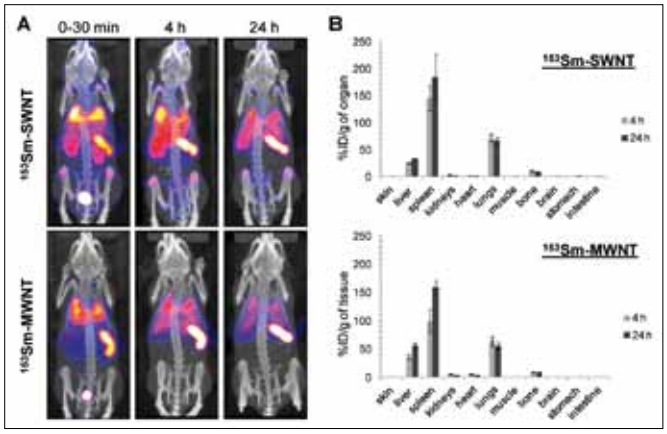
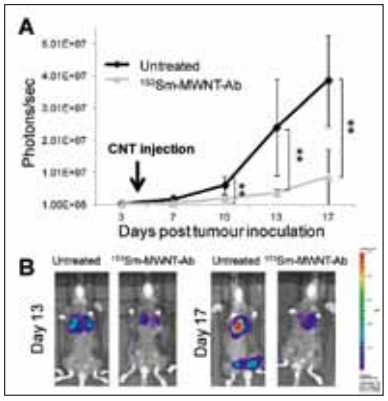


*Fig. 2: In vivo radiotherapy studies of ^{153}Sm-MWNT-Ab in tumour-bearing mice. (A) Tumour growth monitoring over the therapy time course. (B) Representative whole body images of untreated and ^{153}Sm-MWNT-Ab treated mice captured on Day 13 and Day 17 post-tumour inoculation. Bioluminescence signals correspond to luciferase expressing B16F10-EGFR cells in the lung. B16F10-Luc-EGFR tumour-bearing C57BL/6 mice received a single dose i.v. injection of ^{153}Sm-MWNT-Ab (20 MBq, 200 µg) on Day 4 post-tumour inoculation. Results are presented as mean ± S.D. (n=6-9). Significant differences were examined using one-way ANOVA following by Tukey's multiple comparison test. (**p<0.01)*



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ASSESSING EQUIVALENCE OF COMPLEX DRUG PRODUCTS CONTAINING NANOMATERIAL: UPDATE FROM FDA

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FDA and CDER continue to foster innovation and the responsible development of drug products containing nanomaterials. Current review practices and regulatory framework are capable of detecting and managing the potential risks to quality, safety and efficacy due to nanomaterials in drug product. As the nanotechnology field continues to mature, the complexity of nanomaterials within drug products is expected to increase. To address this increasing complexity the Agency is building scientific networks within and outside FDA to enhance regulatory science collaborations and define principles in standardization so that critical quality attributes are linked to clinical performance. This presentation will include updates on emerging trends and contain recommendations for sponsors to guide pre-submission and application activities, dialogue and strategies with consideration of generic drug products containing nanomaterials.

THE BRAIN SHUTTLE: DELIVERING BIOLOGICS ACROSS THE BBB

BARBARA WEISER

Although biotherapeutics have vast potential for treating brain disorders, their use has been limited due to the limited penetration across the blood-brain barrier (BBB) and resulting low brain exposure. This talk will describe a brain delivery technology, which can be engineered onto standard therapeutic antibody and other types of biologics for successful BBB transcytosis.

We have developed a Brain Shuttle module by manipulating the binding mode of an antibody fragment to the Transferrin Receptor (TfR). The Brain Shuttle version of an anti-Abeta antibody, which uses a monovalent binding mode to the TfR, increases beta-Amyloid target engagement in a mouse model of Alzheimer's disease. We provide *in vitro* and *in vivo* evidence that the new TfR binding mode facilitates transcellular transport, while a classical binding mode leads to lysosome sorting. Enhanced target engagement of the Brain Shuttle module translates into a significant improvement in amyloid reduction. These findings have major implications for the development of biologics-based treatment of brain disorders. The Brain Shuttle technology could potentially transport all types of therapeutic molecules into the brain, regardless of their intrinsic ability to cross the blood brain barrier.

SIMULATION OF PEPTIDES IN MEMBRANES AND TOXICITY PROFILES OF GOLD NANOPARTICLES

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We have recently developed very efficient methods to simulate the structure formation and function of peptides in membranes, which I will discuss in two contexts: We propose a novel concept for the folding and self-assembly of the pore-forming TatA complex from the Twin-arginine translocase and of other membrane proteins, based on electrostatic "charge zippers", which led to a novel functional understanding of the translocation processes of fully folded proteins through membranes[1]. To further our understanding of the mechanism of toxicity of nanomaterials to be used in drug delivery we investigated toxitiy profiles for ultrasmall (1.4 nm) AuNPs on the electrophysiology of HEK 293 cells expressing hERG, a standard benchmark for drug safety, depending on ligand composition. In patch clamp experiments phosphine-stabilized AuNPs irreversibly blocked hERG channels, whereas thiol-stabilized AuNPs of similar size had no effect *in vitro*, while neither particle blocks the channel *in vivo*[2]. We conclude that safety regulations may need to be re-evaluated and adapted to reflect the fact that the binding modality of surface functional groups becomes a relevant parameter for the design of nanoscale bioactive compounds.

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SUPER STEALTH LIPOSOMES FOR DRUG DELIVERY

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Stealth polymers, such as polyethylene glycol (PEG), are frequently used for drug delivery applications to reduce nanoparticle opsonization and subsequent clearance by the mononuclear phagocyte system. However, the shielding effect of these polymers is usually incomplete and transient, due to loss of nanoparticle integrity upon intravenous administration. In this study, we have developed super stealth liposomes that incorporate unique PEG-dendron-phospholipid constructs. Specifically, these constructs consist of a β-glutamic acid dendron anchor that attaches a PEG chain to four phosphoethanolamines, as opposed to conventional stealth liposomes that have a PEG chain attached to a single phospholipid. The dendron structure increases the phospholipid/PEG attachment ratio, consequently ensuring a more stable interaction between PEG chains and the vesicular surface. *In vitro* results demonstrated superior liposomal stability compared to conventional stealth liposomes, while *in vivo* results showed prolonged circulation half-life and decreased liver and spleen accumulation. In conclusion, this study demonstrates a novel way of improving the performance of a commonly used polymer in drug-delivery. Moreover, the results suggest that super stealth liposomes have the potential to greatly improve the pharmacokinetics and performance of liposomes.

BIOLOGICAL RECOGNITION OF BIOMOLECULAR CORONA

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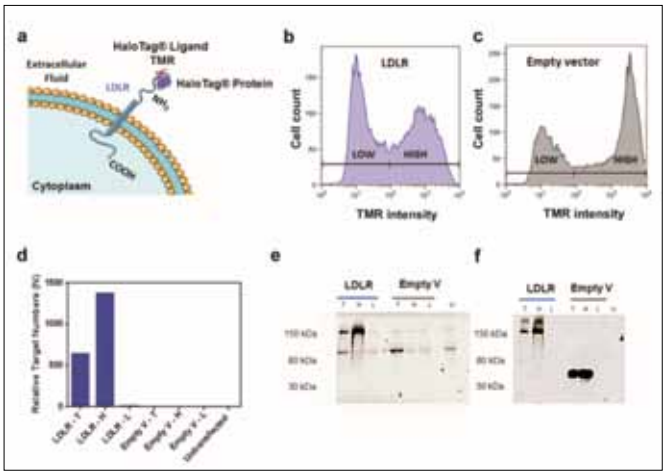
When the nanoparticle surface comes in contact with a biological milieu, adsorption processes may lead to a well-defined surface-assembly of biomolecules derived from the environment. Appropriately presented, and sufficiently long-lived ('hard corona') biomolecular motifs presented at the surface would define how a nanoparticle first interacts with and is recognised by cells[1-5]. Besides their fundamental importance, such questions likely define many key impacts on safety and efficacy of nanoparticle-based therapies, including their bio-distribution and nanomedicine targeting. For instance, well-defined active processes driven by receptors on the specialized

cells of the liver could drive nanoparticle accumulation. However, little is known of the molecular organisation of various proteins in the corona. Hence, the detailed molecular interactions between the presented protein motifs and receptors are yet to be elucidated. Here we present a systematic method to advance such hypotheses, by direct investigation of nanoparticle-receptor interactions in biological milieu. Thus, combining host-cell fusion receptor-protein expression systems (Figure 1) with tools to detect epitope presentation on the nanoparticle surface6, we are able to study specific receptor recognition in appropriate biological milieu. In addition, this approach overcomes the limitations that some receptors cannot be isolated in a functionally relevant form, as their expression levels are interdependent in endogenously expressed cells.

Figure 1. Expression of human LDLR in HEK-293T cells. a) Scheme of LDLR fused with a HaloTag® protein at its N-terminus. The HaloTag® protein forms a covalent bond with a fluorescent HaloTag® ligand, TMR. b) Expression of LDLR-HaloTag® fusion protein in cells transfected with LDLR vector. c) Expression of HaloTag® protein in cells transfected with empty vector. The protein expression was measured by flow cytometry after incubation with TMR. Cells were sorted to two populations (High and Low) based on the TMR intensity. d) Quantification of LDLR mRNA level by RT-qPCR after transfection with LDLR vector in the total cell population (LDLR-T), sorted High TMR (LDLR-H), and Low TMR (LDLR-L) subpopulations, as well as equivalent samples after transfection with empty vector. e and f) Western blot analysis of LDLR expression (MW 95KDa) probed by using anti-LDLR monoclonal antibody (e) and anti-HaloTag® antibody to detect HaloTag® protein fusion (MW 33KDa) (f). T, total cell population; H, subpopulation of high TMR intensity; L, subpopulation of low TMR intensity; and U, untransfected cells.



In this presentation, we will illustrate the recognition of biomolecular corona by a couple of key receptors, low-density-lipoprotein receptor (LDLR) and Fc-gamma receptor I (FcγRI), which are abundant in the liver. Our results suggest that the 'labelling' of nanoparticles by biomolecular adsorption processes allows for ubiquitous nanoparticle multi-pathway involvement in biological processes, which governs the liver accumulation of nanoparticles.

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A "BENCH TO BEDSIDE" NANOPLATFORM TO OVERCOME LOW GEMCITABINE CHEMOSENSITIVITY: PRECISION DESIGN AND PERSONALIZED APPLICATION

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Due to diagnosis difficulty at an early stage, 20% of the patients suffering from pancreatic cancer are considered surgically resectable at the time of diagnosis. On the other hand, 80% of the patients with advanced pancreatic cancer undergo vigorous radiation therapy that provides little benefit to patients; therefore the optimal treatment is chemotherapy. However, the objective response rate is only 9.4% after the current first-line regimen, gemcitabine (GEM) single agent treatment, which indicated that low chemosensitivity against GEM exists in most patients and seriously restricts the therapeutic effect of GEM.GEM has a complex process of metabolism in cancer cells, and there are many mechanisms that can affect GEM cytotoxicity, such as human equilibrative nucleoside transporter-1 (hENT1, the major GEM transporter across cell membrane), deoxycytidine kinase (dCK, the rate-limiting enzyme in the activation of GEM to triphosphate), two subunits of ribonucleotide reductase (RRM1 and RRM2, overexpression of this enzyme induces high level of the dNTP pools which can competitively inhibit cytotoxicity of GEM). To determine key factors associated with the GEM chemosensitivity in clinical treatment, we retrospectively enrolled all consecutive pancreatic cancer patients who received radical operation at Tianjin Cancer Hospital between January 2005 and June 2011. According to whether to accept GEM-based adjuvant chemotherapy after operation, these patients were divided into two groups: non-GEM group and GEM group. Using immunohistochemical evaluation and Kaplan-Meier curves for survival analysis, we found that the low hENT1 protein expression (hENT1^{low}) or high RRM2 protein expression (RRM2^{high}) was significantly associated with worse overall survival in GEM group, however not in non-GEM group (Figure 1). These results suggested that the low hENT1 and high RRM2 protein expression respectively played an important role in poor GEM chemosensitivity in pancreatic cancer.

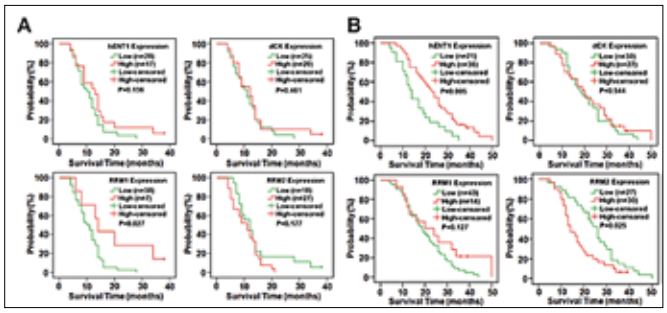

Figure 1. Kaplan-Meier curves were used for overall survival analysis in the non-GEM group (A) and GEM group (B) according to different expression levels of four biomarkers. The P values were obtained using the log-rank test.

To overcome the low GEM chemosensitivity in pancreatic cancer cells associated with hENT1^{low} and RRM2^{high}, we used a DOTAP-

based cationic liposome (approved by the US Food and Drug Administration for clinical trials, NCT00059605) to encapsulate GEM into its hydrophilic core, while the negatively charged siRNA against RRM2 (siRRM2) was absorbed on to the surface of the cationic nanoparticle by electrostatic forces. This nanoparticle exhibited excellent ability for drug delivery to tumor tissue *in vivo*, in particular for siRNA delivery (Figure 2).

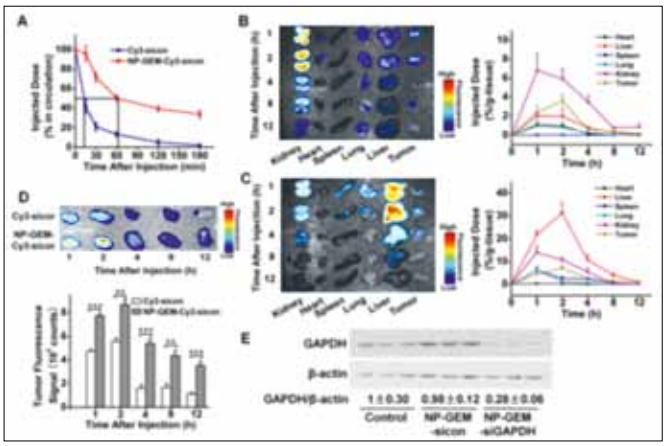

*Figure 2. Characterization of siRNA-delivery activity and knockdown effect in vivo (A) Quantification of sicon circulating in the blood-stream at various time points after intravenous injection of naked Cy3-sicon and NP-GEM-Cy3-sicon. (B, C) In vivo biodistribution of (B) naked Cy3-sicon and (C) NP-GEM-Cy3-sicon upon intravenous administration. (D) Tumor homing effect in vivo. (E) The knockdown effect in vivo. Mice bearing subcutaneous Panc1 xenografts were injected intravenously with saline, NP-GEM-sicon and NP-GEM-siGAPDH. After 48 h, tumors were harvested, and proteins were extracted and studied using western blot. **, P<0.01. ***, P<0.001.*

Next, to evaluate whether our nano-drug formulation can overcome the low GEM chemosensitivity, we used a lentiviral system to create two Panc1 cell phenotypes, that is, one with stable hENT1-specfic shRNA expression (Panc1-shENT1#1) and the second with stable RRM2-overexpression (Panc1-RRM2). The western blot results are represented in Figure 3. Due to the specific uptake pathway of nanoparticles and gene-silencing effect of siRNA, our nano-drug formulation significantly overcame the low gemcitabine chemosensitivity in genetically engineered Panc1 cells with low hENT1 and high RRM2 expression, respectively (Figure 3).

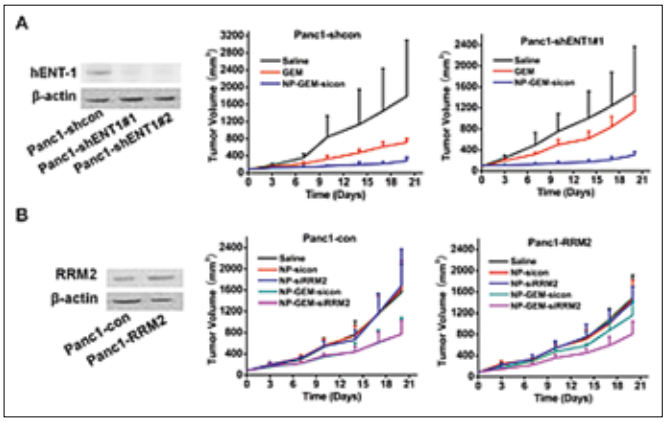

Figure 3. Confirmation of stable hENT1 knockdown in Panc1-shENT1#1 cells (A) and RRM2 overexpression in Panc1-RRM2 cells (B) using western blot. The anti-tumor effect of our nano-drug formulations in Mice bearing subcutaneous xenografts is shown.

To predict the subpopulation of patients who would benefit from our nano-drug formulation, several primary patient-derived pancreatic cancer cells (PDPCs) were employed in this study. Compared to established tumor cell lines, primary patient-derived cancer cells may possess superior power in translating cancer therapeutics from the laboratory to the clinic. Our findings indicated that the enhancement of anti-tumor effect of NP-GEM-sicon related to GEM was higher in PDPCs with hENT1^{low} in comparison to hENT1^{high} (Figure 4). In addition, compared to NP-GEM-sicon, the benefit from NP-GEM-siRRM2 was also correlated with the expression level of RRM2 (Figure 4). These results highlight the importance of personalized application in nanomedicine, and further suggest that the expression level of genes targeted must be detected before application.

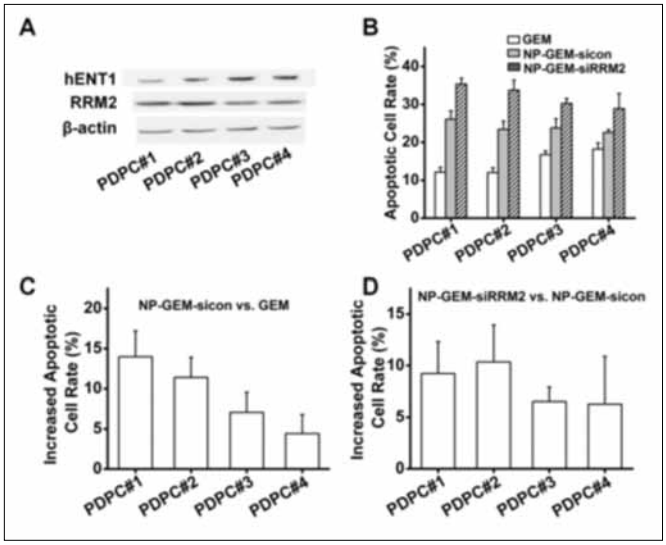

Figure 4. The benefits from NP-Gem-sicon and NP-GEM-siRRM2 were related to the expression levels of hENT1 and RRM2 in PDPCs, respectively. (A) The expression levels of hENT1 and RRM2 in PDPCs were determined by western blot. (B) The apoptotic cell rates after GEM, NP-Gem-sicon and NP-GEM-siRRM2 treatments in PDPCs. (C) The increased apoptotic cell rates after NP-GEM-sicon treatment related to GEM treatment in different PDPCs. (D) The increased apoptotic cell rates after NP-GEM-siRRM2 treatment related to NP-GEM-sicon treatment in different PDPCs.

In summary, we demonstrated that the essential factors of GEM chemosensitivity in pancreatic cancer patients in North China were associated with the expression levels of hENT1 and RRM2. Our synthesized nano-drug formulation provides a method to enhance gemcitabine chemosensitivity, and therefore may potentially be used as a model design for personalized nanomedicine.

CANCER IMMUNOTHERAPY: STRATEGIES FOR PERSONALIZATION AND COMBINATION APPROACHES

ALFRED ZIPPELIUS

Cancer immunotherapy targeting immune checkpoints such as CTLA-4 and PD-1 has shown major successes in multiple cancer types during the last years and is now considered one of the pillars of cancer therapy. Numerous additional immunomodulatory pathways as well as inhibitory factors expressed or secreted by myeloid and stromal cells in the tumor microenvironment are potential targets for complementary therapeutic combinations with immune checkpoint blockade. Given the breadth of potential targets in the immune system, critical questions to address include which combinations should move forward in development and which patients will benefit from these treatments. We provide evidence that the antibody-drug-conjugate (ADC) T-DM1 is particularly effective in eliciting anti-tumor immunity in poorly immunogenic, aggressive malignancies such as HER2-positive breast cancer, rendering it susceptible to CTLA-4/PD-1 blockade. This finding potentially expands the applicability of immunotherapy to HER2-positive breast cancer, but also to a broad range of other cancer types targetable by ADCs. To address this important question, we took advantage of the prospective, multi-center, controlled, phase II/III WSG-ADAPT trial, which is one of the first new generation (neo)adjuvant trials dealing with individualization of (neo)adjuvant decision-making in early

breast cancer. Equally important, we utilized an orthotopic and syngeneic mouse model of human HER2 oncogene-driven mammary carcinogenesis. Although combined CTLA-4 and PD-1 checkpoint blockade did not result in a survival benefit and significantly enhanced anti-tumor immune responses, the combination with T-DM1 resulted in the cure of the vast majority of mice. Mechanistically, the combination was capable to trigger innate and adaptive immunity, as revealed by massive T-cell infiltration, activation, and Th1-polarization in the preclinical tumor model. Tumor rejection in mice treated with the combination therapy was accompanied by a massive increase of CD4+/CD25+/FoxP3+ regulatory T-cells, which are commonly hypothesized to play a causal role in promoting cancer development via the suppression of anti-tumor immune responses. Therefore, unexpectedly, the CD8 to regulatory T-cell ratio, which is widely assumed to increase during successful immune-mediated long-term cure, significantly dropped as compared to the control group. Depletion of regulatory T cells in mice receiving the combination therapy partially decreased treatment efficacy and resulted in severe autoimmunity. Our findings emphasize the importance of understanding how conventional cancer therapies such as targeted agents and standard of care chemotherapeutic agents contribute to the success of cancer immunotherapies, either additively or synergistically, to extend the depth and duration of response. We need to invest into efforts in understanding the effects of these drugs on the tumor immune infiltrate and in identifying strategies for combining these drugs with immunotherapeutic agents to maximize therapeutic efficacy.

INTRATYMPANIC DELIVERY OF NANOMATERIALS FOR THE INNER EAR THERAPY AND THE ASSOCIATED PATHWAY AND SAFETY

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Background: The intratympanic delivery of therapeutics and contrast agents is a currently used approach in clinical practice because systemic adverse effects can be avoided and the method introduces the minimum amount of agents to the individual. Engineered nanomaterials are holding the future in the inner ear therapy due to the high drug loading capacity and molecular targetability. Among these, liposome nanocarrier (LPNs), polymersomes (PMs), and lipid core nanocapsules (LCNs) are major engineered nanomaterials (ENMs) suitable for inner ear therapies.
Materials and Methods: Rats were selected for the evaluations. The delivery methods include standard transtympanic injection, the targeted tympanic medial wall administration using a high-performance polyimide microlumen, and automatic sustained delivery to the middle ear cavity using a miniature osmotic pump. Tropomyosinrelated kinase B (TrkB)-ligand and tetanus toxin-1 (Tet1) peptide were used as targeting molecules. Distributions of the ENMs in the inner ear were observed using confocal microscopy and MRI. Impact of inner ear distribution of the ENMs on the auditory system was analysed using auditory brainstem response (ABR). Impact of the ENMs on the biological barrier function was evaluated using gadolinium-enhanced MRI. Potential glycosaminoglycan accumulation in the cochlea were analysed using periodic acid Schiff's staining light microscopy. Hyaluronic acid secretion and expression of the binding receptors CD44 and toll-like receptor 2 (TLR2) in the cochlea was analysed using immunofluorescent confocal microscopy. Potential changes in the neural elements of neurofilament-200 (NF-200), synaptophysin, and ribbon synapse were analysed using immunofluorescent confocal microscopy. Potential apoptosis in the cochlear cells were evaluated using terminal transferase (TdT) to label the free 3'OH breaks in the DNA strands of apoptotic cells with TMR-dUTP (TUNEL staining).
Results: All the tested ENMs entered the inner ear from the middle ear efficiently administered using all approaches. Both the round and oval windows were involved in the transportation of ENMs. TrkB-ligands-functionalized LPNs displayed targeted distributions in the cochlear modiolus. Tet1-functionalized PMs demonstrated targeted distributions in the cochlear nerve. LPNs did not affect the biological barrier function of the external ear canal skin, middle ear mucosa, and the inner ear. There was no changes in the ABR of rats after LPN administration. There was no accumulations in the glycosaminoglycan and hyaluronic acid in the rat cochlea exposed to LPNs. Administration of LPNs neither change the expressions of CD44 and TLR2 nor induce apoptosis in the rat cochlea. LCNs neither cause hearing loss nor change the expressions of NF-200, synaptophysin, and ribbon synapse in the rat cochlea.
Conclusions: LPNs, PMs, and LCNs are effective nanocarriers for the targeted inner ear therapy. Both the round and oval windows are involved in the transportation of the ENMs from the middle ear into the inner ear. Administrations of LPNs and LCNs to the middle ear are safe for the inner ear and might be used in the clinic practice of Otology.
Keywords: nanomaterials, focal drug delivery, targetability, biocompatibility, hearing, animal, peptide, imaging, pathway, extracellular matrix, receptor

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ABSTRACTS POSTERS

TARGETED ALBUMIN COATED IRON OXIDE NANOPARTICLES FOR PH SENSITIVE TUMOR THERAPY AND DIAGNOSTICS

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During the past decades iron oxide nanoparticles have found many applications in medicine, chemistry and other fields of science. Due to unique combination of magnetic properties, variety of coating, high colloidal stability and biocompatibility such nanoparticles are proposed as potential dual agents for tumor diagnostics by MRI and as drug carriers for therapy. In this work we have developed novel type of water soluble, biocompatible nanoparticles which have shown their efficiency visualization and therapy of brain and breast tumor models on animals.

One of the main factors that affect properties and stability of iron oxide nanoparticles is type of coating that is used for creation of protection layer that prevents aggregation and allows drug immobilization. In our work we have used as a coating serum albumine which was immobilized on surface by adsorption with further crosslinking by glutaric dyaldehyde. Such modification leads to formation of iron oxide nanoparticles with 35-40 nm diameter. Results of HAADF STEM analysis presented on Figure.1 shows that individual nanoparticles are embedded in protein shell and form agglomerates.

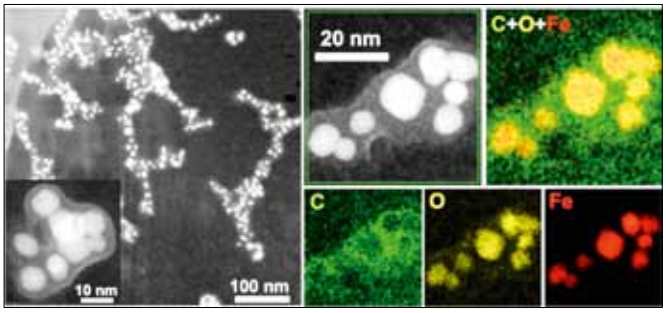

Figure 1. HAADF STEM and EDX analysis of iron oxide nanoparticles coated by serum albumin.

Further investigation revealed that this nanoparticles are stable in PBS buffer system at least for 2 weeks and we haven't observed any change in size and PDI values of suspensions. Toxicity experiments on 4T1 mouse breast cancer, C6 rat glioma and primary rat astrocytes cell lines have shown that iron oxide nanoparticles doesn't show any significant toxicity at concentrations of iron up to 1 mg/ml. T2 relaxivity was measured on ClinScan 7T MR-tomograph and was equal to 160 $s^{-1}mM^{-1}$ which is enough to use tham as a contrast agnet for MRI. To increase delivery potential of nanoparticles we have covalently conjugated to their surface monoclonal antibodies to vascular endothelial growth factor (VEGF) - a protein which is highly expressed on tumor cells. ζ-potential of nanoparticles was equal to - 30±4 mV. This observation in addition to fact that pure albumin in body serves as many xenobiotics and drug carrier allows ous to propose that this nanoparticles can serve not only as a contrast agents for MRI but also as a nanocarrier for amphiphylic positively charged drugs such as doxorubicine. It was shown that loading occurs mainly due to electrostatic interaction between negatively charge surface of nanoparticles and positively charged amine group of doxorubicine. This process leads to increase of surface ζ-potential and loss of colloidal stability of nanoparticles after loading. However with loading values up to 8% no significant changes were observed. Also release of drug from nanocarriers occurs in pH dependable manner. At pH 7.4 during 24 h only 20% of doxorubicine was released, however at pH 6.5 and 5.5 this value reached 65 and 80% respectively.

Experiments on tumor visualization was performed on animals bearing C6 glioma (Figure 2) and 4T1 breast cancer models. In both cases intravenous injection of targeted nanoparticles with following MR - imaging in SWI sequence allowed to clearly visualize borders and body of tumors.

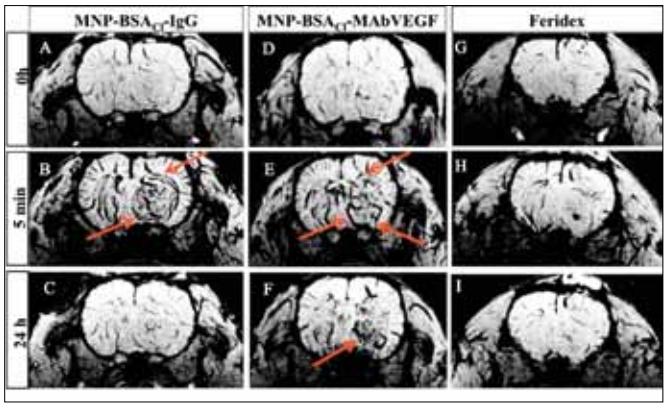

Figure 2. SWI MR images of rats bearing experimental glioma C6 before injection of contrast agents (A, D, G). MR images of gliomas using contrast agents MNP-BSACl-IgG (left column), MNP-BSACl-mAbVEGF (middle column), Feridex (right column): 5 min (B, E, H) and 24 hours (C, F, I) after i.v. injection.

Therapy of 4T1 mouse breast cancer models was also performed. Survival rate for animal received iron oxide nanopartilces conjugated with monoclonal antibodies to VEGF was equal to 39,5 days. On the other hand survival rates for animal that received PBS and pure Dox were 25 and 26 days respectively.

In conclusion we can say that we have developed new type of nanoparticles, which can be used in dual tumor therapy as drug carriers and tumor diagnostics by MRI.

This work was supported by grant of President of Russian Federation MK-6371.2016.7, RFBR grant 16-33-60180 and RSF grant № 14-15-00698 (Nanoparticles synthesis).

DELIVERY OF ANTIBODIES THROUGH BLOOD BRAIN BARRIER FOR EFFECTIVE VISUALIZATION OF CNS ABNORMALITIES

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Contrast-enhanced magnetic resonance imaging is an effective tool to detect brain abnormalities, such as tumor and demyelination. Conjugation of contrast agents with targeting moieties leads to better visualization of pathological lesions. To improve T1-weighted MRI diagnostics of the CNS pathologies we synthesized macromolecular Gd-based contrast agent conjugated with specific antibodies to Cx43 for visualization of glioma or with specific antibodies to GFAP for visualization of demyelination.

We modified poly-L-lysine (PLL) with diethylenetriaminepentaacetic acid (DTPA), and conjugated with mAb to Cx43/mAb GFAP at different molar ratio [DTPA]:[Lys]:[mAb] followed by Gd(III) complexation. Obtained targeted contrast agent had higher T1-relaxivity values (6.5 mM-1s-1) in comparison with commercially available agent Magnevist (3.4 mM-1s-1) as detected by 7T MR-tomograph

ClinScan (Bruker). Cellular uptake of specific T1-contrast agent was more than 4 times higher compared to the non-specific IgG-contrast agent, as detected by flow cytometry and confocal analysis (Figure 1). MRI experiments showed that the obtained agents could markedly enhance visualization of glioma C6 and cuprizone-induced demyelination *in vivo* after its intravenous administration. Fluorescence imaging of brain slices confirmed notable accumulation of specific conjugates in the peritumoral zone (Cx43-contrast agent) or demyelinated area (for GFAP-contrast agent) compared to non-specific IgG-conjugates at 24 h after i.v. injection(Figure 2). Accordingly, T1-contrast agent based on polylysine and specific monoclonal antibody was successfully synthesized and characterized. This agent has a high relaxivity and high affinity to Cx43 and GFAP antigens *in vitro* and *in vivo*.

This work supported by grants of RFBR 16-34-00373 (synthesis of GFAP-contrast agent) and RSF grant №14-15-00698 (MRI scan).)

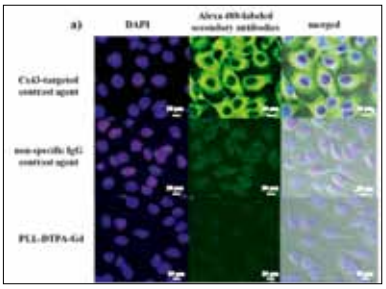

Figure 1. Confocal microscopy of the Cx43-targeted contrast agent on glioma C6 cells.

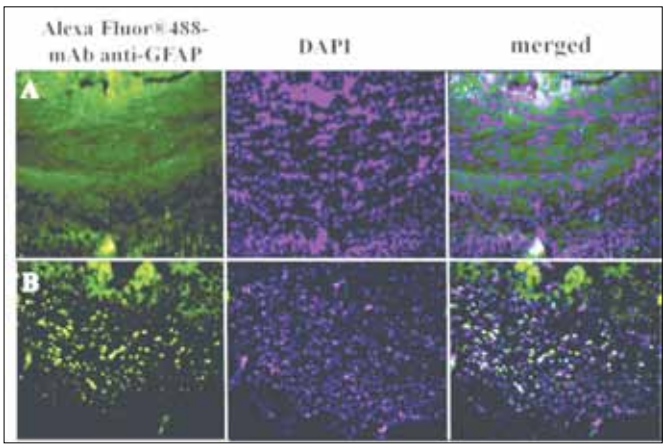

Figure 2. Fluorescent analysis of intravenously injected mAb anti-GFAP-Alexa Fluor®488 in normal (A) and demyelinated (B) corpus callosum.

NON-INVASIVE OPTICAL IMAGING FOR THE VALIDATION OF NOVEL CANCER NANOMEDICINES

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Functional Validation and Preclinical Research (FVPR) is a specific area of CIBBIM-Nanomedicine devoted to the validation of novel nanomaterials with applications in biomedicine. FVPR receives nanomaterials from different laboratories in order to test their safety, efficacy and biological function by using standardized cellular and animal models and with the special aid of non-invasive optical imaging.

Preclinical development of nanotechnology formulated-drugs shares many features with the development of other pharmaceutical products. However, there are some relevant differences that should be carefully studied when planning *in vitro* and *in vivo* proof-of-concept assays. Indeed, nanoparticulated therapeutic systems have challenges related to their production, physicochemical characterization, stability and sterilization, but offer special advantages regarding drug solubilization, bioavailability and biodistribution[1].

Hence, taking advantage of the bioluminescence imaging approaches, at FVPR we have developed novel clinically-relevant animal models which mimic natural tumor progression and metastatic dissemination of several human cancers[2]. Moreover, the *in vivo* tumor-accumulation and whole-body biodistribution of nanomaterials is carried out using fluorescence. Such basic *in vivo* pharmacokinetic and biodistribution assays aid researchers to optimize the design of the nanomedicines, rapidly identifying nanoparticles with good or bad pharmacokinetic profiles. For instance, in case of nanoparticles designed for intravenous administration, aggregation of nanoparticles within the bloodstream causes a significant accumulation of the nanosystem within the lungs and other reticulo-endothelial system (RES) organs such as liver and spleen (Figure 1). Similarly, expected or unexpected excretion of the imaging agent (Figure 1, bottom panel) could also indicate that the nanoconjugate has been metabolized rapidly *in vivo* as we have demonstrated for Gd labeled gold glyconanopaticles[3].

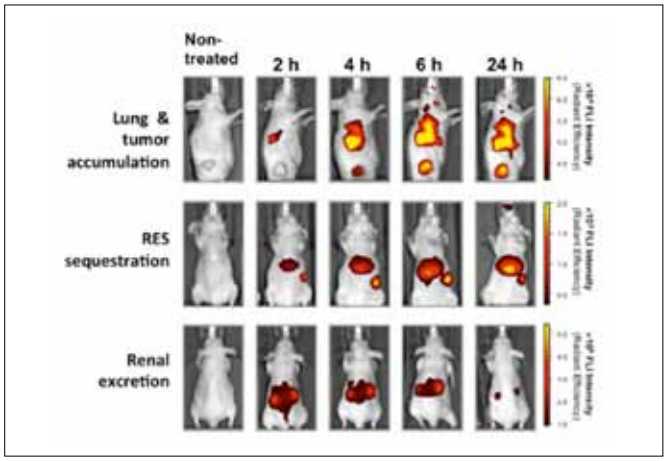

Figure 1. Biodistribution of fluorescently labeled nanoparticles along time in cancer mouse models after intravenous administration. In vivo imaging can rapidly identify nanomedicines accumulating in tumors due to EPR (first row), but can also serve to recognize nanoparticle formulations causing lung aggregates (first row) or high RES sequestration (second row). Nanoparticle metabolization and excretion through hepatic or renal routes (third row) is also easily detected.

In addition, bioluminescence and fluorescence imaging applications also allow the visualization of key processes in tumor progression and treatment response *in vivo*, such as angiogenesis, apoptosis, etc. by means of optically-labeled probes. In conclusion, optical imaging helps to accelerate both academic and pharmaceutical preclinical research across multiple therapeutic and drug discovery areas, providing valuable molecular information to bridge between preclinical and early clinical studies. Importantly, this latter fact emphasizes the importance of having research areas or centers such as FVPR specifically oriented to the biological evaluation of nanomaterials and working in the interphase of material science, molecular biology and clinicians.

ACKNOWLEDGEMENTS:

This work has been partially financed by Fundació Marató Tv3 (PENTRI project 337/C/2013) and Euronanomed research initiative (DiamESTar project). The Spanish Ministry of Science and Innovation supported NG-A and SM as laboratory technicians (Subprograma de Personal Técnico de Apoyo 2013).

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IMMOBILIZATION OF A NS1 DENGUE PROTEIN SPECIFIC ANTIBODY ON A MAGNETIC (FE_3O_4) NANOPARTICLE FOR MAGNETIC PURIFICATION AND IMMUNOLOGICAL DETECTION

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The hyper-endemicity of dengue fever in tropical and sub-tropical zones have led it to become the most common mosquito-borne viral disease in humans. Super paramagnetic iron oxide nanoparticles (SPION) were synthesized and functionalized with a hydrophilic coating. Monoclonal antibodies for the antigen NS1 (DENV-1) were immobilized on their surface. This conjugated complex was prepared as a means to purify and later detect dengue fever virus. The goal of this work was to create the SPION/antigen complex and confirm its antibody binding abilities as means to bind the NS1 antigen and magnetically purify increasing ease of current detection techniques. This research is designed to act as the base from which new diagnostic testing techniques can be developed for dengue and potentially for other diseases as zica and zhincongunya.

INTRODUCTION

The dengue fever is a viral disease spread by Aedes aegypti, a mosquito that lives in both tropical and subtropical areas. Infecting up to 50 million people per annum with 2.5 billion living in areas susceptible to the disease, this virus is an already large global issue.[1] The lack of a vaccine for dengue makes the only preventative measure controlling mosquito populations in vulnerable regions. This creates a crucial need for efficient cost effective diagnosis systems against the virus.[2]

Dengue fever and the more severe case of dengue hemorrhagic fever have dramatically increased since the 1960s. Once an individual has contracted one of these diseases, their symptoms may include: high fever, severe headache, pain behind the eyes, muscle and joint pains, nausea, vomiting, swollen glands and rash. These symptoms can last for up to one week and usually occur between 4-10 days after being bitten. The disease proliferates in tropical/subtropical regions.[7]

Using these diagnostic techniques in DENV surveillance of mosquito vectors is an expensive task requiring special reagents, equipment and laboratory facilities. There is a current demand for a simpler process that would require less instrumentation and trained professionals. The NS1 a nonstructural RNA binding protein of the dengue viral genome in conjunction with a magnetic-nanoparticle antigen complex demonstrates great potential to provide this diagnostic edge.

Magnetic-nanoparticle conjugated with NS1 particles opens up new opportunities of manipulation and separation. The synthesis of MNPs (specifically magnetite in our case) normally follows one of three synthesis reactions: a co-precipitation starting from a mixed F_eSO_4/ F_eCl_3, a reduction–precipitation route, or an oxidation–precipitation method.[11] The use of bifunctional cross-likers (dextran, albumin or polyethylene glycol) and methods of bio-conjugation allows biomolecules to be covalently bonded to the surface of the MNP.[10,12] To confirm surface coverage by the biomolecule along with purity, capillary electrophoresis is performed. This technique allows small samples to be tested due to its high sensitivity.[10]

NS1 plays an important role in viral replication of the flavivirus and has been observed to be secreted by DENV-infected mammalian cells. The release of NS1 can also be detected in the blood stream (specifically the plasma) of infected individuals.[5] Antibody kits exist to allow the binding and detection of these proteins by use of reverse transcription-polymerase chain reaction (RT-PCR).2 Problems with this technique exist where laboratories are scarce.

As dengue is an increasing international health concern, preemptive measures need to be taken to lower cost while raising diagnostic ease. The goal of this work was to create the SPION/antigen complex and confirm its antibody binding abilities as means to bind the NS1 antigen and magnetically purify increasing ease of current detection techniques. This research is designed to act as the base from which new diagnostic testing techniques can be developed for dengue and potentially for other diseases as zica and chincongunya.

METHODS

The prepared magnetite nanoparticles (MNPs) by co-precipitation method were stabilized and functionalized with 3-aminopropyltriethoxysilane. After, the black, MNPs suspension was chemically modified on the surface by salinization with a solution 3-aminopropyl-trimethoxysilane (97%). 25mgs of the MNPs were weighed dry and placed in 5ml of the solution APTMS. This solution was sonicated for 5 minutes. The solution was then incubated at 60 °C for 4 hours. After, the solution was washed with toluene and sonicated again, finally being washed 3 times with ethanol. Subsequently, drying was performed.[13]The final step was the attachment of the monoclonal antibodies. The antibody used was anti-dengue virus NS1 glycoprotein antibody [DENV2]. The final testing stage was to make sure that after immobilization of the antibodies on the nanoparticles they would still possess functionality and be able to capture the NS1 antigen. Characterization of the MNP/antibody complex was performed by FTIR, SEM, TEM, UV-visible absorption spectroscopy. Antibody-antigen conjugation was verified by ELISA test. The final stage of the experiment was to qualitatively observe the presence of the SPIONs. To do this we utilized a colorimetric test with Prussian blue.

RESULTS

The final antibody sandwich test was consistent indicating that the antibodies were attached to the SPION and still possessed the ability to recognize the NS1 antigen. The experimental findings may be extrapolated and scaled to lead to future developments of simple, quick, and inexpensive diagnostic tests.

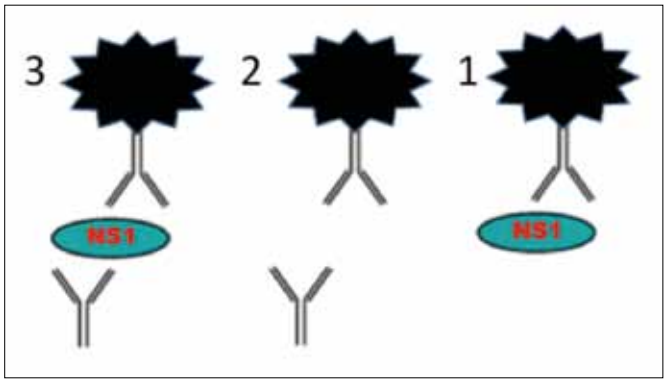

Figure 1: 3 expected outcomes for the SPION complex. (1) Represents the sandwich binding HT case we expected to see in the wells were both antibodies are present and bind to the antigen. (2) Represents H0 where the NS1 would not be recognized by the SPION complex. The MOCK represents this case where NS1 is not present and the nanoparticle/ antibody should not bind to the plate. (3) Shows H1 the case

where no ab41616 is present and again the nanoparticle should not bind.

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EFFICIENT DRUG DELIVERY SYSTEMS FOR RETARDED RELEASE OF BORTEZOMIB FROM CALCIUM PHOSPHATE CEMENTS

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Previous studies have shown that dendritic glycopolymers based on dendritic polyamine scaffolds are highly promising hemo- and biocompatible materials in the field of biomedical applications.1 Besides their use as drug delivery systems[2-4] these materials are also usable as polymeric drugs[5] and as diagnostics[6], but also as anti-amyloidogenic and anti-prion agents[7,8]. For the local multiple myeloma therapy calcium phosphate cement (CPC) are used as bone graft substitute. The CPC provides crucial advantages, such as osteoconductivity, biodegradability and the potential drug loading. Though, it lacks retarded drug release for short-/long-term treatment due to the free diffusion of small molecules through the micropores in the CPC. From this there is need for searching suited drug delivery system being able to undergo retarded release of drugs (Figure 1).

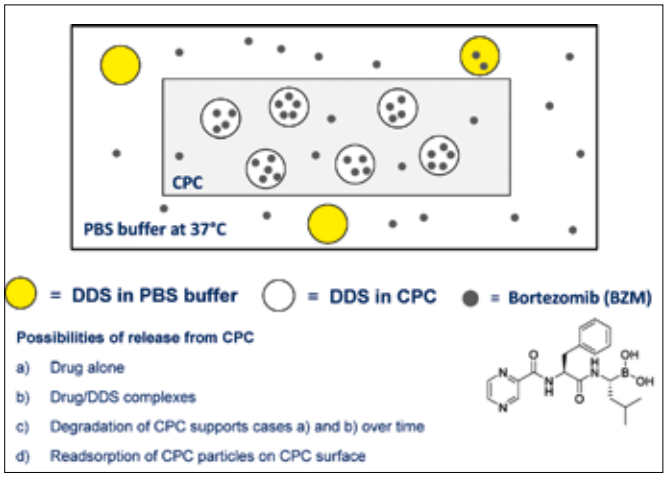

Figure 1. Schematic presentation for the integration of bortezomib/ drug delivery system (DDS) in calcium phosphate cement.

Here we present dendritic glycopolymers (DG) consisting of poly(ethylene imine) (PEI) decorated with oligo(glutamic acid) (PGlu) and/or maltose and maltotriose (Mal; Mal-III), respectively, as nanocarriers for the proteasome inhibitor bortezomib (BZM) in CPC [9].Thus, in aqueous solution the drug delivery systems exhibit a sufficiently high drug uptake of 54% for PEI-Mal and 73% for PEI-Mal-III, but only 35% for PEI-PGlu-Mal, while a significant retarded BZM release from DG/CPC composite is determinable. This has been observed with different polymer/drug ratios. In the table BZM release values at 37 °C are shown for 1 g CPC containing 50 µg BZM and 100 µg GD. PEI-PGlu-Mal provide the most suitable release profile for BZM.
At high polymer concentrations the mechanical and morphological properties of the bone substitute are not influenced by the DG. The compressive strength remains at 27-29 MPa for the CPC with and without DG. Moreover biocompatibility of the DG was tested by lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) activity. The DG do not affect the cell proliferation and differentia-

tion of hMSC.10 Besides this, first in-vitro applications showed that BZM/DDS complexes in CPC (Figure 1) are effective materials to kill various multiple myeloma cells. Concluding the results CPCs loaded with BZM complexed by the DG are promising materials for bone reconstruction in terms of short-/long-term treatment of cancer damaged bones.

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MULTIFUNCTIONAL AND -RESPONSIVE POLYMERSOMES AS PLATFORM FOR SYNTHETIC BIOLOGY, MIMICKING CELL COMPARTMENTS AND LAB-ON-CHIP DEVICES

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Over the last years, huge efforts have been undertaken to develop feasible polymer-based systems for biomedical applications and synthetic biology[1]. Polymeric capsules and polymersomes among other have been proven to be promising candidates for such purposes. Compared to their biological counterpart, the liposomes, the membrane from polymersomes is considerably thicker and shows increased mechanical and chemical strength[2]. In this context, our efforts were directed to establish pH-stable polymersomes over a broad pH range by the incorporation of two different photo-crosslinkable moieties in the membrane. This allows us for undergoing reversible switching of polymersome´s membrane to trigger the uptake and release of small molecules under various pH values und to squeeze out dendritic glycopolymers under shear forces[3-4].

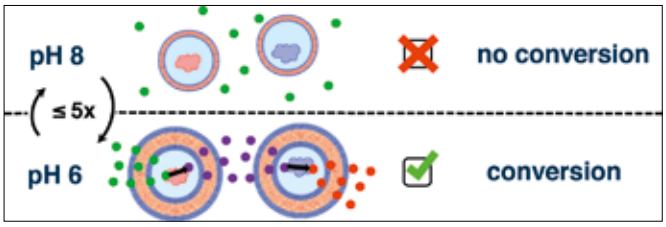

Figure 1. pH-controlled multienzymatic conversion triggered by pH-sensitive and –permeable polymersomes[6].

For developing even more complex polymersomes usable in biomedical applications and synthetic biology, where post-non-covalent conjugation steps and/or de-conjugation/displacement steps are required, we will report further progress on pH- and size-controlled diffusion processes and post-conjugation processes[5]. Results are presented and discussed in respect to pH-dependent (multi-)enzymatic conversion steps[3,6] (Figure 1) as well as enhanced folic acid-tailored uptake of doxorubicin-enclosed polymersomes by folic acid-sensitive cells[7]. Furthermore, examples are presented, where non-covalent steps by using adamantane-β-cyclodextrin inclusion complexes were established to perform simultaneously and sequentially pH-controllable modification of the outer shell of polymersomes[5] and polymersome´s lumen. Such post-loading events also enabled us to carry out pH-dependent displacement steps where surface-tunable and versatile polymersomes can be used in future synthetic biology, cell engineering processes or lab-on-chip devices.

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CHARACTERIZATION OF NATURAL LDL- AND NON-TARGETED NANOPARTICLE-UPTAKE IN BREAST CANCER STEM CELLS OF TRIPLE NEGATIVE ORIGIN

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Triple negative breast cancer (TNBC) patients have a higher rate of metastatic recurrence and a poorer prognosis than women with hormone receptor expressing breast cancer subtypes [1], [2], [3], [4]. The current standard therapy for patients with TNBC is limited to chemotherapy, which leaves a clear need to establish new approaches for therapy [5]. Cancer mortality is mainly due to metastatic spread to vital organs and gain of therapy resistance. Breast cancer stem cells (BCSCs) and their progenitors (BCSCPs) play a key role in metastatic dissemination of solid tumors. These dormant disseminated tumor cells may reside adjacent to microvasculature of the lung, the bone marrow and the brain [6]. Thus, it is becoming evident that if advanced cancers have to be eliminated, it is necessary to find novel agents, which target the BCSCs [7] and perhaps more importantly, the BCSCPs that survive as micrometastases in order to prevent disease recurrence. It has been shown that increased LDL metabolism in the cancer cells and especially in TNBC indicates an additional component contributing to higher malignancy [8]. Thus, LDL-like nanoparticles have emerged as potential drug delivery systems for anti-cancer drugs and for targeting different types of tumors. The goal of this study was to examine the uptake efficacy of actual LDLs that target the LDL receptor (LDLR) via its ApoB100 protein, as well as non-targeted stealth nanoparticles (NPs) in normal breast stem cells and BCSCs. These two systems were chosen to evaluate the two uptake pathways, of clathrin-dependent, receptor-mediated endocytosis (for LDL) and clathrin independent, non-receptor mediated endocytosis, (for the stealth nanoparticles).
We report that receptor- mediated uptake of LDLs through the ApoB100 protein, was equal or lower in BCSCs compared to non-BCSCs and normal, non-tumorigenic breast stem cells. Non-targeted NPs, displayed an overall lower uptake efficacy compared to LDLR-targeted NPs by a factor of four. However, they showed higher specificity for BCSCs compared to normal breast stem cells. In conclusion, LDLs targeting the LDL-receptor via their ApoB100 protein resulted in higher uptake than passive targeting using stealth nanoparticles. However, these passive targeting NPs conferred greater

selectivity for BCSCs versus normal breast stem cells. Therefore, BCSCs show an increased tendency for non-clathrin dependent uptake compared to non- stem cells.

The future work will focus on LDLR-peptide-ligand and folate-ligand targeting of pure drug-loaded NPs for selective uptake via the Extravasation Permeation and Retention (EPR) effect, suitably for small NPs. Obviously, this will all depend on the level of drug-dosimetry achieved at the tumor site, and intra-cellularly in the BCSCPs, but it may have interesting applications in the smaller, distant metastases that have a leaky, and perhaps more permeable neovasculature.

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NOVEL IODINATED CONTRAST AGENTS FOR TARGETED BIOMEDICAL IMAGING: STUDYING THEIR BIODISTRIBUTION AND PHARMACOKINETICS BY X-RAY CT

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ABSTRACT SUMMARY

This study deals with the development of new generations of contrast agent for biomedi-cal imaging based on nano-emulsions templates. Compared to common and clinical contrast agents, nano-emulsions brings new real advan-tages like a long circulation in blood, the control of the biodistribution and pharmacokinetics in function of their chemical nature and the size, and the absence of toxicity.

INTRODUCTION

In spite of the progresses of the imagers' efficiency, notably X-ray and optical modality, their use and potentials are still dramatically limited by the low efficiency and toxicity of contrast agents.[1] This study presents the development of new contrast agents overcoming these limitations, based on non-toxic nano-emulsions highly loaded in contrasting materials, intended to fluorescence tomography and/or computed tomography (CT) preclinical imaging. The success of the formulation of such contrast agents relies on several interdependent challenges: (i) Designing efficient and cost-effective contrast that are easy to synthesize and that can be loaded at high concentrations in nanoparticles. (ii) Developing formulations of the contrast agents without organic solvents and specific mechanical device. (iii) Adjusting the nanoparticle surface to allow high stability of the nanoparticles (at least several months), good bioavailability and efficient targeting. (iv) Mini-mal toxicity of the contrast agent.

EXPERIMENTAL METHODS

2,3,5-Triiodobenzoic acid, α-tocopherol, 4-dimethylaminopyridine, N,N'-dicyclohexylcar-bodiimide, dichloromethane, ethyl acetate, cy-clohexane were purchased from Sigma Aldrich, France. Non-ionic surfactant (Cremophor ELP®) from BASF (Ludwigshafen, Germany).

The 2,3,5-triiodobenzoic acid (5 g, 0.01 mol), 4-dimethylaminopyridine (0.18 g, 0.0015 mol) and N,N'-dicyclohexylcarbodiimide (2.3 g, 0.011 mol) were sequentially added at room temperature to a solution of DL-α-tocopherol (3.5 g, 0.008 mol) in dicholoromethane (250 mL). The reaction mixture was stirred overnight at room temperature and the dicyclohexylurea and other precipitates were removed by filtration. The organic phase was then washed twice with saturated aqueous $NaHCO_3$, once with saturated NaCl solution and dried with anhydrous Na_2SO_4. The solvent was removed in vacuum and the oil was then purified by the gradient elution method on silica gel using cyclohexane and ethyl acetate as an eluent. Reaction yields were around 80%. The resulting product was a light, yellowish viscous oil with a high iodine content of around 41.7%. Nano-emulsions of iodinated α-tocopherol were for-mulated by the spontaneous nano-emulsification method, as described previously. In short, pure α-tocopheryl 2,3,5-triiodobenzoate (0.75 g), was firstly mixed with the non-ionic hydrophilic surfactant (0.5 g), and maintained at room temperature. Phosphate buffered saline (PBS), used as an aqueous phase (1.88 g), was then added to the surfactant/oil mixture under gentle magnetic stirring. This optimized formulation was chosen to give a compromise between the nano-emulsion size and monodispersity, and the iodine content of the suspension. As a result of the process optimization, this compromise led to a droplet diameter of around 85 nm, with the following formulation parameters: surfactant/ oil weight ratio (SOR) 1/4 40%, and (surfactant/ oil)/water weight ratio (SOWR) 1/4 40% (see Ref. [2] for details on the formulation process). The α-tocopheryl 2,3,5-triiodobenzoate content in the nano-emulsions (i.e. injectable product) was about 24 wt.%. The schematic represen-tation of a nano-emulsion droplet is reported in Fig. 1. Finally, nano-emulsions were sterilized by filtration (0.22 mm membrane, Millex-GP, polyethersulfone (PES) membrane, Millipore, Molsheim, France) before intravenous administration.

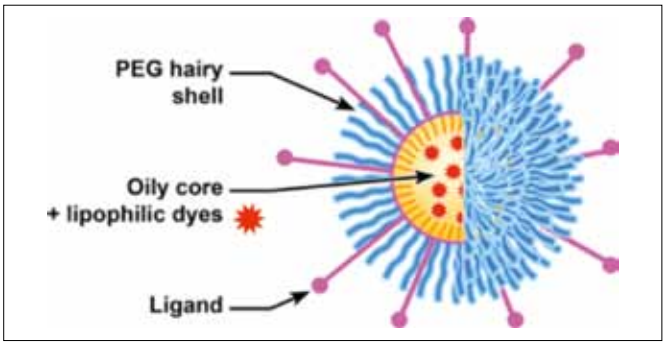
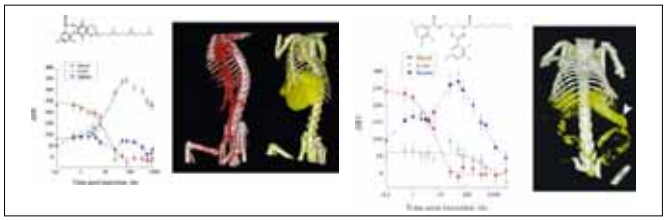


Figure 2: MicroCT: In vivo follow-up in mice of the contrast enhancement in X-ray imaging (no fluorescence imaging here), induced by injection of iodinated nano-emulsions (from Ref. [2]). These nano-emulsions are similar in composition and only differ in the chemical nature of the oil composing the droplet core: iodinated vitamin E (left) and iodinated glyceryl monocaprilate (right).

RESULTS AND DISCUSSION

Contrast agents were formulated as lipid nano-emulsions that consisted of a lipid core, surrounded by a non-ionic surfactant layer (see Fig. 1). The lipid core comprised lipophilic molecules either grafted with iodine compounds for X-ray contrast, and/or solubilized fluores-cent dyes with high loading ratio. The surface of the nano-droplets was fully covered by a hydro-philic polymer, like PEG, aiming at reducing the recognition by immune system, increasing the circulating time in the blood stream, and thus allowing a better control of the *in vivo* behavior. Moreover, we have developed several appro-aches (described below) to functionalize the droplet surface by grafting ligands. Our prelimi-nary results regarding the CT scan on mice are summarized in Fig. 2, showing the pharmacokinetics in blood (red curves), liver (open symbols) and spleen (blue curves) of nano-emulsions composed of iodinated vitamin E (left) and iodinated glyceryl monocaprilate (right). Though these nano-emulsions only differ in the composition of the core, their pharmacokinetics is strongly different as one targets the liver, and the other the spleen.



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CONCLUSION

Nano-emulsions is a simple system that presents a great potential as contrast agent. In the present abstract, we showed that nano-emulsions are not only very suited for the formulation of CT contrast agents, but also that changing simple parameter like the nature of oil, we can target the imaging properties.

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EURONANOMED, AN ERA-NET TO BRING TOGETHER NANOMEDICINE RESEARCH FUNDING OF EUROPEAN ACTORS AND BEYOND

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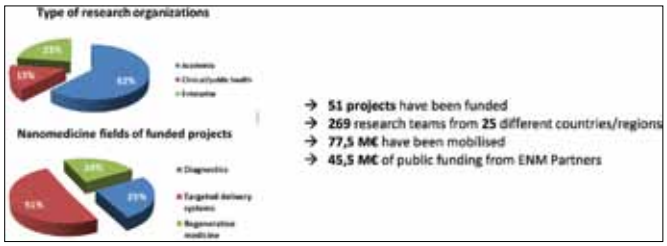
EuroNanoMed (ENM) is an ERA-NET on Nanomedicine established since 2008 as a platform for funding agencies and ministries to coordinate research programmes with the goal of creating and funding collaborative research projects that can convert research in nanotechnology into practical gains in medicine. EuroNanoMed II began in November 2012, attracting 20 partners from 17 countries/regions, and continues until the end of October 2016. During the period 2009–2015, EuroNanoMed has successfully launched 6 joint calls for proposals thematically aligned with the strategic priorities of the European Technology Platform on Nanomedicine (ETPN): drug therapy, diagnostics and regenerative medicine. These joint calls for proposals allowed ENM to fund 51 transnational research projects involving 269 partners from 25 countries/regions, with about € 45.5 million from the ENM funding agencies. Our 7th joint call for proposals, in 2016, is open and 67 proposals are under evaluation.



A first analysis of the outcomes of the EuroNanoMed funded projects already completed show a good impact in the field:

- High dissemination level → 136 peer review articles published
- Employment → 11 new permanent contracts
- Continuation of these projects with other funding resources → 24 proposals submitted for the continuation of ENM-funded research
- 13 patents were submitted during the lifetime of the projects
- 2 start-up companies were created → UAB-Ferentis in Lithuania (from I-CARE project) and Adjuvatis in France (from iNanoDCs project)
- Patented results of Nanosplice projects were transferred to a company founded by Swedish partners

The third phase of EuroNanoMed, EuroNanoMed III, has recently been approved for funding by the European Commission, and the project will start by the end of 2016. A joint transnational call co-funded by the EuroNanoMed III partners and the European Commission will be launched at the beginning of the project. This co-funded call potentially boast a war chest of ~15 million euros for 2017. Participation in past EuroNanoMed calls by category has stood at 62% from academia, 23% from companies, and 15% from clinical or public health organizations. The majority of groups have been from academia and this is a challenge for the next phase, where EuroNanoMed III aims at more industry involvement, to help bring research results to clinical applications or closer to the market. Upping industry participation is a challenge and EuroNanoMed III has attracted new funding partners that fund industry, including Science Foundation Ireland; the Technology Foundation STW from The Netherlands; Center for the Development of Industrial Technology (CDTI) from Spain; TUBITAK from Turkey; and the German Ministry BMBF through an agency which only funds industry.
In terms of successfully funded projects, Portugal, Spain and France have stood out up to now, with Israel also prominent in many calls. Norway is to step up its involvement in the third phase. The new phase also welcomes Quebec, Canada and Taiwan.
EuroNanoMed III partners

EuroNanoMed II also sought collaborations and teamed up with ETPN to craft a strategy document that will provide direction for the long term: the Nanomedicine Strategic Research and Innovation Agenda.[1] Other collaborations with European initiatives are planned in the third phase, including collaboration with ENATRANS

to spotlight EuroNanoMed funded projects with greatest potential impact or real-world application.
Finally, other important tasks have been accomplished during ENM II: training on regulatory aspects, actions to increase the participation of young researchers, collaboration with other EU initiatives (ETPN, ECRIN, EATRIS) and dissemination of ENM II activities. ENM II will organize a training workshop in regulatory aspects for funded researchers in October 2016, back to back with a review seminar, where funded researchers will present the main outcomes of the funded projects.
[1]www.etp-nanomedicine.eu/public/press.../Nanomedicine%20SRIA%202016-2030.pdf

NANOFACTURING: SCALE UP OF ULTRA-SMALL GLYCAN COATED GOLD NANOPARTICLES

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A number of nanomedicine formulations have enabled, or been shown to hold considerable potential for enabling more effective and less toxic therapeutic interventions. However, progress to date in translating these initiatives to commercial success has been limited. One of the main reasons for this bottleneck is due to the inability of researchers and stakeholders to manufacture batches of the nanomedicine product at the required scale and according to Good Manufacturing Practice (GMP) requirements. The NANOFACTURING project will focus on- facilitating Access to required infrastructures and expertise - creating GMP pilot lines for up-scaling manufacturing- addressing the current developmental and production gaps - taking nanomaterials already successfully produced at proof-of-concept/milligram levels and facilitating their scale-up to sub-kilogram quantities- providing large-scale and GMP production for clinical trials and nanomedicine translation. The NANOFACTURING project, through a consortium of 9 partners, will develop the synthetic processes, process control methods, analytical assays for QA/QC, functional specifications, and best practices, interfacing existing R&D centres of excellence, transfer organisations and private GMP manufacturing facilities (including SMEs) to ensure efficient translation from discovery through to first in man, proof-of-concept studies and beyond to Phase III according to industrial and regulatory standards. Specifically, the NANOFACTURING project aims to create a platform process for early, mid- and large-scale manufacturing of glycan-coated gold nanoparticles (GNPs), a widely researched and developed class of self-forming nanoparticles with impressive promise for future therapeutic drug development.

TECHNOLOGY

Nanofacturing's core technology relates to Midatech's glycan-coated gold nanoparticles (GNP). Gold nanoparticles comprise of a core of gold metal atoms surrounded by a layer of glycans (sugars) to which various ligands can be attached. The small size of Midatech's gold nanoparticles creates several potential critical qualities for GNP-based drugs (Figure 1):

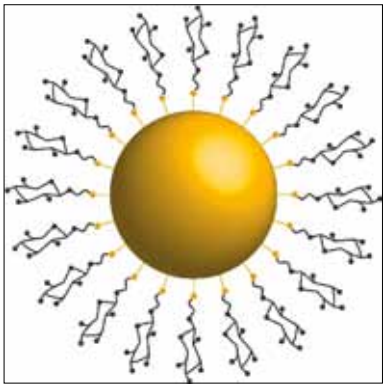

Figure 1.: Schematic Representation of glycan-coated gold nanoparticles (GNP)

- Multivalency: ability to attach several therapeutic and targeting molecules to single GNP
- Solubility: can bind and transport non-soluble therapeutic compounds to sites of disease.
- Releasability: can release the active compound inside the cell.
- Mobility: small size (2–5 nm) means they may cross membranes and other difficult to reach places
- Targetability: configured to seek out specific tumour surface biomarkers.
- Stability: peptides and nucleic acids may be stabilised
- Excretability: eliminated via the kidneys and liver.

The ability to engineer new nanopharmaceuticals based on this patent protected platform technology, developed by Midatech Pharma España, S.L.U. (Project Coordinator), will have inherent advantages over existing treatments for multiple therapeutic areas as endocrine, central nervous system, antiviral and oncology.[1]
[1] For more information, visit: www.nanofacturing.eu

SONOPORATION IMPROVES THE ACCUMULATION AND PENETRATION OF LIPOSOMES IN TUMORS WITH LOW EPR

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INTRODUCTION

Passive drug targeting to tumors is based on the highly variable Enhanced Permeability and Retention (EPR) effect. Over the years several pharmacological and physical strategies have been evaluated to overcome physiological barriers to enhance EPR. Sonoporation, which is based on the combination of ultrasound (US) and microbubbles (MB), is able to permeabilize membranes and open endothelial tight junctions. Here, using two different types of MB (phospholipid soft-shell/polymeric hard-shell), we systematically analyze the impact of sonoporation on the accumulation and penetration of liposomes in two tumor models (A431/BxPC3), both characterized by low levels of EPR.

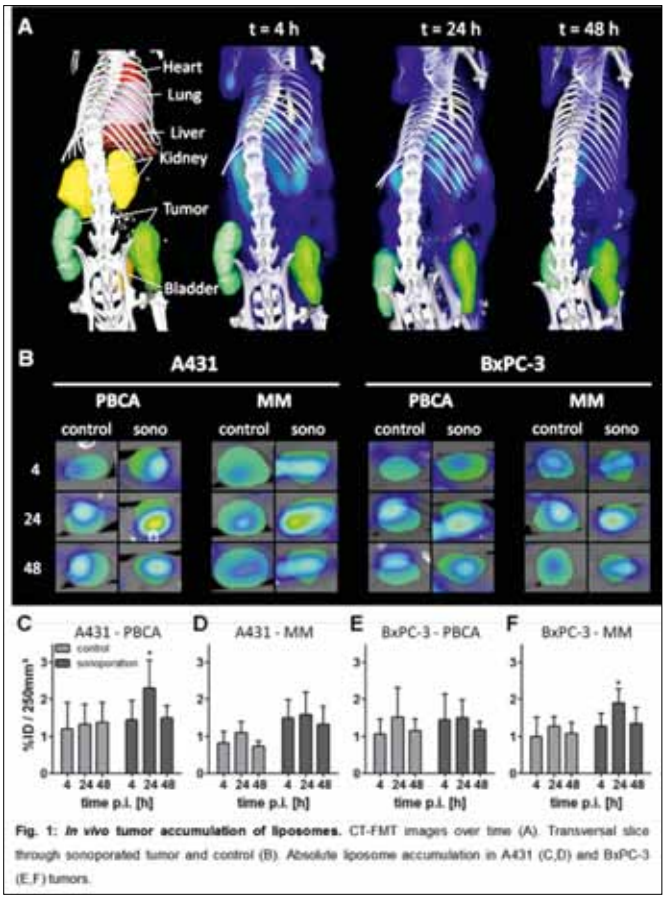

Fig. 1: *In vivo* tumor accumulation of liposomes. CT-FMT images over time (A). Transversal slice through sonoporated tumor and control (B). Absolute liposome accumulation in A431 (C,D) and BxPC-3 (E,F) tumors.

METHODS

Mice bearing highly cellular A431 epidermoid tumors or highly stromal BxPC-3 pancreatic tumors on both flanks were co-injected

with MB and double-fluorophore-labeled liposomes. MB were locally destroyed in the tumors on the right flank, by exposure to destructive Power Doppler US pulses. Using hybrid computer tomography - fluorescence molecular tomography (CT-FMT), the tumor accumulation of the liposomes was monitored over time. Findings were verified and extended by fluorescence reflectance imaging (FRI) and two-photon microscopy (2PM), to evaluate the impact of sonoporation on tissue penetration and intratumoral distribution.

RESULTS

A clear tendency towards increased liposome accumulation upon sonoporation was observed (Fig. 1).

This was particularly obvious at 24 h post liposome administration, with increases of up to 100%. 2PM confirmed these findings and also showed that liposome penetration out of the vessels into the tumor interstitium was enhanced upon sonoporation, with significantly larger amounts of liposomes present within deeper tumor compartments (Fig. 2).

Some differences between the two tumor models and MB types were observed, but because of the relatively small group size (n=5) and the relatively high inter- and intra-individual variability (likely caused by variability in the underlying EPR effect), these differences were not significant.

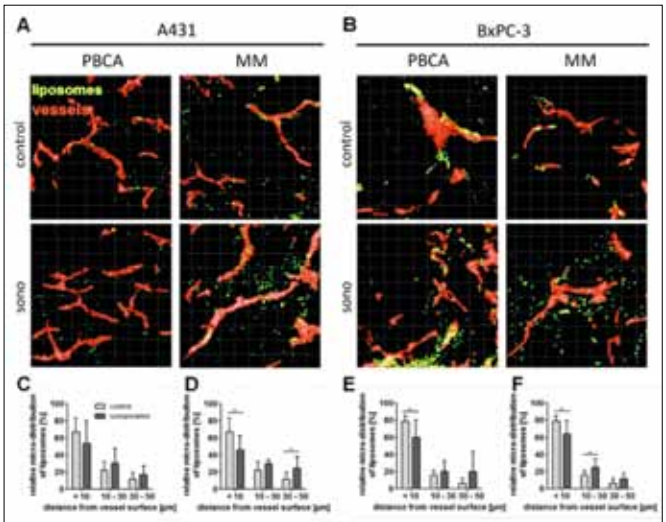

Fig. 2: Sonoporation improved liposome penetration, exemplified by 2PM images of A431 (A) and BxPC-3 (B) tumors. Relative distribution of liposomes upon PBCA (D,E) and MM (D,F) mediated sonoporation.

CONCLUSION

Our findings show that sonoporation enhances the accumulation and penetration of liposomes in tumors with low EPR. These results support recent achievements in the use of sonoporation in patients (for improving drug delivery to pancreatic tumors and across the blood-brain barrier). They should be intensified and expanded in the future, to optimize the US settings and the dosing regimens, and to evaluate how sonoporation efficiency can be maximized.

Acknowledgements:

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UNVEILING THE DARK SIDE OF THE PROTEIN CORONA: A STUDY ON ITS CELLULAR UPTAKE AND EVOLUTION

FILIPPO BERTOLI, David Garry, Marco P. Monopoli, Anna Salvati, Kenneth A. Dawson

Nanomaterials (and specifically nanoparticles), as a result of their unique properties (like small size and their large surface area), offer great promise for drug delivery systems and medicine therapies[1, 2]; therefore, in last ten years, a great scientific effort has been employed in determining how nanoparticles interact with biological systems and, specifically, with cells. Different studies have showed how nanoparticle - cell interactions are governed by different parameters, the main being the layer of proteins and other biomolecules adsorbed on the nanoparticle surface from the surrounding biological media (protein corona)3. Subsequently to membrane interactions most nanoparticles are known to follow the endo-lysosomal pathway, progressing from early to late endosomal structures until they localise in the lysosomes. Although the composition and extracellular stability of the protein corona of various nanoparticles have been characterized and studied[3] as well as the uptake and cellular processing of different nanomaterials, little is still understood about the intracellular uptake and evolution of the corona layer. Indeed, if the original corona is partially retained on the nanoparticle surface, the proteins in this layer may play an important role in determining subsequent cellular processing. As a first step to unveil these questions here we fluorescently labeled the protein corona formed on nanoparticles with different surface properties (amino and carboxyl modified polystyrene nanoparticles, silica nanoparticles and silver nanoparticles) and we follow its intracellular uptake and evolution (as well as the core particles, Figure 1) with different fluorescence based techniques. We then emphasize the fundamental role the properties of the core nanomaterials play in the corona final destiny (Figure 2); specifically showing how different surfaces leads to different kinetics in the persistence and processing of the corona proteins by the cells. To achieve a better understanding of the cellular processing of the corona proteins subcellular fractionation techniques have been employed to isolate the organelles in which the corona localized and a multi-technique (including fluorescent microscopy, flow cytometry, 1 D SDS PAGE and mass spectrometry) approach has been using in identifying the corona proteins and assess their final intracellular fate. The findings show that, when corona proteins reach the lysosomes, they are there degraded by the lysosomal proteases. We then compare the amount of proteins the nanoparticle bring into the lysosomes with the amount of serum internalized in normal conditions (Figure 3), and we have found that the same serum proteins can exhibit a different intracellular processing when they are carried inside cells by nanoparticles as components of their corona, comparing to what is observed when they are free in the extracellular medium. The potential of nanoparticles to carry a diverse (material-dependent) cargo of proteins that is atypical in endogenous processes has many potential implications. Many of these processes have not been studied as yet in detail, and where poorly understood phenomena have been reported, the role of the intracellular co-transported proteins has not been considered as a potential mechanism. Finally, the approach outlined here allow a complete molecular analysis of all components within these intermediate time periods. This could also, in principle, be linked to the physiochemical microstructural organization of biomolecules on the nanoparticle surface, and the enzymatic processes that act upon them.

Figure 1: Uptake of fluorescently labelled corona: A549 cells were exposed to a pulse of 100 µg/mL labelled corona-nanoparticle complexes for 2 hours in unlabelled complete medium and then chased for different times. a: Schematic representation of the experimental design: briefly, nanoparticles dispersed in fluorescently labelled serum in order to form a fluorescent hard corona (the corona-nanoparticle complexes were then isolated through centrifugation and resuspended in unlabelled serum before being exposed to cells (left)

where the internalization and localisation of the corona could be determined by fluorescence imaging and flow cytometry in a time resolved manner (2, 8 and 16 hours respectively from left to right). b: Confocal images of single A549 cells after exposure to nanoparticles (left panel in green : corona channel green, blue : nanoparticles, right panel, in red, green: corona channel, blue: nanoparticles, red lysosomal channels, scale bar : 5 µm). The results show that labelled corona proteins enter the cells and reach the lysosomes, colocalising with the nanoparticles. After 8 hours, fluorescent corona proteins on the nanoparticles decrease. At later times (16 hour) the majority of nanoparticles are inside the lysosomes and the fluorescent signal associated with the corona is almost vanished (highlighted in red in the pictures). It is also possible to observe non-lysosomal localisation of corona-nanoparticle complexes at all time points (highlighted in green in the pictures).

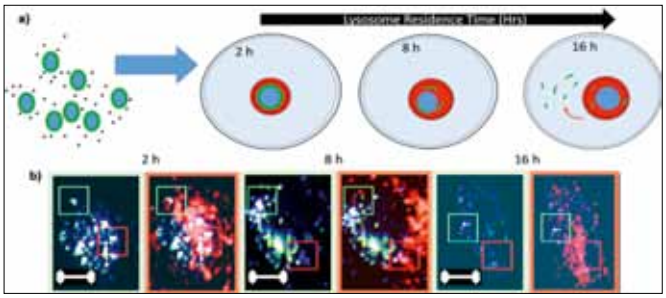


Figure 2: Uptake of fluorescently labelled corona adsorbed on different nanoparticles: A549 cells were exposed to a pulse of 100 µg/mL labelled corona-nanoparticle complexes for 2 hours in unlabelled complete medium and then chased for different times. a: Single cell confocal images of A549 cells after exposure to different nanoparticles (green : corona channel, blue : nanoparticles and red lysosomal channels; left panel in green: corona and nanoparticles, right panel in red: previous imaged merged with lysosomes , scale bar : 5 µm). The results show that labelled corona proteins enter the cells and reach the lysosomes in all cases (highlighted by a white rectangle in the pictures). b: Fluorescence distributions as obtained from flow cytometry analysis of live cells treated as described above (b). It is possible to note that the distributions correspond to the fluorescent images, suggesting that, while it is possible to distinguish internalization in all cases, the fluorescence associated with the corona proteins internalized by the cells is related to the nanoparticle type. c: Plot of the mean fluorescence obtained from c, confirming that the signal associated with the corona proteins is present in all treated samples, but highlighting how the higher internalization happened with polystyrene amino modified nanoparticles (PS-NH_2).

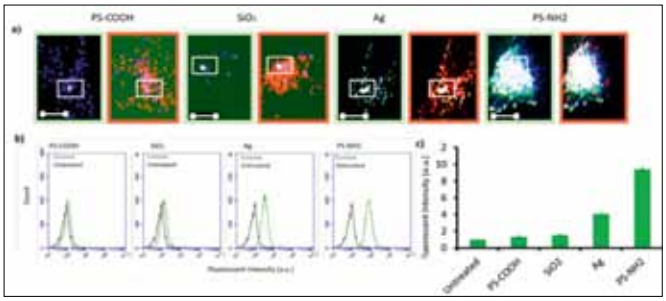
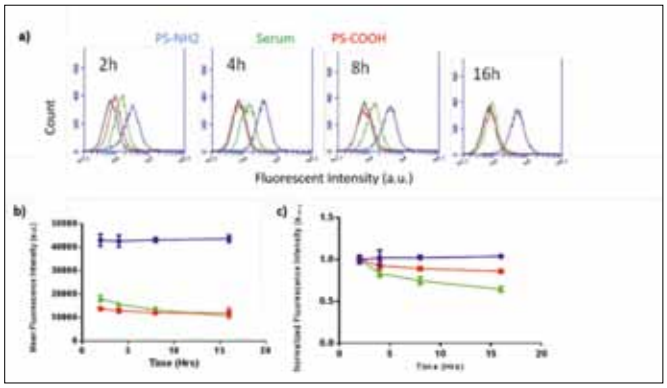


Figure 3: Comparison of uptake of labelled corona and serum inside cells. a: Mean fluorescence intensity distributions obtained by flow cytometry on cells exposed for 2 hours to the different nanoparticle corona complexes (PS-COOH and PS-NH_2) and cells exposed to cell culture medium containing 5% labelled serum followed by replacement with unlabelled (nanoparticle-free) medium and further growth for different times. Overlap of the distributions clearly shows that the highest uptake of fluorescent proteins is obtained when cells are exposed to labelled corona formed on PS-NH_2, while this is much smaller in the case of PS-COOH, and at a comparable level for cells exposed to simple labeled serum. At later times the proteins (or fragments of them) of the corona on the PS-NH_2 nanoparticles seem to persist inside the cells, while the proteins associated with the other samples have either completely degraded or exported. b: Mean cell fluorescence intensity as a function of time, from data shown in panel a, clearly showing how PSNH2 corona proteins are internalized in high amount by the cells in comparison to other particles or free proteins. c: Normalized evolution of the fluorescent signal associated with nanoparticle corona and free serum proteins, showing that the proteins associated with the nanoparticles are processed with different kinetics in comparison to what observed for free (labeled) serum proteins.



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USING LABORATORY µCT FOR SURGICAL RESEARCH ON THE RABBIT ANIMAL MODEL

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The rabbit, oryctolagus cuniculus, has been important in biomedical research due to its role as "bioreactor" with the most prominent application being the production of antibodies. Nevertheless, it has also significant potential as an animal model owing to its increased phylogenetic closeness to humans compared to the most common laboratory animals such as rodents, fishes, and nematodes. The rabbit model ideally bridges the gap between small laboratory mammals, most prominently mice and rats, and large laboratory mammals including canines, sheep, goats and pigs. Especially for the case of surgical research, the rabbit animal model offers the additional practical advantage of adequate size for surgical manipulations that can be more easily extrapolated to humans, as well as a closer resemblance of physiology, especially in domains such as the cardiovascular system. This explains the increased number of studies in atherosclerosis and lipid metabolism as well as studies on orthopedics, bone regeneration, and cranio-maxillofacial surgery. Towards a comprehensive three-dimensional anatomical characterization of this animal model for research related to neurosurgery, we have performed high-resolution micro computed tomography (µCT) of perfusion-fixed New Zealand rabbit heads. For this task, we used the laboratory system nanotom® m. Performing a time-efficient, three-hour scan of the entire rabbit head, we were

able to visualize a volume of around 1,000 cm^3 with a pixel length of 35 µm and sufficient contrast to clearly depict the fine details of the cochlea and the trabeculae of spongy bone. Using the commercial visualization and image processing software VGStudio Max 2.1, a 3D rendering of the bone structure was performed, allowing both for complementing anatomical dissection images of the posterior fossa and especially the cerebellopontine angle, as well as for performing accurate spatial measurements of structures such as the meatus acousticus internus (MIA) and the internal acoustic channel (IAC), that cannot be accessible non-destructively and are important for an experimental model of facial nerve injury that is to be established. Owing to its three-dimensional, non-destructive nature, we are convinced that µCT will ideally complement the standard histological and surgical procedures used in other rabbit models, including those for modified access techniques, cranial and facial bone regeneration as well as cerebral ischemia/reperfusion and cranial nerve regeneration.

Keywords: Micro computed tomography, surgical research, New Zealand rabbit, three-dimensional imaging, micrometer resolution, cranial bone, facial bone.

SMALL AND BIOCOMPATIBLE COATINGS OF IRON OXIDE NANOPARTICLES FOR IMPROVED DETECTION AND TREATMENT OF LYMPH NODE METASTASES

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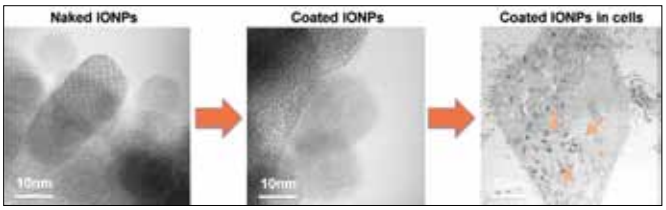
Lately, iron oxide nanoparticles (IONPs) have been the focus of intense clinical research. One very promising application is their use in theranostics where IONPs act both as contrast agents for magnetic resonance imaging (MRI) and as heating sources for tumor elimination. As their *in vivo* performance strongly depends on their stability in aqueous solutions, we developed biocompatible coatings to prevent their aggregation in unbuffered water. Current coatings are made of large molecules such as sugars (e.g. dextran) or polymers, which are known to accumulate in the liver and spleen, diminishing the IONPs' access to the tumor and exerting toxic effects. Here, we report the results of *in vitro* screening of 11 biocompatible coating strategies based on small molecules. Small coating sizes increase the number of IONPs which can be loaded inside small volumes, for instance metastases in lymph nodes (< 3mm), and thus improve the efficiency of hyperthermia treatment as well as increase the MR signal. In order to target this particular type of tumors, we chose coating molecules with at least one functional chemical group for further coupling with targeting ligands (e.g. antibodies). This specific and exclusive location of IONPs inside tumors not only removes false negatives in MR images (tumors which appear as healthy spots), but also avoids serious secondary damage of healthy body tissues during the hyperthermia treatment. The 11 coated-IONPs were fully characterized, revealing the presence of the coating molecules arranged in 2 to 3 layers longitudinally to the IONPs. As the biological environment responds very differently in contact with IONPs of different sizes and surface charges, the hydrodynamic diameters and zeta potentials of coated-IONPs were determined by dynamic light scattering (DLS) to predict their *in vivo* behavior. In addition, qualitative and quantitative analysis of the chemical groups available for further functionalization of the IONPs with targeting ligands was done by X-ray photoelectron spectroscopy (XPS). Besides the physico-chemical characterization of the coated-IONPs, we investigated their *in vitro* behavior in the presence of different cell lines and showed very low toxicities and coating-dependent cellular uptake.



Transmission electron microscopy micrographs of naked and coated IONPs in the absence and presence of lymph node metastases cells.

NANOBIO INTERACTIONS FOR ULTRASMALL NANOPARTICLES: WHAT ABOUT THE CORONA?

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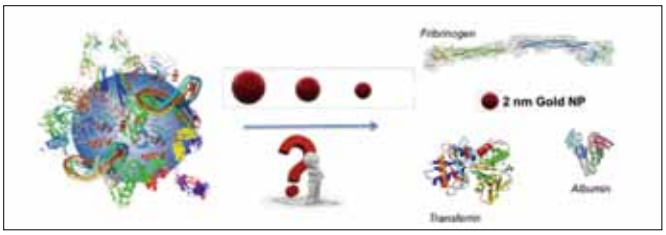
Over the recent years, there is an increase interest in the nanotechnology for medical applications. This new field of research, known as nanomedicine, is developing new promising alternatives to drug delivery, diagnosis and bio imaging. Nanoscale sized particles have unique potential of interacting with DNA, proteins, membranes, organelles, cells and so on. When in contact with the biological medium, the nanoparticles (NPs) interact with biomolecules such as proteins which adsorb onto their surface, forming the so-called "corona". The protein-nanoparticle complexation leads to the alteration of the surface, which gives a new biological identity that can determine the final fate of NPs in the human body.[1] Although the very promising role of NPs in the future medical field, a considerable concern is still caused by the possible long term toxicity of nanomaterials, due for example to the demonstrated tendency of NPs to accumulate in specific organs, as liver or spleen, in an uncontrolled fashion, observed particularly in the size range of 6-100 nm.[2] A different behaviour have been observed for the ultrasmall nanoparticles (USNPs), in the size range of 1-3 nm.[3] Recent biodistribution in-vivo studies in mice showed efficient renal clearance of ultrasmall glutathione coated gold particles and a very low concentration of these USNPs in the liver.[4] USNPs represent therefore a new promising tool in nanomedicine, not limited by unwanted organs accumulation. Nevertheless their interactions with biological media are still unclear because their small size makes the characterization and the proteomics study extremely challenging.



Most of the plasma/serum proteins present a hydrodynamic diameter of about 3–15 nm, so till 5 times the size of the USNPs; can we still speak about corona for USNPs? If yes, this can be dramatically different in respect of the classical model described for bigger nanoparticles, possibly leading to very different biological behaviour.
The aim of this project is to understand the role and the nature of USNPs-proteins interactions obtaining fundamental insights toward a potential application of this class of nanoparticles in nanomedicine.

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RADIOTHERAPY ENHANCES THE THERAPEUTIC EFFICACY OF MMP-SENSITIVE LIPOSOMES IN HEAD AND NECK CANCER.

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Most chemotherapeutic agents lack specificity and target any rapidly dividing cell. Nano carriers such as liposomes can overcome this barrier by delivering the drug payload specifically to target tissues, and thereby minimize side effects in non-target tissues and organs. However, drug bioavailability after tumor accumulation remains a general challenge in existing drug delivery systems. Incorporation of a site-specific trigger in the nano carrier can provide release of the payload to the target tissue in a controlled fashion, and enhance the efficacy of the drug.We have designed a matrix metalloproteinase (MMP)-sensitive liposomal drug delivery system, wherein high local expression of MMPs in the tumor microenvironment converts a PEGylated anionic liposome to a dePEGylated cationic liposome that is internalized more readily by cells. A lipopeptide containing a MMP-2 and MMP-9 cleavable sequence provide the tumor-specific enzymatic dePEGylation. Upon cleavage the dePEGylated cationic liposome is internalized efficiently by cells via electrostatic interaction with the negatively-charged cell membrane. Inside the cell, the cationic charge of the liposome can interact with the negatively charged lipids in the endosome membrane to mediate a higher escape of the drug into the cytosol. In this study, we encapsulate oxaliplatin, which elicits its cytotoxic effect by intercalating into the DNA, and blocks DNA replication and transcription. The conceptual design is illustrated in Figure 1.A, whereas Figure 1.B shows Cryo-TEM of the MMP-sensitive liposomes. Figure 1.C presents the Zeta Potential before and after enzyme cleavage, demonstrating the charge-reversal properties. Figure 1D shows a tumor-growth-delay experiment in MMP-positive CT26 tumor-bearing mice.

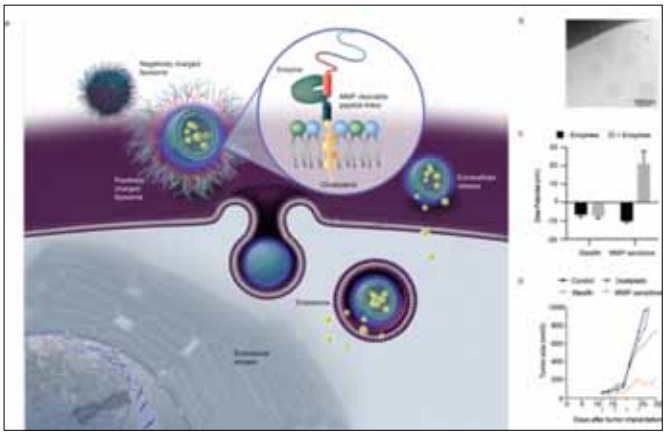

Figure 1 illustrates the triggered release mechanism of the MMP-sensitive liposomes (A), MMP-sensitive liposomes subjected to Cryo-TEM (B), Zeta Potential measurements of MMP-sensitive liposomes and Stealth liposomes in the absence or presence of enzyme to demonstrate charge-reversal properties of the MMP-sensitive liposomes (C), and a tumor-growth-delay experiment in MMP-positive CT26 tumor-bearing mice, median (D).

We wanted to test the efficacy of this drug delivery system in combination with other treatment modalities. Since chemoradiotherapy is a well-established treatment paradigm for multiple tumors, we investigated the strategy of using the oxaliplatin loaded MMP-sensitive liposomes in combination with radiation using head and neck cancer as a disease model. Cisplatin is one of the most commonly used chemotherapeutic agents for head and neck cancer. However, oxaliplatin is associated with less toxicity and better tolerability, which is potentially further lowered with a tumor specific drug delivery system such as the MMP-sensitive liposomes.
To study the combined effect of MMP-sensitive liposomes and radiation (RT), we conducted a tumor-growth-delay experiment in the MMP-positive xenograft model, FaDu, a head and neck cancer cell line. We have previously shown the enhanced efficacy of the MMP-sensitive liposomes over oxaliplatin loaded Stealth liposomes, which are liposomes with the same lipid composition as the clinically approved liposomal formulation Doxil. In this study MMP-liposomes were compared to free oxaliplatin in the absence or presence of RT. Mice were given equal amounts of oxaliplatin encapsulated in the MMP-sensitive liposomes or as free drug every fourth day for a total of 4 doses. The tumors were subsequently radiated 6 and 24 hours after administration of oxaliplatin and MMP-sensitive liposomes, respectively, using a 15mm cone to minimize radiation to the surrounding tissue. We observed an enhanced antitumor effect with this approach as shown in Figure 2, in which the MMP-liposomes + RT had a much greater tumor growth inhibition compared with free oxaliplatin, MMP liposome alone and free oxaliplatin + RT.

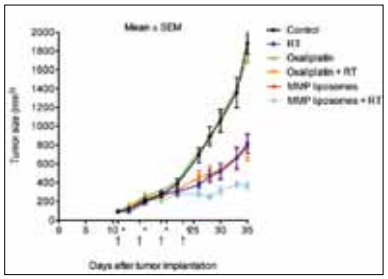

Figure 2 In vivo evaluations of MMP-sensitive liposomes in combination with radiation (RT) in the MMP-positive human head and neck cancer cell line FaDu.

In these studies we have used oxaliplatin, however this drug delivery platform can be employed to carry and deliver numerous anticancer agents into the microenvironment of an MMP-positive tumor. The two key features of the platform, the MMP-sensitization and the subsequent surface charge reversal (from anionic to cationic), provide the ability to deliver the payload in a controlled fashion to the tumor tissue and thereby enhance the efficacy of the carried anticancer agent. Results from the experiment presented here additionally suggest that the MMP-sensitive liposomes hold potential for clinical translation in combination with radiation.

TAILORING SHEAR-SENSITIVE LIPOSOMES AS VASODILATORS FOR ATHEROSCLEROSIS TREATMENT

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Nowadays, up to 50 % of people struck by heart attack die before arriving at the hospital. In diseased human coronary arteries the blood flow, and as a consequence the shear stress at the blood vessel walls, differs from that one being in normal arteries. Thus, we introduce mechano-sensitive liposomes filled with vasodilator drugs for local drug release [1]. To specifically tailor the properties of the liposomes, the morphological differences between seriously constricted and healthy human coronary arteries have to be known. Micro computed tomography (µCT) has been used to visualize hard and soft tissues of blood vessels, providing the basis for flow simulations [2]. In parallel, the behavior of the liposomes has been investigated by means of combining microfluidics with small-angle X-ray scattering (SAXS). This combined technique is a valuable tool to determine changes of the liposomes, such as deformation and breaking, by tuning the shear stress according to the values found in the flow simulations on the actual human coronary artery morphologies. The suitable geometries of the microfluidic device allow for mimicking healthy and diseased parts of selected blood vessels. The device is prepared using poly(di-methylsiloxane) (PDMS), UV-curable adhesive material and polyimide film [3]. To visualize the shear rate profile along the microfluidic device at a certain flow rate, flow simulations have been performed [4].

Keywords: Phospholipid liposome, drug carrier, microfluidics, small-angle X-ray scattering, micro computed tomography, flow simulation.

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ALPHA INTERFERON LOADED CHITOSAN NANOPARTICLES FOR THE OPTIMIZATION OF ITS THERAPEUTIC USE

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INTRODUCTION

Interferon alpha (IFNα) is a protein drug used to treat oncological diseases and viral infections. As it presents a short half-life, it must be administered frequently in order to maintain therapeutic effectiveness. However, its low therapeutic index and fluctuations in drug plasma levels can lead to severe adverse effects [1]. In this context, the design of a suitable drug delivery system (DDS) able to protect and control the release of the cargo would permit to reduce the frequency of administration. In this first stage, we designed and characterized a DDS. For this, IFNα2b was encapsulated in chitosan nanoparticles (CHT NPs) which were subjected to physicochemical and biological characterizations.

MATERIALS AND METHODS

CHT NPs were prepared by ionotropic gelification method between the positively charged amino groups of CHT (Low molecular weight CHT, Sigma-Aldrich, USA) and the negative groups of tripolyphosphate (TPP, Sigma-Aldrich, USA) [2]. Briefly, a TPP solution was added dropwise to a CHT solution pH 5.5 under constant magnetic stirring. The resulting nanosuspension was then left to gelify for 15 min. To prepare IFNα loaded CHT NPs, a suitable amount of drug was dissolved into de CHT solution. CHT and CHT NPs were characterized by infrared spectroscopy using a Nicolet Spectrometer (Nicolet 380 ATR/FT-IR spectrometer, Avatar Combination Kit). The particle size (D_h), size distribution (PDI) and zeta potential (Z-Pot) of blank and loaded CHT NPs were measured with a Zetasizer Nano-ZS (Malvern Instruments, UK). Unreacted CHT was quantified in the freshly nanosuspension by a colorimetric method reported elsewhere [3] using alizarinsulfonic acid as dye. The encapsulation efficiency (EE) was determined by an indirect method. For this, the concentration of free IFNα was determined with an ELISA commercial kit (Affimetrix eBioSciences). The %EE was calculated according to the following Equation: $\%EE = [(D_o - D_f) / D_o)] \times 100$, where Do is the initial amount of IFNα used and Df is the amount of free IFNα (non-encapsulated drug) (n = 3). CHT NPs stability was physically determined at 4 and 25°C. For this, Dh, PdI and Z-Pot were monitored as a function of time for 30 days, using the technique described above (n = 3). The antiviral activity was determined by measuring the inhibition of the cytopathic effect of Vesicular Stomatitis Virus (VSV) on MDBK cells. MDBK cells (20,000 per well of a 96-well plate) were infected with VSV at a MOI of 0.1. After incubation with soluble IFN or encapsulated IFN, cells were fixed and stained with crystal violet. Absorbance was measured at 590 nm. Absorbance of uninfected control cells was set as 100%.

RESULTS AND DISCUSSION

Infrared spectra of CHT NPs and CHT evidenced the interaction between CHT and TPP, supporting the formation of CHT NPs. The mean Dh was 381.7 ± 35.2 nm and 353.0 ± 31.2 nm, the PdI 0.472 ± 0.030 and 0.407 ± 0.010 and the Z-Pot 31.4 ± 4.6 mV and 31.8 ± 1.7 mV for blank and IFNα-loaded CHT NPs, respectively. The Z-Pot value suggests not only a net positive surface charge due to an excess of CHT but also physical stability of the DDS. The higher the zeta potential, the stronger the repulsion, the more stable the system becomes. In fact, both blank and IFNα-loaded CHT NPs were stable at 4 and 25°C over a period of 30 days, according to DLS results. The amount of CHT that formed NPs determinated colorimetrically was 95.5% and the encapsulation efficiency was 99.9%. The antiviral activity of encapsulated IFN was significantly higher than that of commercial soluble one (Figure 1A). Furthermore, slopes of dose-response curves are significantly different (lineal regression, p<0.0001; Figure 1B).

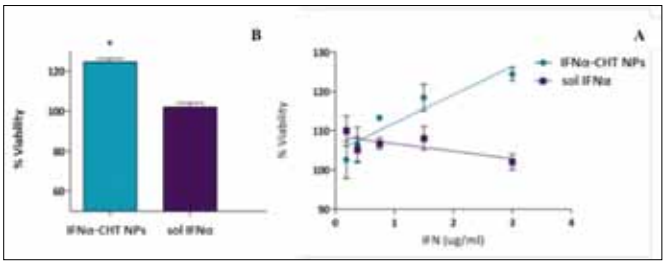

*Figure 1. Antiviral assay. (A) Percent viability of MDBK cells after being treated with IFNα loaded CHT NPs and soluble commercial IFN. *Statistically significant increase of cell viability after being treated with IFNα loaded CHT NPs when compared to soluble commercial drug (Mann Whitney test, p< 0.05). (B) Percent viability of MDBK cells for 2-fold dilutions of both treatments; slopes are significantly different (lineal regression, p<0.0001).*

CONCLUSIONS

This data outlined a straightforward method for encapsulate IFN with mild conditions in order to preserve its structural integrity and functionality. This DDS showed not only high encapsulation efficiency and stability but also a significantly greater antiviral activ-

ity compared with the commercial drug. These promising results lead to initiate further investigations to establish the release profile and safety of the DDS in order to obtain a suitable system able to optimize the therapeutic use of IFN.

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INTERACTIONS BETWEEN GRAPHENE AND BIOMOLECULES: ENABLING BIONANOSCIENCE STUDIES OF GRAPHENE

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Since its discovery in 2004 graphene has attracted great research and technological interest due to its unique properties as, among the others, high conductivity, flexibility and high resistence.[1] It is a common belief that graphene materials could enable the development of multifunctional biomedical devices and methods in many branches of life sciences, including drug delivery, cancer therapies and biosensing. There are especially high expectations for its potential applications in the field of sensors and biomedicine.[2,3] Nevertheless, the interaction of graphene materials with biological components is still unclear and sometime controversial.[4,5] The understanding of such interactions will be fundamental in order to enable the study graphene impact on health and environment, as well as the role of the interface in diagnostic and other medical application.
Until now, most of the biological studies focus on graphene oxide, that thanks to its hydrophobicity offer a good colloidal dispersability in water. In the case of graphene a central issue that have to be faced at this point is how to disperse it in a meaningful and reproducible manner in water and biological media. The hydrophobicity of the graphene surface, indeed, make necessary the use of molecules, polymers, surfactants or ionic liquids in order to stabilize the graphene flakes in water. It is well known that when a nanomaterial is in contact with biological medium, its surface get covered by biomolecules forming the so-called corona, which provide a new identity to the material and determine its final fate in the cellular uptake.[7, 8] The use of agents therefore should be avoided not only because of the possible intrinsic toxicity, but also because it can promote differences in the properties at the interface, leading to confusion in the biological outcomes.
C:\Users\Valentina Castagnola\Documents\VALENTINA CBNI\CONFERENCE\CLINAM 2016\Picture1.png
Here we propose a simple one-pot green synthesis of graphene flakes exfoliated in complete serum, resulting in an extremely stable water dispersion.
Out of the complex protein portfolio in serum, we identified those proteins which assist graphene exfoliation by strongly binding onto the surface. These protein layer represent, therefore, the final biological identity of the material. This work is the fundamental starting point for every toxicological study on graphene giving insights on the possible internalization and trafficking pathways when the material is in contact with cell.

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ATTRIBUTES AFFECTING SITE SPECIFIC NANO-THERAPEUTICS

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In today's era we feel an urgent need to face unsolved problems in medicine and the healthcare industry. Increasing global population and deadly diseases, only contribute to it. The field of nanomedicine has shown great hope in this regard and commercial efforts worth billions have been made and are seen on an upward trend.
Nanotheraputics can be enhanced using "NanoRams", nano entities capable of sensing, signalling, information processing and thus enabling ultra-specified local drug delivery and defence against hostile particles. The NanoRams are programmable nano robotic devices capable of differentiating substances, based on their sensing of chemical structures etc and labelling. These may prove useful outside of medical field but so also suited for tumor tissue targeted actions as these find potential use in changing our approach in the treatment of Malignancy, Malaria, HIV, CRF, diabetes and even vaccine development.
The concept talks about creating the ability to steer right upto the lesion and deliver treatment to the target. A roadmap includes the journey from identifying exact nano sensors–testing them for specific molecular structures/pathogens–intelligence programming and control analysis—in lab testing and further on.
Probably now is the time we need to take a step back, look upon the clues that nature provides us in even the possibly smallest living particles, and add to our approach. NanoRams, put forward an effort to change fundamentals and conventional ways of making decisions for innovations in this ever emerging field of nanomedicine. It should be noted that the concept needs further research and analysis under the collaborative efforts of different wings of nanotechnology to provide an integrative result. There may be no immediate return on investment but this may be a sustainable innovation in the long term to reduce the burden of global healthcare. The least this paper can do is to trigger a novel thought process in the most able minds or throw open a new ray of light to those stuck onto similar objectives!

ANTI-POLYETHYLENE GLYCOL IGG AND IGM ANTIBODIES IN HEALTHY HUMAN DONORS

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Poly(ethylene glycol) (PEG) is often attached to nanoparticles to confer "stealth" properties for increased serum half-life and bioavailability. However, the presence pre-existing anti-PEG antibodies in naive individuals may impact the clinical efficacy and safety of PEGylated medicines by altering their pharmacokinetics, biodistribution or safety profile. In the present study, we developed chimeric human anti-PEG IgG and IgM reference standards to facilitate robust assay of anti-PEG IgG and IgM antibodies in human serum samples. Chimeric antibodies were generated by selection of intermediate anti-PEG binders from a panel of anti-PEG monoclonal antibodies (AGP3, AGP4, rAGP4, E11, 3.3 and 6.3) previously developed in our lab.[1-4] The chimeric antibodies (c3.3-IgG and cAGP4-IgM) have fully human constant regions that can be specifically detected with anti-human IgG or IgM secondary antibodies to facilitate direct comparison of the antibody standard curves with patient samples (Fig. 1). A robust direct ELISA was developed to measure anti-PEG responses in up to 4% human serum with linear log-log responses of 15 to 4000 ng/mL for c3.3-IgG and 3 to 1000 ng/mL cAGP4-IgM (Fig. 2). Assay of serum samples from 1004 naive normal donors revealed a high prevalence of pre-existing anti-PEG IgM and IgG antibodies as well as specific correlations of anti-PEG IgG or anti-PEG IgM with donor sex and age. Our study provides important information on the widespread prevalence of pre-existing anti-PEG antibodies and suggests that additional studies on the clinical impact of pre-existing anti-PEG antibodies on the therapeutic efficacy of PEGylated nanomedicines are warranted.

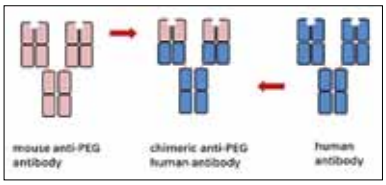

Figure 1. Chimeric human anti-PEG antibodies. Chimeric antibodies were generated by combination of the antigen-binding domains of mouse anti-PEG IgG or IgM monoclonal antibodies with the constant regions of human IgG or IgM antibodies.

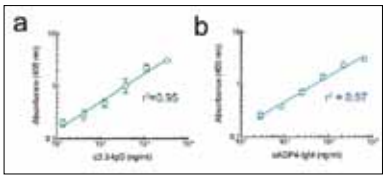

Figure 2. Chimeric antibody standard curves. Linear regression fit of forty eight c3.3-IgG (a) or cAGP4-IgM (b) standard curves transformed to a log-log plot performed on eight separate days over a two month period. Bars, SD.

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CONSTRUCTION OF PEI-CHOLESTEROL LIPOPOLYMER WITH LIPID MICROBUBBLE AS GENE DELIVERY SYSTEM AND IT'S PROTECTIVE EFFECT OF MIF SIRNA ON ACUTE LUNG INJURY IN MICE

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Although less efficient than gene delivery by viral vector, non-viral gene transfer still offers great promise for gene therapy because of its safety profile compared with its viral counterpart. The polyethyleneimine-cholesterol cationic lipopolymer (PC) as gene carries has been constructed and its gene transfer efficiency *in vitro* with lipid microbubbles was presented in this paper. PC lipopolymer was synthesized by linking cholesteryl chloroformate to the amino groups of branched poly(ethylenimine) (PEI) of 1800Da. The structure and molecular weight of PC were confirmed by 1H-NMR and MADI-TOF-MS (Matrix-assisted laser desorption /ionization time-of-flight tandem mass spectrometry) respectively. The average molecular weight of PC was approximately 2000 Da. The gene delivery system of bubble/PC/DNA was constructed by mixed PC/pDNA(N/P 10:1) complexes with lipid microbubbles (2~8μm) which was prepared by DPPC, DSPE-PEG_{2000} and perfluoropropane with the thin-film evaporation technique. pEGFP (enhanced green fluorescent protein) was used as reported gene to investigate the DNA condensing ability of PC lipopolymer by agarose gel electrophoresis. And their cytotoxicity and *in vitro* transfer efficiency of different complexes were compared each other in A549 and MCF-7. The results indicated PC lipopolymer can condense plasmid DNA when N/P ratio up to 4, PC complexes and bubble/PC/DNA complexes were nontoxic to A549 and MCF-7 when formulated at the N/P ratio of 10/1 as determined by MTT assay. This bubble/PC/DNA delivery system provided good transfer efficiency with other desirable characteristics such as against-precipitation of plasma proteins.

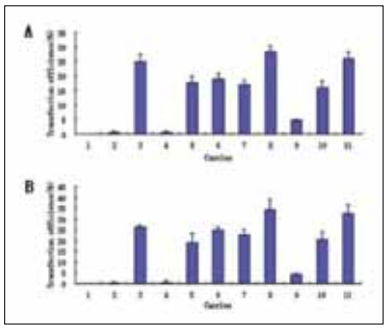

Figure 1. Transfection efficiency in cultured A549(A) and MCF-7 (B) cells after transfection with different carriers complexes. Naked DNA, PEI1.8K/DNA and Lipofectamine2000/pDNA(5:1) complexes were used for comparison. n=3.

1 Control ; 2 Naked DNA ; 3.Lipofectamin2000/pDNA(5:1); 4.PEI1.8K/DNA(270.4ng/200ngN/P=10) ;5 .PC/DNA(304ng/200ng:N/P=10); 6 .lipo(5μg)/PC/DNA(304ng/200ng:N/P=10) 7 .lipo(5μg)/PC/DNA(304ng/200ng:N/P=10)/ultrasound 8. bubble(5μg)/PC/DNA(304ng/200ng:N/P=10)/ultrasound 9. PC/DNA(304ng/200ng N/P=10) /in 10%FCS 10.lipo(5μg)/PC /DNA(304ng/200ng:N/P=10) /in 10%FCS 11.bubble(5μg)/PC/DNA(304ng/200ng:,N/P=10)/in10%FCS

To investigate the protective effect of MIF siRNA mediated by ultrasound lipid microbubbles gene transfer system and evaluate the effectiveness of this gene transfer system. Construction of acute lung injury modal induced by lipopolysaccharide (LPS) in mice which were randomly divided into 4 groups: normal control group (Con), LPS stimulation (LPS), LPS + PC + MIF siRNA treatment group (PC + MIF siRNA), LPS + WP + MIF siRNA treatment group (WP + MIF siRNA). Transfection of MIF siRNA by microbubbles ultrasound using following methods, including EMSA, Western-Blot, ELISA and HE stained for histopathological examination were used, to observed the expression of NF-κB and IκB-α, to detect the secreted level of inflammatory mediators of TNF-α, IL-1β, IL-6, and to assessed the

pathological changes. Result shows MIF siRNA- carried and transferred by WP + MIF siRNA can up-regulated the expression of IκB-α in cytoplasm and significantly inhibited the expression of NF-κB in nuclear, then inhibited the secreted level of inflammatory mediators of TNF-α, IL-1β, IL -6 and improved the pathological changes. But the PC+ MIF siRNA treatment group has no any improvement on acute lung injury in mice. In conclusion, the bubble/PC/DNA complexes is a novel non-viral delivery system and MIF siRNA can be effectively transferred by lipid microbubbles gene transfer system and have protective effect on acute lung injury in mice.

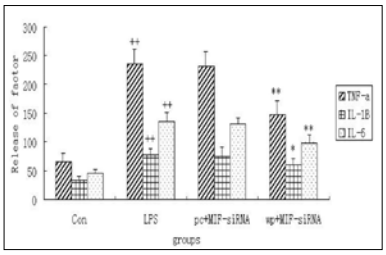

Fig 2 Release of lung homogenates inflammatory mediators in different groups.

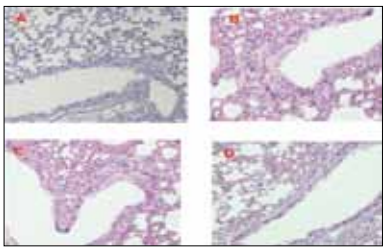

Fig 3. Pathological changes of mice lung tissue in different experiment groups. A:control group. B: LPS stimulation, C: PC+MIF siRNA treatment group, D: WP+MIF siRNA treatment group.

Key words: lipid microbubbles, MIF siRNA, Polyethyleneimine-cholestero, acute lung injury, gene delivery systems.

Acknowledgements: This research was supported by grants from the National Natural Science Foundation of China (NO.81373339 and NO.81173013)

BIOCOMPATIBLE DEXTRAN-COATED SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES (SPIONS): A NEW, SAFE CONTRAST AGENT FOR MAGNETIC RESONANCE IMAGING.

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BACKGROUND

Iron oxide-based contrast agents have been in clinical use for MRI of lymph nodes, liver, intestines and the cardiovascular system, serving mainly as a T2 signal-reducing agents, although they can shorten both T1 and T2/T2* relaxation processes in absorbing tissues. Despite the documented efficacy, no intravenous iron oxide-containing agents are currently approved for imaging applications. Our aim was to characterize a new type of dextran-coated SPIONs (SEONdex) with ultrahigh-field magnetic resonance imaging (MRI) and to analyze the hemocompatibility and immuno-safety of these nanoparticles *in vitro* and *in vivo*.

METHODS

7T MRI of the agarose-embedded nanoparticle samples was performed. The key parameters related to nanoparticle hemocompatibility and immuno-safety were investigated *in vitro* and ex vivo. To address the concerns associated with the hypersensitivity reactions to injectable nanoparticulate agents, we analyzed the complement activation-related pseudoallergy (CARPA) upon the intravenous administration of SEONdex in a pig model.

RESULTS

In this study, we developed an ultrahigh-field MRI protocol to characterize a new type of dextran-coated SPIONs (SEONdex) and analyzed their relaxation properties in T1-, T2- and T2*-weightings in a phantom. SEONdex had a highly significant effect ($p<0.001$) on the relaxation times of all three different weightings in comparison to the agarose control (Fig. 1A-B). As expected, the T1-, T2- and T2*-weighting intensities were declining with increasing SEONdex concentration. In particular in T2*-weighting, SEONdex concentration correlated highly with the relaxation time (R2 = 0.9513).

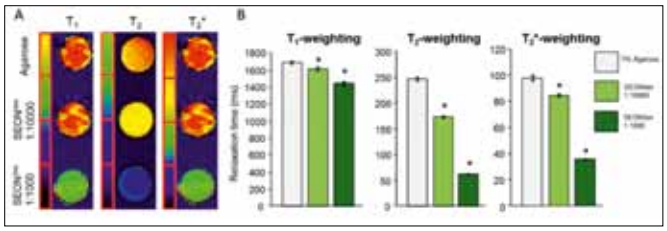

Figure 1. Relaxation times of SEONdex concentration series. The 0.2 mL microcentrifuge tubes were filled with 0, 1:1000, 1:10000 SEONdex (7.57 mg Fe/mL) in 1% agarose gel and embedded in 5% agarose gel. (A) Example images of agarose-embedded SEONdex show reduced T1, T2 and T2-times with the increasing iron content. (B) Relaxation times; T1-, T2- and T2*-weighted MRI was done five times consecutively. ROIs were selected manually in OsiriX. Data are expressed as mean ± SD. *P<0.05 vs agarose control; two-sided, unpaired t-test.*

We further showed that SEONdex nanoparticles *in vitro* did not induce complement or platelet activation, did not affect plasma coagulation or leukocyte procoagulant activity, and had no relevant effect on endothelial cell viability, motility or endothelial-monocytic cell interactions. SEONdex had moreover excellent stability in the whole blood and was characterized by a very low internalization in non-phagocytic cells. The *in vivo* studies in a pig model of CARPA demonstrated that intravenous administration of SEONdex did not evoke the hypersensitivity reaction even at 5 mg Fe/kg, indicating a non-immunogenicity of these nanoparticles (Fig. 2).

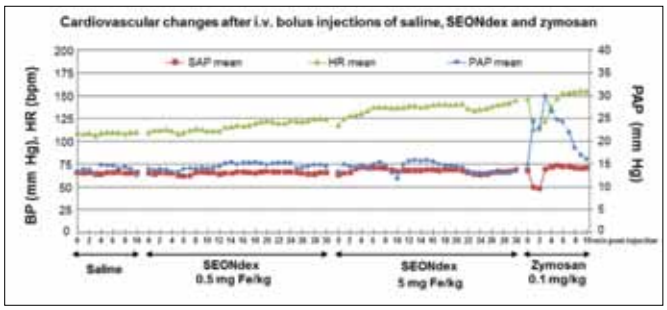

Fig. 2. Cardiovascular reaction to SEONdex in a pig model of CARPA. Saline (negative control) and SEONdex (0.5 mg Fe/kg); followed by 5 mg/kg SEONdex and zymosan (positive control), were injected in the pigs in bolus (<10 s) via the left external jugular vein. Pulmonary arterial blood pressure (PAP), systemic arterial blood pressure (SAP), and hear rate (HR) were continuously monitored for up to 30 min. Means of 2 independent experiments are shown.

CONCLUSIONS

Our findings suggest that due to their superb safety profile, low internalization by non-phagocytotic cells and size-tunability, SEONdex particles represent a suitable candidate for a new generation MRI contrast agent, offering the possibility of repeated administration without inducing hypersensitivity reactions.

FUNDING

This work was supported by the EU ("NanoAthero" project FP7-NMP-2012-LARGE-6-309820), the DFG (CI 162/2-1), the Bavarian State Ministry of the Environment and Consumer Protection and the Cluster of Excellence Engineering of Advanced Materials (EAM).

NANOSYSTEMS FOR INTRAVASCULAR APPLICATIONS: FUNCTIONAL EFFECTS ON HUMAN ENDOTHELIAL AND MONOCYTIC CELLS

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BACKGROUND

The potential clinical impact of nanotechnology in terms of detection and management of cardiovascular diseases is enormous, but no specific nanoparticle-based system has been approved for diagnosis or therapy of atherosclerosis in humans. To ensure clinical safety, the diagnostic and drug-delivery systems intended for intravascular applications must firstly be subject to a close toxicologic scrutiny *in vitro*. Thus, the purpose of this work was to investigate the effects of different types of nanoparticles on endothelial (EC) and monocytic cell functions.

METHODS

Long-term effects of nanoparticles on EC viability were assessed by real-time cell analysis and live cell imaging. ECs grown in bifurcating slides were exposed to chronic laminar or non-uniform shear stress for 20h in the presence or absence of different nanoparticle types, followed by stimulation with TNF-α (2.5 ng/mL) and dynamic monocyte adhesion assay. Endothelial migration and chemotaxis of THP-1 monocytic cells towards MCP-1 were determined. A pilot study in pigs was done to assess the complement activation-related pseudoallergy (CARPA) upon the intravenous administration of liposomal nanoparticles.

RESULTS

We investigated diverse nanosystems, comprising liposomes, lipid nanoparticles, polymer and iron oxide nanoparticles. Some of the nanosystems contained P-selectin targeting agent (fucoidan), contrast agent (gadolinium chelate) or anti-inflammatory glucocorticoid (budesonide). The majority of tested nanoparticles were well tolerated by ECs up to the concentration of 100 µg/mL in static, and up to 400 µg/mL in dynamic conditions. Fucoidan-coated polymer nanoparticles inhibited EC migration (Fig. 1A-B), but had a beneficial suppressive effect on monocytic cell recruitment under non-uniform shear stress (Fig. 2A).

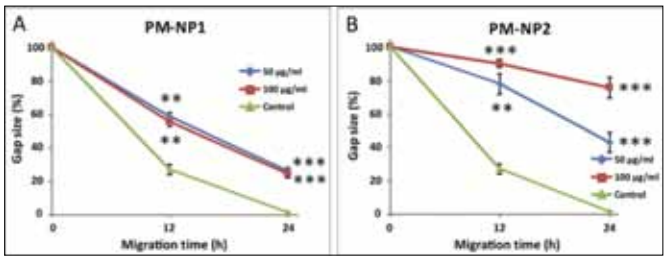

*Fig.1: Effect of polymer nanoparticles coated with fucoidan on endothelial cell migration. HUVECs were pre-treated with (A) PM-NP1, or (B) PM-NP2 at 0, 50 or 100 µg/mL overnight. A gap between two cell layers was created using a cell culture insert. After removal of the insert, cell migration was monitored for 24h. Data are expressed as mean ± SEM; n=3. **p<0.01, ***p<0.001 versus untreated control.*

Lipid nanoparticles also dose-dependently reduced monocytic cell adhesion to ECs under non-uniform shear stress (Fig. 2B). No significant effects of dextran-coated iron oxide nanoparticles on EC migration or monocytic cell recruitment were observed, but lauric acid and albumin-coated iron oxide nanoparticles inhibited EC migration and monocytic cell chemotaxis. Liposomal nanoparticles had no effect on cell migration, but slightly induced monocytic cell recruitment under non-uniform shear stress.

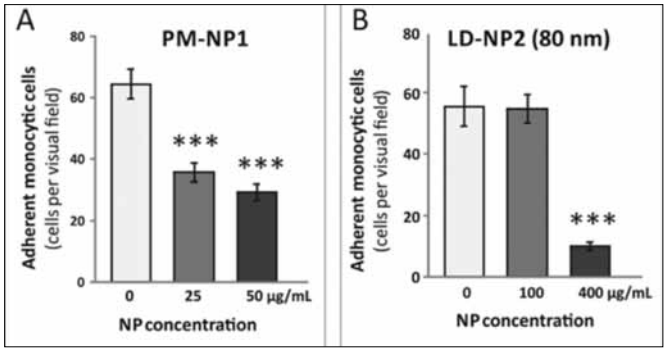

*Fig. 2: Effect of circulating nanoparticles on monocytic cell recruitment. HUVECs grown in bifurcation flow through slides were perfused with (A) Polymeric nanoparticles (PM-NP1), or (B) lipid nanoparticles of 80 nm size (LD-NP2) at 100 or 400 µg/mL for 18 h, followed by stimulation with TNF-α (2h). (A) Adherent THP-1 cells were quantified after 1h of dynamic adhesion assay in at least 8 microscopic images per experiment (non-uniform region, 10x objective magnification), n=3. Data are expressed as mean ± SEM, ***p<0.001 versus untreated control.*

CONCLUSIONS

All lipid based nanoparticles, as well as dextran-coated iron oxide NPs had no effect on endothelial cell migration. However, lauric acid and albumin-coated iron oxide NPs and both polymer nanoparticle formulations significantly decreased EC migration and induced an endothelial growth- and motility-arrest state. Liposomes, lipidots and polymer NPs induced a concentration-dependent decrease in monocytic cell chemotaxis. Monocytic cell recruitment under non-uniform shear stress was inhibited by polymer nanoparticles and lipidots, and increased upon treatment with both liposomal nanoparticle types, whereas iron oxide NPs had no effect.
Taken together, reduced monocytic cell recruitment and chemotaxis can be considered beneficial in terms of vascular inflammation. All of the tested nanosystems show sufficient *in vitro* safety and potential for use in cardiovascular applications, but the observed effects must be carefully taken into account.

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MESOPOROUS SILICA NANOPARTICLES SURROUNDED BY LIPID BILAYER AS DRUG DELIVERY SYSTEM.

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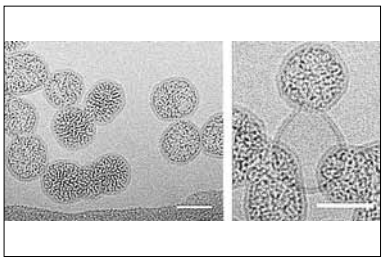

Figure 1 Cryo-TEM measurements show (left) MSNP+@SLB- with a size of 62.3 ± 6.5 nm, and bilayer thickness oaf ~4.7 ± 0.6 nm (right) an intermediate stage where a liposome is in the process of fusing with MSNP+. Scale bar: 50 nm.

Vectors for efficient and targeted delivery of drugs are actively sought for both parenteral and nonparenteral routes of administration for next-generation drug delivery systems (DDS). Vectorization of drugs has typically relied on the use of soft materials, e.g. lipid-based and polymeric nanocarriers. The gamut of options for DDS also includes porous inorganic materials, which however face an inevitable trade-off between the material advantages and potential toxic effects.1 The material advantages of the inorganic vectors are the stability within diverse biological environments, the flexibility to include any desired drugs or combinations and the ability to load different contrast agents. Moreover, multiple processes handle to control the drug-matrix interactions and release kinetics.2 Nevertheless, porous inorganic vectors do not have an efficient way to prevent undesired release, and rendering less control over the ensuing release kinetics of the encapsulated drugs. A relevant measure towards addressing this trade-off would be to combine the molecular functionalities offered by organic carriers and the flexibility and stability offered by the inorganic vectors. To this end, mesoporous silica nanoparticles coated with supported lipid bilayers were attempted in literature,3 with intention to combine favorable material attributes of silica as well as the biomimetic lipid bilayer coatings. Despite high promise, the approach met with a key challenge related to colloidal stability of the particles, due to an incompletely formed supported lipid bilayer.4 Our work addresses this challenge using an ultrasonication mediated liposomal fusion onto positively charged mesoporous silica nanoparticles (MSNP+) to form stable and defect free coatings of negatively charged supported lipid bilayers (SLB-). Thus obtained MSNP+@SLB- with a diameter of 62.3 nm +/- 6.5 nm, showed favorable characteristics towards drug-delivery functions, including:

- ability to load different molecules with high efficiency,
- release them in a controlled manner,
- to carry the payload within cells,
- to be non-cytotoxic to blood cells with and without human plasma, even at high concentrations of nanoparticles.
- SLB- coatings was found to be robust and less influenced by osmotic forces or due to ultrasonication, unlike SLB- coatings formed using literature approaches, that show significant aggregation

The talk would focus on the synthesis and characterization of the MSNP+@SLB-, loading the vectors with model drugs, investigation of their release behavior in biologically relevant environment, investigation of uptake and release within breast cancer cells and evaluation of their compatibility towards RBCs and PBMC cells.

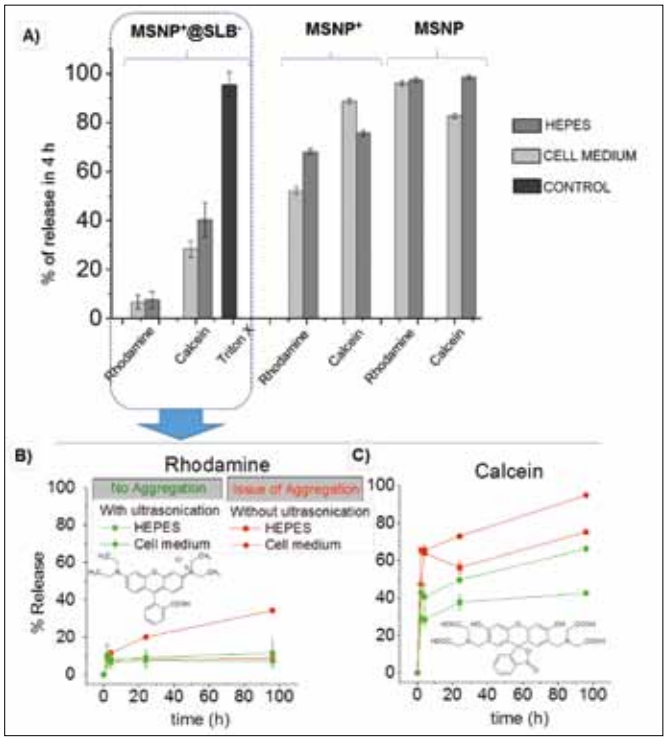

Figure 2 (A) Comparison of probe release profile for MSNP, MSNP+ and MSNP+@SLB- at 4h of incubation in physiological media: HEPES, cell culture medium and Triton X-100 as positive control shows excellent gating behavior of MSNP+@SLB- (B,C) Comparison of (B) Rhodamine and (C) Calcein release profile for MSNP+@SLB- produced by liposomal fusion mediated by ultrasonication and liposomal fusion mediated by electrostatic assembly3 shows better gating behavior for particles obtained via ultrasonication.

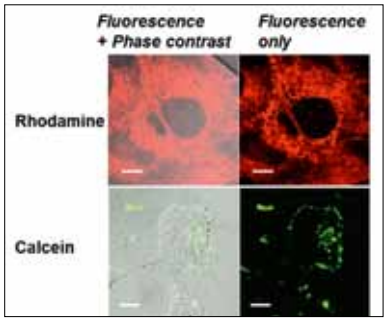

Figure 3 Confocal microscopy pictures of MCF-7 cells incubated for 4h hours with fluorescent MSNP+@SLB – at 0.5 mg/mL. Scale bars indicate 10 µm

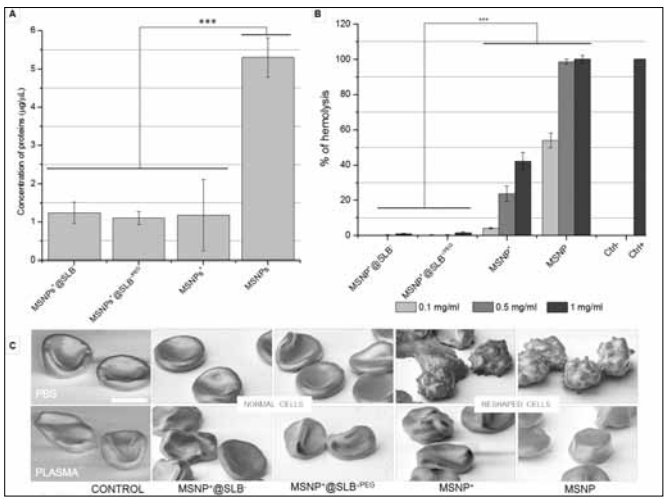

Figure 4 (A) NP were incubated in human plasma for 1 h. This graph shows the quantification of adsorbed plasma proteins on the

*surface of MSNP+@SLB-, pegylated coating of MSNP+@SLB-/PEG, MSNP+ and MSNP. Data are mean ± SE. The comparison between columns provide a *** p < 0.001 performed on three independent experiments. (B) Quantification of released hemoglobin expressed in percentage of hemolysis in PBS buffer. Height of the columns corresponds to the mean values ± SE. The comparison between the population provide *** p < 0.001 (Carried out on four independent experiments). (C) SEM pictures with high-magnification of red blood cells in PBS or human plasma in contact with 0.1 mg/mL of NP of: MSNP+@SLB- and pegylated coating of MSNP+@SLB-/PEG; MSNP+ and MSNP.*

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SYNTHESIS AND CHARACTERIZATION OF A SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLE (SPION) COATED WITH CITRIC ACID FOR MR IMAGING: EFFECT OF TIME AND TEMPERATURE

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INTRODUCTION:

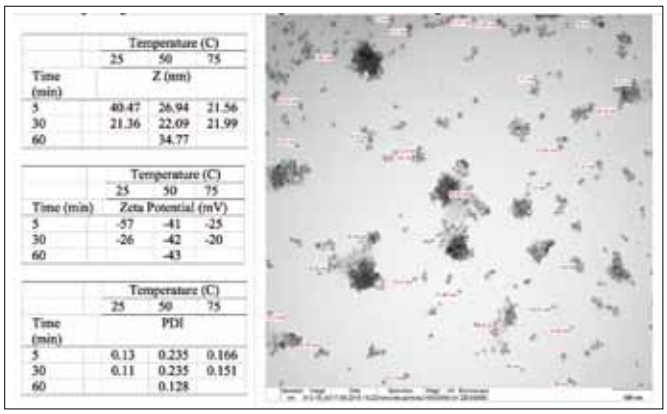
Superparamagnetic iron oxide nanoparticles (SPION) are extensively used for biomedical applications such as magnetic resonance imaging, drug delivery, tissue repair, hyperthermia, etc [1-2]. Numerous methods can be used to synthesize magnetic nanoparticles, including co-precipitation, sol-gel, microemulsion, thermal decomposition, and mechanical synthesis [3-4]. The co-precipitation technique is the simplest and most efficient way to obtain a large amount of magnetic particles. Here, SPION coated with citric acid (hydrophilic agent) were synthesized by co-precipitation method, and the effect of reaction time, reaction temperature, pH, stirring speed, sonication and centrifuge on particle size and distribution was investigated. The particle size was determined and compared with transmission electron microscopy (TEM) and dynamic light scattering (DLS) every two weeks. Finally, the application of these magnetic nanoparticles for MR imaging was investigated.

METHODS:

Magnetic nanoparticles coated with citric acid were synthesized using Fe(II) and Fe(III) salt chemical co-precipitation with aqueous ammonia solution under vigorous stirring and sonicated and centrifuged. The samples were prepared at different reaction times (5, 30 and 60 min), reaction temperatures (25, 50 and 75 °C), pH (8-13), stirring speed (600-1200 rpm), sonication (0-20 min) and centrifuge speed (7000-14000 rpm). Iron concentration was measured by means of the phenanthroline assay. MR Relaxometry of the SPION was performed on a clinical 3T whole-body MR scanner (Philips Achieva) using a knee coil (SENSE-flex-M; Philips) at room temperature.

RESULTS:

The optimum values for pH, stirring speed, sonication and centrifuge speed were obtained from different tests and were fixed at 12, 800 rpm, 20 min and 14000 rpm, respectively. Fig. 1a shows the DLS results for different samples prepared at different times and temperatures. Fig. 1a indicates that SPION coated with citric acid prepared at 25 °C and with reaction time 30 min have the smallest size with narrow size distribution. TEM micrography of this sample showed a core size around 10 nm (Fig. 1b). The size of particles was checked every two weeks and the DLS results showed that there was no significant change in particle size and zeta potential for this sample even after 6 months.



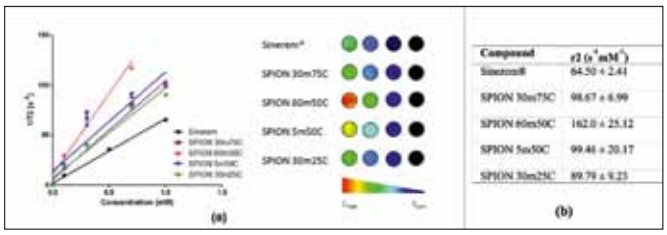
	Temperature (C)		
	25	50	75
Time (min)	Z (nm)		
5	40.47	26.94	21.56
30	21.36	22.09	21.99
60		34.77	

	Temperature (C)		
	25	50	75
Time (min)	Zeta Potential (mV)		
5	-57	-41	-25
30	-26	-42	-20
60		-43	

	Temperature (C)		
	25	50	75
Time (min)	PDI		
5	0.13	0.235	0.166
30	0.11	0.235	0.151
60		0.128	

Fig. 1. DLS results of the samples at different times and temperatures (a, left) and TEM micrograph of the sample prepared at 25 °C and 30 min (b, right).

Transverse relaxation rates were measured in TSE scan mode using multi-slice sequences and calculated by fitting an exponential curve to the signal amplitudes for each segmented region using the Imalytics Preclinical Software (Fig.2a). Furthermore, for several selected samples, relaxivity values (r2) were calculated on the basis of relaxometry measurements, and these were found to be higher than the relaxivity of the commercial SPION MRI agent Sinerem® (Fig. 2b).



Compound	r2 ($s^{-1}mM^{-1}$)
Sinerem®	64.50 ± 2.41
SPION 30m75C	98.67 ± 6.99
SPION 60m50C	162.0 ± 25.12
SPION 5m50C	99.46 ± 20.17
SPION 30m25C	89.79 ± 9.23

(a) (b)

Fig. 2. Transverse relaxation rate of SPIONs (1/T2) as a function of Fe concentration at 3T MRI (a) and Relaxivities of synthesized SPIONs (b) (Control: Sinerem®).

CONCLUSION:

We investigated several operational parameters including reaction time, reaction temperature, pH, stirring speed, sonication and centrifuge speed on particle size and size distribution of magnetic nanoparticles. We obtained SPION with small size, low polydispersity index and excellent MR imaging properties.

NANOPARTICLE-LOADED MESENCHYMAL STEM CELLS FOR ELIMINATION OF CANCER STEM-LIKE CELLS

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Despite progress made in cancer treatment, it is commonly accepted that standard methods are not effective enough. It was noticed that similar to healthy tissue malignant tissue is heterogeneous and comprises of the cells with diverse differentiation level. A small subpopulation of tumor cells that is responsible for tumor initiation, growth and metastasis was found. These cells were called cancer stem-like cells (CSCs). CSCs maintain tumor growth and possess chemo- and radioresistance. Therefore, conventional therapies eliminate differentiated less aggressive cancer non-stem cells, while CSCs survive the treatment (Fig.1.)[1]. Due to CSCs, cancer relapse is such a common incidence.

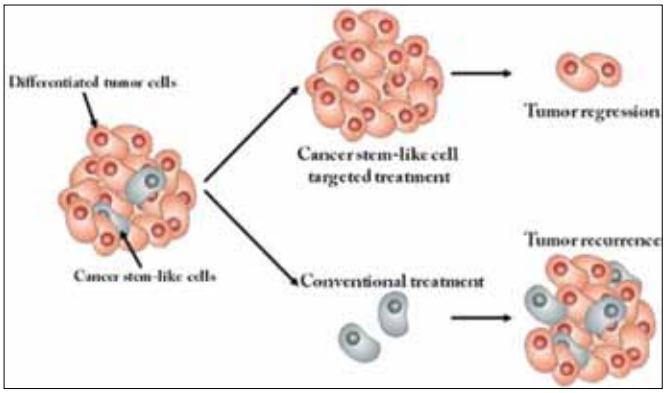

Fig. 1. Cancer stem-like cell specific and conventional cancer therapies.

It was suggested that nanomedicine could be a solution to permanent elimination of CSCs. Due to nanosize, materials gain useful properties: gold nanoclusters and semiconductor nanocrystals begin to fluoresce, iron oxide nanoparticles become superparamagnetic, etc. Because of these properties and large surface area, which enables conjugation of various therapeutic molecules, nanoparticles quickly gained much attention in oncology[2].

First, it was claimed that nanoparticles should accumulate passively in the tumors due to enhanced permeability and retention (EPR) effect – defective and leaky tumor vessels should allow easier penetration of nanoparticles into the tumor than into a healthy tissue, while poor lymphatic drainage should keep nanoparticles „locked" inside the tumor[3]. However, passive accumulation of nanoparticles lacks specificity to tumors[4; Dapkute et al., unpublished data]. As a result, functionalization of nanoparticles using various targeting molecules – ligands, peptides, antibodies, etc. – became a natural choice [1]. However, the lack of tumor-specific antigens made this strategy more complicated.

Therefore, it was suggested to use cellular nanoparticle vehicles, which are naturally attracted to tumor tissues. Due to their inherent tissue regeneration function, mesenchymal stem cells (MSCs) migrate toward inflammations and wounds. As tumors secrete high concentrations of similar chemokines as a damaged tissue, MSCs intensively migrate toward tumors. Therefore, it was proposed to use MSCs for tumor-specific delivery of drugs and nanoparticles[5].

In our study, it was investigated whether MSCs could transport quantum dots (QDs). QDs have wide fluorescence excitation and narrow emission spectra, large absorption coefficients, high brightness and photostability. Due to these optical properties, these nanoparticles could be used for the early cancer diagnostics. QDs also have a large surface area that can be easily modified with drugs, photosensitizers, antibodies and/or other targeting molecules. Therefore, QDs can also be used for cancer treatment[1, 6]. Combining both diagnostic and therapeutic functions QDs could become a multifunctional nanoplatform for the imaging and eradication of cancer. QDs used in the experiments were commercially available Invitrogen Qdot® 625 ITK™ Carboxyl QDs. Although other authors mostly use MSCs extracted from bone marrow or adipose tissue, in our experiments MSCs from donor skin tissue were used. The successful application of dermal MSCs for the delivery of nanoparticles would be very advantageous as skin tissue has the widest area and the easiest accessibility in the human body. In addition, after some surgeries skin tissue is considered a waste and therefore could be used for cost-effective appliance in cancer treatment.

In our experiments, we first determined the optimal conditions for maximum QDs loading into MSCs. Firstly, lactate dehydrogenase (LDH) cytotoxicity test was used to determine the maximum non-toxic QDs concentration. Then we incubated MSCs with QDs for various time intervals, analyzed the cells with BD Accuri C6 flow cytometer and determined that after 6 hours of incubation the saturation of QDs uptake is reached. Nikon Eclipse TE2000-S, C1 Plus confocal microscope revealed the intracellular localization of nanoparticles. QDs enter the cell via rapid endocytosis and are localized in the vesicles spread all over the cytoplasm (Fig. 2).

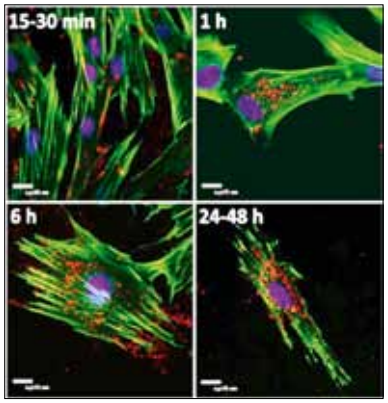

Fig. 2. Confocal fluorescence microscopy images showing intracellular localization of QDs in MSCs during different incubation time intervals. Blue – nuclei (Hoechst), green – actin (Alexa Fluor 488® Phalloidin), red – QDs.

For MSCs to be used as vehicles of QDs, nanoparticles should not diminish migratory properties of the cells. Therefore, we determined the effect nanoparticles have on both *in vitro* and *in vivo* migration of MSCs. To evaluate *in vitro* migration we used cell culture inserts with 8 µm pores. We tested the ability of QD-loaded and unlabeled MSCs to migrate through the pores of insert toward various chemoattractants – growth medium supplemented with 20% fetal bovine serum (FBS), human breast cancer cell line MDA-MB-231, which possess CSCs properties, human breast cancer non-stem-like cells MCF-7, and non-cancerous human breast epithelial cells MCF-10A. The results revealed that MSCs migrate toward positive control (growth medium with 20% FBS) and CSCs but the migration toward MCF-7 cancer cells and non-cancerous cells is the same as the undirected random migration of MSCs. In addition, QDs did not reduce the migration of MSCs. In vivo migration studies were performed on CB17 SCID immunodeficient mice. The mice were injected with breast CSCs (MDA-MB-231) into mammary fat pat and after a month the human tumor xenografts were formed. QD-loaded MSCs were injected above the tumor. The mice were sacrificed after a week and the organs were both cut into slices using microtome and homogenized into single cell suspension for flow cytometric analysis. The results showed that MSCs were able to migrate selectively to the tumor and metastatic tissue but not to healthy organs. Both *in vitro* and *in vivo* experiments show nanoparticle-loaded MSCs specificity to CSCs.

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A NEW GMP-COMPATIBLE RADIOLABELLING METHOD ENABLES LONG TERM IN VIVO PET TRACKING OF PREFORMED LIPOSOMAL NANOMEDICINES

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INTRODUCTION

Quantitative imaging methods for tracking liposomal drug nanocarriers *in vivo* are of high interest in nanomedicine. In this context, PET imaging, with its excellent quantification properties, could be used to predict treatment efficacy and the stratification of patients into different treatment regimes [1,2]. To date, however, methods to radiolabel liposomal drugs with metallic PET isotopes have relied in the introduction of chelators to the lipid bilayer or by co-encapsulation with the drug. Both methods represent a significant barrier for clinical translation of preformed liposomal drugs.
We hypothesised that it should be possible to radiolabel preformed liposomal drugs, without modification of their components, if the encapsulated drug has metal-chelating properties (Fig. 1). Here we demonstrate this method based on metastable cell labelling agents and its application for monitoring and quantifying drug biodistribution using PET in two murine cancer models (Fig. 2).

METHODS

Preformed liposomal nanocarriers (liposomal alendronate (PLA) and liposomal doxorubicin (Doxil/Caelyx)) where labelled with ^{89}Zr ($t_{1/2}$ = 3.2 d, 23% β^+) and ^{64}Cu ($t_{1/2}$ = 13 h, 17% β^+) using cell membrane metal ionophores (hydroxyquinolines). Radiolabelling yields and *in vitro* stabilities were calculated using size exclusion chromatography. PET/SPECT-CT imaging was performed in murine models of breast cancer (MTLn3E-hNIS) and ovarian cancer (SKOV3). Ex vivo biodistribution studies were performed at the end of the imaging studies.

RESULTS

Radiolabelling yields of up to >98% with specific activities in ranges as high as 100 GBq/µmol of encapsulated drug (^{89}Zr) were achieved. Empty liposomes, with the same phospholipid composition and hydrodynamic size as PLA/Doxil, did not radiolabel. *In vitro* stabilities in human serum were >85-95% after 48 h at 37°C. ^{89}Zr/^{64}Cu-PLA were imaged in murine tumour models of breast (MTLn3E-hNIS) and ovarian cancer (SKOV-3) for up to 7 days (^{89}Zr-PLA) or 2 days (^{64}Cu-PLA). Radioactivity at the end of the studies was mainly found in the spleen, liver, primary tumour (5-10% ID/g) and blood (8-10% ID/g). Interestingly, in the MTLn3E-hNIS model, uptake in metastatic organs such as the sentinel lymph nodes, ascertained by SPECT reporter gene imaging, was significantly higher (16% ID/g) than in non-metastatic and control lymph nodes (6% ID/g).

CONCLUSIONS

A new, highly efficient and stable method to radiolabel preformed liposomes with PET radiometals has been developed. Liposomes radiolabelled using this method can be tracked *in vivo* using PET imaging for at least 7 days allowing quantification and biodistribution measurements of liposomal drugs. Our technology is GMP-compatible and we are working towards translating it for human use in conjunction with clinically approved liposomal anti-cancer drugs.

Figure 1: (a,b) Schematic representation of our novel drug nanocarrier radiolabelling method. Note that in the absence of drug, radiolabelling is not successful. (c) Radiolabelling with Zr-^{89} and Cu-^{64} is radionuclide-, concentration- and drug-dependent.

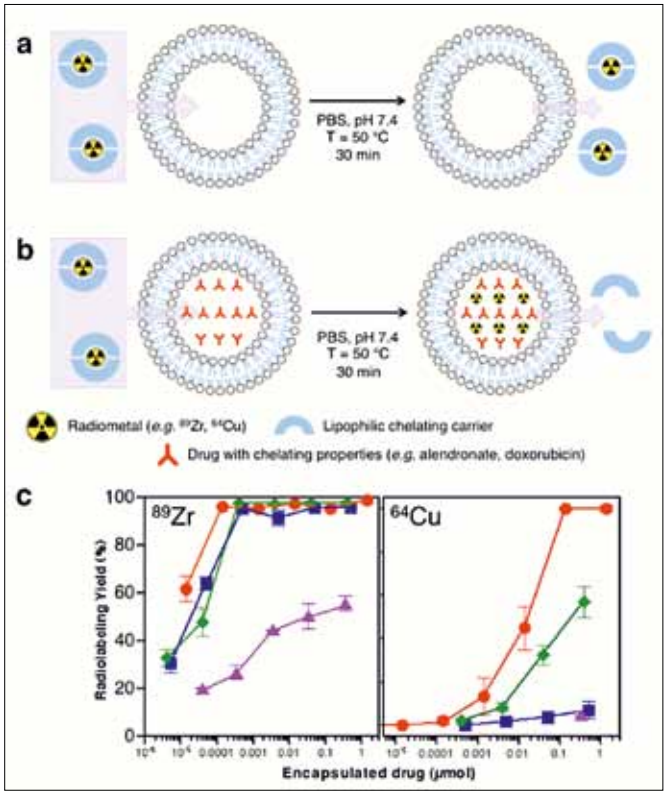
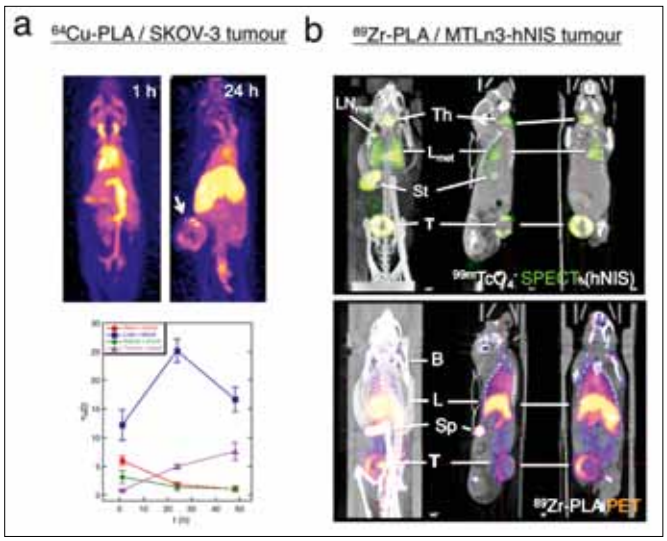


Figure 2: In vivo imaging: (a) MIP images SKOV-3 tumour and image-based biodistribution; (b) MTLn3E-hNIS tumour (top) SPECT reporter gene imaging showing primary tumour (T) and metastatic organs (Lymph nodes and lungs) (bottom) ^{89}Zr-PLA PET imaging of same mouse and timepoint (72h)



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prehensive Cancer Imaging Centre funded by the CRUK and EPSRC in association with the MRC and DoH (England).

THIRD GENERATION ABRAXANE: LM-101 – AN INJECTABLE PHOSPHOLIPID-COATED NANO-PARTICLES LOADED WITH PACLITAXEL. COMPOSITION AND METHOD OF PREPARATION, ELECTROCHEMICAL CHARACTERIZATION AND DISSOLUTION STUDIES

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Introduction: Successful paclitaxel nanoparticles formulations include Abraxane (an albumin bound nanoparticle paclitaxel) and Genexol-PM (a polymer bound nanoparticle paclitaxel). Previously, we have reported on the development of Genexol-PM as 2nd Gen Abraxane. The development of Genexol-PM was a significant step forward in manufacturing with utilization of a one pot synthesis technique using a biodegradable di-block copolymer composed of methoxy-poly(ethylene glycol)-poly(lactide) to form nanoparticles with paclitaxel containing hydrophobic core and a hydrophilic shell. (Fig.1) However, clinical hypersensitivity and instability in serum/plasma remain problematic. Here we report on the one pot synthesis of paclitaxel nanoparticle formulations using phospholipids which retains the desired plasma instability of Abraxane and the PBS stability of Genexol-PM. Due to the use of naturally occurring phospholipids in this 3rd Gen Abraxane, hypersensitivity should not be an issued.

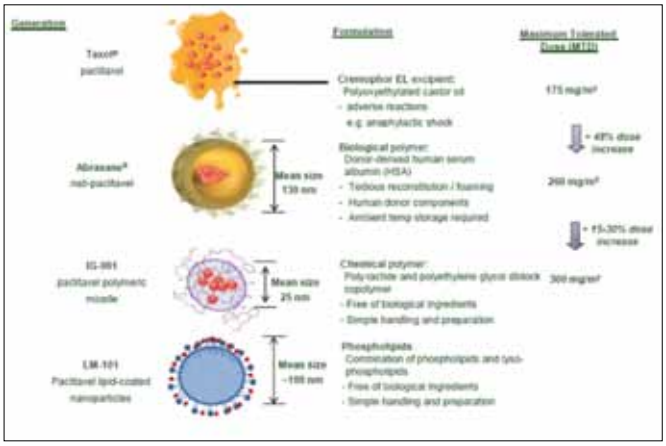

Fig. 1: Evolution of the paclitaxel nanoparticle

METHODS

Nanoparticle synthesis was conducted using two methods; Method 1: microfluidization-solvent evaporation (similar to Abraxane method) and Method 2: thin film hydration (one pot method similar to Genexol-PM method). Briefly, in method 2, phospholipids and paclitaxel were dissolved in ethanol and subjected to rotary evaporation until a thin film was formed and all the solvents were evaporated. The film was then hydrated using deionized (DI) water to produce paclitaxel loaded phospholipid nanoparticles. Nanoparticle size and zeta potential were measured using a Malvern ZS DLS system. The formulation was subjected to serial filtration using 1.2µm, 0.8µm, 0.45µm and 0.2µm syringe filters. The drug incorporation/loading in phospholipid nanoparticles was measured using ELISA. Electrochemical properties of the formulation were measured using screen printed carbon nanotube electrodes from DropSens and a PGSTAT204 Autolab station from Metrohm. Testing for dissolution of nanoparticles in human plasma, 50 mg/mL human serum albumin (HSA) solution, PBS and DI water was performed by measuring nanoparticle size using dynamic light scattering.

RESULTS

A series of phospholipids and lyso-phospholipids were tested for assembly of paclitaxel nanoparticles. The findings were as follow: short chain lipids such as PC 10 (1,2-didecanoyl-sn-glycero-3-phosphocholine), PC 12 (1,2-dilauroyl-sn-glycero-3-phosphocholine), Lyso PC 10 (1-decanoyl-2-hydroxy-sn-glycero-3-phosphocholine) and Lyso PC 12 (1-lauroyl-2-hydroxy-sn-glycero-3-phosphocholine) produce particles with good drug loading, optimal size and stability. Method 2 produced nanoparticles with higher drug loading and higher stability as compared to Method 1. The electrochemical property of formulations synthesized using method 1 and method 2 were different with one being negatively charged and the other being positively charged. Each formulation exhibited distinct cyclic voltammetry (CV) scan (Fig. 2). The difference in charge on the particles produced by two different methods is indicative of different organization of lipids around the particles produced by method 1 and method 2.

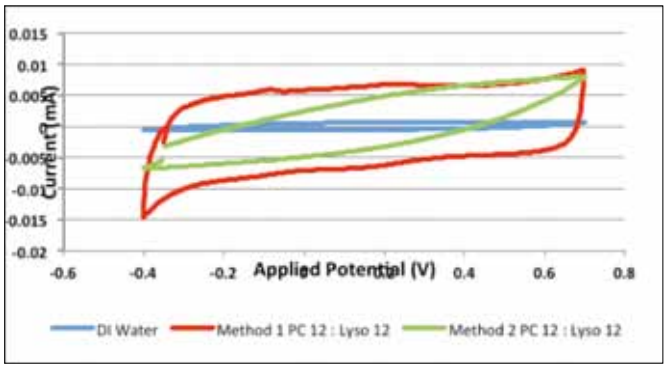

Fig. 2: Electrochemical measurement (cyclic voltametry) of the two formulations of PC 12; Lyso-PC 12 synthesized using method 1 and method 2.

The phospholipid nanoparticles produced using Method 2 rapidly disintegrated in human plasma showing that it has dissolution properties similar to Abraxane and Genexol-PM (Fig. 3). Like Genexol-PM, the nanoparticles remained intact in DI water and PBS solution even at very low paclitaxel concentration (1-10 ug/ml). This would allow LM-101 to be administered as PBS-diluted solution into peritoneal cavity for treatment of ovarian cancer or distilled into the bladder for treatment of bladder cancer. The high plasma/serum instability of Genexol-PM was eliminated and LM-101 acquired behavior similar to that of Abraxane in serum/plasma. This would allow LM-101 to be administered intravenously with PK properties of Abraxane for the treatment of breast, lung, pancreatic, and melanoma cancers.

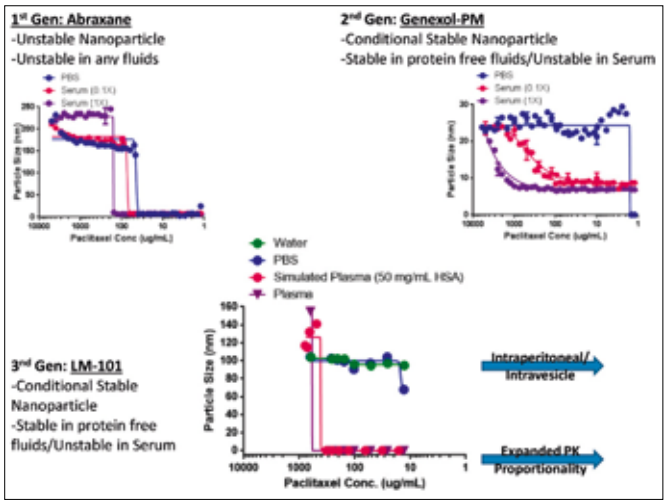

Fig.3: Dissolution of paclitaxel nanoparticles formulation (LM-101) in human plasma, simulated human plasma (50 mg/mL human albumin solution), PBS and DI water.

CONCLUSIONS

A new nanoparticle paclitaxel formulation was formulated using phospholipids, which shows similar dissolution behavior as Abraxane and Genexol-PM. We are planning to put this into bioequivalence trial for regulatory approval against reference listed Abraxane. This regulatory pathway pioneered by us for Genexol-PM and is currently being used widely by other developers of nanoparticle paclitaxel formulations. Clinical trials conducted with Genexol-PM demonstrated significant antitumor activities comparable to historical data for Abraxane and across three cancer indications (Metastatic Breast Cancer, Non-small-cell lung carcinoma, and Pancreatic Cancer). We are expecting similar results with the phospholipid coated paclitaxel nanoparticle described here

DIFFERENTIAL LIGHT SCATTERING (DLS) FOR SIZE MEASUREMENT OF IRON SUCROSE: A VALIDATED METHOD

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INTRODUCTION

Iron sucrose (IS) is a colloidal drug used to increase iron blood levels in iron deficiency anemia. During last decade, several intended copies of IS were approved following the generic paradigm approach. However, in 2011, clinical data provided evidence that patients receiving either IS or one of its copies were showing different clinical outcomes1. In order to assess the pharmaceutical quality of both IS and its copies, the European Directorate for the Quality of Medicines & HealthCare (EDQM) installed the Non-biological complexes (NBC) working party. One of the main tasks of the working party is to draft a monograph to prove unequivocally the quality of IS. In this perspective, we suggest an ICH validated analytical procedure based on the use of dynamic light scattering (DLS) to determine the hydrodynamic diameter of these particles.

MATERIALS AND METHODS

IS underwent a 50-fold dilution in Milli Q water starting from the concentrated solution (20 mg Fe/mL) as previously described2. Hydrodynamic diameters of IS were successively determined as size distribution in Number using a Zetasizer Nano S (Malvern, UK). The scattering angle was set at 173° and the He-Ne laser beam was used at λ=633 nm. The refractive index was set at 1.33413. Each sample was introduced into a disposable polystyrene cell and the test was carried out after an equilibration time of 60 seconds.

RESULTS AND DISCUSSION

The hydrodynamic diameter of IS was identified as equal to 7.0 ± 0.1 nm (n=3).
In order to prove the reliability of the assay, a validation of the analytical procedure was performed. Following ICH guideline Q2 (R1)4, the specificity of the procedure was assessed using negative controls, positive controls and different stress tests. The results of the validation are reported in the following table.

*Table 1: Summary of the ICH validation for the DLS procedure (n=3). *RSD=Relative standard deviation*

	Assay	Acceptance Criteria	Results	Pass/Fail
Negative controls	Milli Q water	Absence of particles in IS range	No particles detected	Pass
	NaCl 10mM		No particles detected	Pass
	KNO_3 10mM		No particles detected	Pass
	Sucrose pH=11		No particles detected	Pass
Positive controls	Ferric carboxymaltose	Different size compared to IS	17.2 ± 0.4 nm, RSD= 2.3%	Pass
	Iron polymaltose		26.3 ± 0.5 nm, RSD= 2.0%	Pass
	Iron dextran		9.9 ± 0.5 nm, RSD= 5.3%	Pass
	Sodium ferric gluconate		9.4 ± 0.2 nm, RSD= 2.1%	Pass
Stress conditions	IS 0.4 mg Fe/mL pH=2.5	None	Degradation of the complex	Pass
	IS 0.4 mg Fe/mL pH=11		7.0 ± 0.7 nm, RSD= 10.2%	Pass
	IS 0.4 mg Fe/mL -20°C/24h		7.3 ± 0.1 nm, RSD= 1.0%	Pass
	IS 0.4 mg Fe/mL 37°C 80 rpm/24h		6.9 ± 0.1 nm, RSD= 1.0%	Pass
	IS 0.4 mg Fe/mL 100°C/24h		10.3 ± 0.5 nm, RSD= 5.1%	Pass

Following ICH requirements, negative controls were represented by samples that do not contain the analyte. We proved the absence of any particles in the IS size range in three relevant media: Milli Q water, NaCl 10mM and KNO3 10mM. Moreover, we proved the absence of particles in an alkaline solution of sucrose.
In addition, four iron sucrose drugs, which show a closely related structure to IS, where used as positive controls. Sodium ferric gluconate, iron dextran, ferric carboxymaltose and iron polymaltose showed larger sizes than IS. The RSD value was always lower than 15%.
Stress tests were carried out with attention to the behavior of IS in acidic/alkaline environment. Successively the effect of cold/heat stress was evaluated.
We showed the degradation of IS in acidic environment, whereas the solution is stable at alkaline pH. Furthermore, we proved the stability of IS after incubation at -20°C for 24h and after incubation at 37°C under slow agitation (80 rpm). Finally, we evaluated a minor increase in the hydrodynamic diameter of IS when incubated at 100°C for 24h.

CONCLUSION

We successfully established an analytical procedure to determine the hydrodynamic diameter of IS solutions. ICH validation revealed the robustness and reliability of our DLS protocol. Authorities might further use this protocol to characterize IS and evaluate the quality of its intended copies.

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MICROVASCULAR STRUCTURE IN TUMORS AND HEALTHY TISSUES

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Microvascular structure is a key to understand tumor formation and the following invasion of the surrounded tissues. Small differences in morphology and physiological behavior of the angiogenic vessels at a microscopic level have an impact on the treatment efficacy. Moreover, the situation is complicated by the well-known chaotic organization of the proliferative mass. This chaotic structure, which is reflected both by tissue morphology and the vascular architecture, is responsible for the heterogeneous behavior of tumors. The aim of this work is to present a mathematical framework that explicitly considers the spatial variability at a voxel level with the aim to evaluate microvascular structure in tumors and healthy tissues. Such framework consists of two classes of geometrical estimators: the texture class allows estimating the fractal dimension (FD), which is a measure of the self-similarity at different length scales, and the lacunarity (L), which quantifies the relative distribution of substructures within the tissue. The shape class yields measures of the compactness, which describes the deviation

of a mass from spherical symmetry, and the signature, which is a measure of the branching or infiltration of the tumor into the surrounded healthy tissues. We tested this analysis framework in an *in vivo* study using a group of twelve mice injected s.c. with C51 tumor cells. Six mice were treated with a proangiogenic drug (dimethyloxalylglycine, DMOG) and six with a placebo (saline). We analyzed some physiological parameters describing the tumor vasculature: permeability derived from DCE-MRI and perfusion, i.e. blood volume and flow, derived from DSC-MRI. This analysis was complemented by a detailed study of the vascular architecture using vessel size index (VSI) MRI and synchrotron radiation-based microCT. We found significant differences in the FD and L values between treated and non-treated group either for both the permeability and perfusion maps. The FD values increased significantly in response to treatment. A higher level of FD means smaller self-similarity and, therefore, more chaotic structure, which may reflect angiogenesis, i.e. an expansion of the chaotic capillary network. FD values did not change in response to treatment with the vehicle only. The L values remained unchanged in the DMOG group and significantly decreased in vehicle treated mice. The increase in FD values is corroborated by the structural analysis of the tumor vasculature. VSI showed a persistent predominance of capillaries during tumor growth, but no formation of bigger vessels indicating the absence of hierarchical organization. This is in line with the microCT results, which reveal a high number of highly tortuous capillaries in C51. In conclusion, the use of non-biased mathematical methods that account for the heterogeneity of tumor tissue enables the identification of changes that are masked when analyzing volume averaged data sets. The biological basis for the changes observed in FD and L remain to be analyzed.

Keywords: Tumor vascular structure, angiogenesis, texture analysis, fractal dimension, lacunarity.

FOLATE RECEPTOR-TARGETED LIPOSOMES WITH ENCAPSULATED ANTI-SARCOSINE ANTIBODIES FOR ATTENUATION OF PROSTATE TUMORS

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Prostate cancer is the most occurring malignancy in men from USA or Western Europe with 29% of men of 30 to 40 years of age and 64% of men of 60 to 70 years of age suffering from prostate cancer [1]. One of the delineated oncometabolites in prostate cancer is the amino acid sarcosine (N-methylglycine) that plays a substantial role in progression of prostate cancer[2].

In this work, anti-sarcosine antibodies were employed for attenuation of prostate tumours in xenograft mice. The antibodies were modified with CdTe quantum dots for the detection of their intracellular delivery. Free antibodies are unable to enter intracellular space, so to enable internalization of these antibodies in the tumour intracellular space needed for subsequent neutralization of sarcosine, the antibodies were encapsulated in liposomes with loading efficiency of 69.2%. To increase the shuttling efficiency of antibodies-loaded liposomes, their surface was modified with folate, enabling entering of tumour cells via clathrin-mediated endocytosis[3], due to significant overexpression of folate receptors in a wide variety of cancer cells, including prostate cancer[4]. The resulting particles showed an average particle size of 172 nm and zeta potential of +22 mV at pH 7.4 (Fig. 1A). The successful modification with folic acid was confirmed using absorbance (Fig. 1B) and fluorescence (Fig. 1C) spectra of the nanoparticles.

Next, *in vitro* cellular uptake of these liposomal formulations was confirmed using human PC-3 prostate cancer cell line overexpressing folate receptors [5]. The uptake in PC-3 cell line was evaluated by fluorescence microscopy 0; 3; 6 and 12 h from application (Fig. 1D). The obtained results showed willing interactions between PC-3 cells and liposome-encapsulated anti-sarcosine antibodies. Uptake was observable in some cells after 3 h from application and it was increased after 6 h and further after 12 h from application. Moreover, a competitive cellular uptake assay was performed using free folic acid which decreased the cellular uptake of liposomes-encapsulated anti-sarcosine antibodies (Fig. 1E), thus showing that folate receptors play important role in the cellular uptake. Moreover, positively charged surface of liposomes enables them to interact with negatively charged proteoglycans in cellular surface prior to the binding to folate receptors.

In vivo effect of the liposomes-encapsulated anti-sarcosine antibodies on 30 mm^3 PC-3 xenograft tumours was evaluated. The mice were repeatedly intraperitoneally administered with the liposomal formulations for 35 days. The tumour growth was measured twice per week and liposomes-encapsulated anti-sarcosine antibodies showed antitumor activity with 32.5% decrease of tumour weight after the treatment (Fig. 1F).

Using microarray assay, the expression levels of various genes were evaluated (Fig. 1G). The tumours treated with liposomes-encapsulated anti-sarcosine antibodies showed positive regulation ($p < 0.05$) of selected pro-apoptotic genes – BAX, CCND, TEGT, E2F3 or PDGFRB and significant down-regulation ($p < 0.05$) of GAB2, encoding GAB2 scaffolding protein, which serves as a platform for the assembly of signalling systems fundamental for the development of prostate cancer[6]. The administration of liposomes-encapsulated anti-sarcosine antibodies did not cause any significant differences in mice weight and no mice died prior the end of experiment. The treatment of prostate cancer with anti-sarcosine antibodies, especially co-administered with other anticancer drugs, can enhance the treatment efficiency.

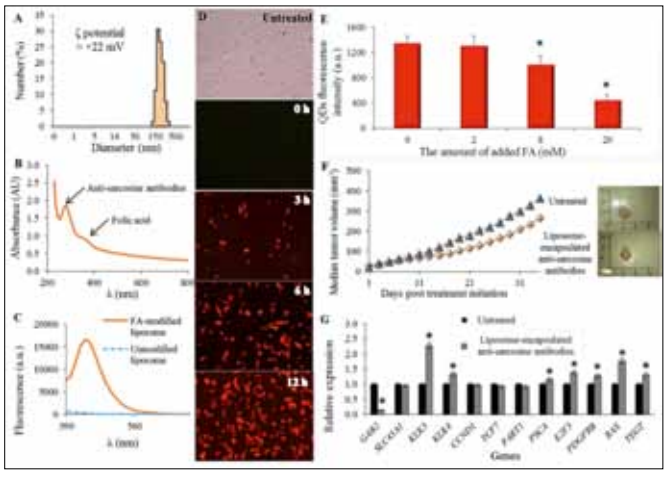

Fig. 1: The preparation of liposome-encapsulated anti-sarcosine antibodies targeted to folate receptors and its influence on prostate cancer cell line and prostate tumours in xenograft mice. A) Characterization of liposomes encapsulating anti-sarcosine antibodies by dynamic light scattering in PBS pH 7.4. B) UV-Vis absorption spectrum of liposome-encapsulated anti-sarcosine antibodies after modification of liposome surface with folic acid (λ = 270 nm corresponds to encapsulated anti-sarcosine antibodies and λ = 365 nm

corresponds to the surface modification with folic acid). C) Fluorescence spectra of liposomes prior and after modification with folic acid obtained using λexc = 360 nm. D) Time dependence of cellular uptake of QDs-labelled anti-sarcosine antibodies encapsulated in folate receptor-targeted liposomes into PC-3 cells obtained by inverted fluorescence microscopy (length of scale bar is 200 µm). Cells were incubated with 10 µM of the complex. E) The competitive inhibitory effects in PC-3 cells. Values represent the mean ± SD of three experiments. Asterisks indicate significant differences ($p < 0.05$). F) Average tumour volume in the nude mice bearing subcutaneous PC-3 tumours. Inset shows the tumour from untreated mice and mice treated with folate receptor-targeted liposomes-encapsulated anti-sarcosine antibodies at the endpoint of experiment. G) Representation of relative expression of genes, specific to any aspect of prostate cancer, found as significant up- or down-regulated in post-treatment tumour tissues. GAB2 - GRB2-associated binding protein 2; SLC43A1 - Solute carrier family 43, member 1; KLK3 - Kallikrein 3 (prostate specific antigen) PSA; KLK4 - Kallikrein 4 (prostase, enamel matrix, prostate); CCND1 – Cyclin D1, alpha polypeptide; TCF7 - Transcription factor 7 (T-cell specific, HMG-box); PART1 - Prostate androgen-regulated transcript 1; PSCA – Prostate stem cell antigen; E2F3 – E2F transcription factor 3; PDGFRB - Platelet-derived growth factor receptor, beta polypeptide, BAX – Bcl2-associated X protein, TEGT - Transmembrane BAX Inhibitor Motif Containing 6. Values are means of three independent replicates (n = 3). Vertical bars indicate standard error. Asterisks indicate significant differences ($p < 0.5$) to the untreated group.

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APTAMER FUNCTIONALIZED IRON OXIDE NANOPARTICLES FOR ACTIVE TARGETING OF PROSTATE CANCER METASTASES

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INTRODUCTION

Detection of early metastases is a key factor in cancer staging and treatment planning to decrease the risk of secondary tumors. Being a prominent marker for lymph node and distant metastases in prostate cancer[1], the transmembrane protein prostate-specific membrane antigen (PSMA) represents a promising option for active targeting of prostate cancer metastases.
We are developing iron oxide nanoparticles actively targeting PSMA. Besides their use as MRI contrast agent, the iron oxide nanoparticles also dissipate heat when exposed to an alternating magnetic field. These two properties make them highly interesting as theranostics by combining MRI diagnostic and successive (co-) treatment by inducing local hyperthermia.
Comparing different ligands attached to the nanoparticle surface, we focus here on a PSMA-targeting RNA aptamer[2]. The aptamer is of smaller size compared to antibodies, which allows faster target recognition, and higher binding affinity compared to small molecules[3] rendering it interesting for specific targeting.

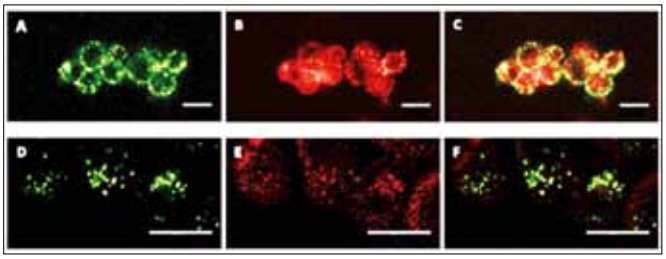
EXPERIMENTAL METHODS

The aptamer was first tested for its targeting ability. For this purpose, the plasma membranes of human, PSMA-positive prostate cancer cells (LNCaP, derived from a lymph nodes metastasis) and PSMA-negative prostate cancer cells (PC3) were stained using ibidi Fuse-It green. After incubation with the aptamer coupled to cyanine 5 (Cy5) and washing of the cells, binding and internalization were followed on living cells by confocal laser scanning microscopy.
Previously coated iron oxide nanoparticles were modified by attaching an azide functional group. The aptamer, coupled to a spacer, was then attached to the coated nanoparticles by copper-free click chemistry in aqueous medium. After purification, each reaction step was followed by changes in hydrodynamic diameter and zeta potential. The functionalized nanoparticles were then tested in-vitro for cytotoxicity by WST-1 cell proliferation assay and for binding to PSMA-positive cells.

RESULTS AND DISCUSSION

Fluorescence imaging shows that the aptamer binds to PSMA-positive LNCaP cells while PSMA-negative PC3 cells remain mainly unaffected (Fig 1). Internalization by LNCaP cells was also shown.

Figure 1: LNCaP (A-C) and PC3 (D-F) cells after incubation with aptamer-Cy5. The plasma membrane of both cell lines is labeled in green (left column, A LNCaP, D PC3), the aptamer-Cy5 in red (center column, B, E). Merging of both channels shows localization of aptamer-Cy5 on the surface of LNCaP cells (C), but not on PC3 cells (F). Scale bar=20 µm.



Azide modification and aptamer coupling to iron oxide nanoparticles were confirmed by measuring the change in surface charge starting from two different coatings, resulting in a negative zeta potential (Fig 2) and a hydrodynamic diameter of around or below 100 nm (data not shown). The resulting nanoparticles form a stable suspension. Cell viability was proven by cell proliferation assay using PC3 cells.

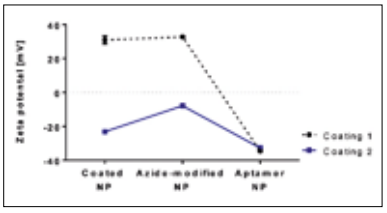


Figure 2: Zeta potential of nanoparticles (NP) after each reaction step (n=3).

CONCLUSION

Iron oxide nanoparticles eligible for theranostic applications were successfully functionalized with a PSMA-targeting RNA aptamer, presenting promising properties for lymph node targeting.
The aptamer was shown to bind to and being internalized by PSMA-positive LNCaP cells while PSMA-negative PC3 cells remain unaffected. In-vivo experiments are in preparation to evaluate the detection of prostate cancer lymph node metastases in a clinical MRI scanner.

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NANOSIZED MATERIALS FOR DRUG DELIVERY: UNDERSTANDING THE MECHANISMS INVOLVED IN NANOPARTICLE UPTAKE

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Nanoparticles are powerful tools for a wide range of applications, including as drug carriers in nanomedicine, because of their ability to interact in new ways with the cellular machinery and their high cellular uptake efficiency. Nanomaterials with different physicochemical properties and design have been investigated for drug delivery and drug targeting purposes, however in many cases a clear understanding of the mechanisms by which they are recognized and processed by cells is still missing. Only with this knowledge it will be possible to design truly targeted nanomedicines and control their localization, uptake and fate inside cells. Furthermore, it has emerged that the environment in which those particles are dispersed confers them new properties that influence remarkably their cellular interactions[1]. Therefore it is essential that the study of the mechanisms involved in uptake and processing of nanosized carriers by cells is performed in relevant biological media such as for instance human plasma.

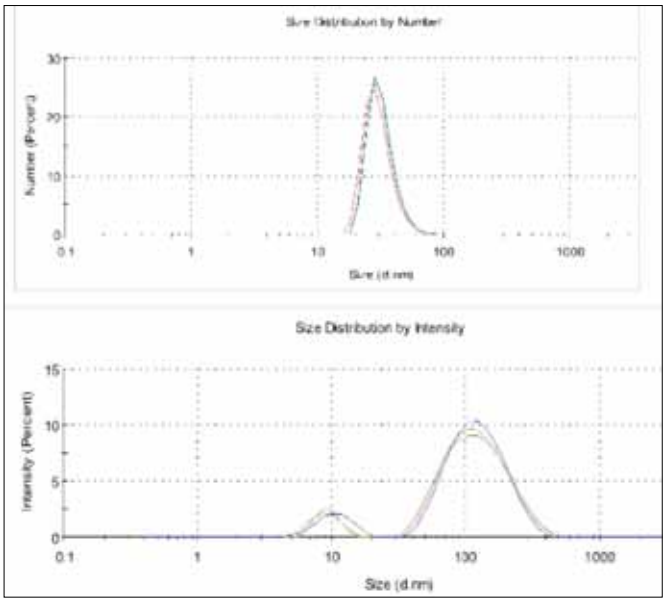

Figure 1 DLS distribution of 50nm silica NPs in water (upper panel) or cMEM (bottom panel) at 37C after 24h of incubation. cMEM: complete medium supplemented with 10% FBS

Within this context, the aim of our study is to characterize nanoparticle-cell interactions and in particular, as a first step, to understand the endocytic mechanisms nanosized objects use to enter cells. For this purpose, fluorescently labeled silica and polystyrene of different sizes are used as model nanoparticles because of their stability and their well-defined properties. Nanoparticles are characterized by Dynamic Light Scattering (DLS) and other similar methods to ensure good dispersions in relevant biological media are applied to cells (Figure 1). Thus, particle uptake is quantified and followed by flow cytometry and fluorescence imaging (Figure 2). In order to determine the role of specific pathways or molecules in the uptake, RNA interference directed toward key proteins involved in different endocytic mechanisms is used, together with commonly known chemical inhibitors for endocytosis. Our investigation includes cell lines typically used to study cellular pathways and nanoparticle-cell interactions, such as A549 - human adenocarcinomic lung epithelial cells - and HeLa - human cervical cancer cells. Together with such model systems, we have optimized protocols to form polarized cell monolayers consisting of Human Umbilical Vein Endothelial Cells (HUVEC), in order to mimic endothelial barriers encountered by nanomedicines (Figure 3). This allowed us to investigate the effect of cell polarization on nanoparticle uptake, in comparison to what observed in isolated non-polarized cells.

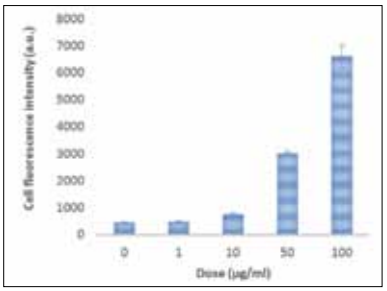

Figure 2 A549 cells were exposed to different doses of 50nm silica nanoparticles in the presence of 10% FBS. Error bars indicate standard deviation of six replicas.

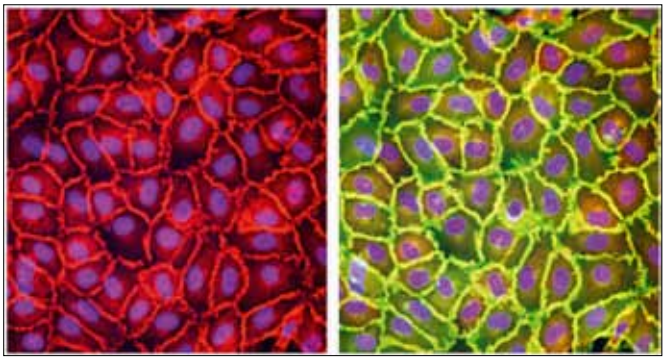

Figure 3 Confocal fluorescence images of HUVEC cells 6 days after seeding. Nuclei are stained with DAPI (blue). CD31 (green), ZO-1 (red).

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IMMUNE SIDE EFFECTS OF POLYMER COATED NANOPARTICLES: PREDICTION AND PREVENTION OF HYPERSENSITIVITY TO PEGYLTED THERAPEUTICAL LIPOSOMES

TAMÁS FÜLÖP

PURPOSE

In order to find a predictive biomarker for complement (C) activation related pseudoallergy (CARPA), a mild-to-severe hypersensitivity syndrome in patients treated intravenously with liposomes, several factors were analyzed in plasma samples of subjects treated with small unilamellar liposomes for a chronic inflammatory disease: C factor H (FH), SC5B-9 (complement terminal complex), anti-FH (IgG against FH) and anti-PEG IgM and IgG. Furthermore, hematological tests were done on patients' plasma to see differences in the levels of blood cells, platelet and CRP level.

METHODS

The samples were obtained from patients treated with liposomal drugs in the Netherlands, the blood was taken before and after administration of the nanomedicine via infusion. The patient samples

were compared to five (untreated) human plasma control via ELISA assays of FH, SC5B-9 and anti-FH. Anti-PEG IgM and IgG ELISAs are set up for human plasma measurements according to previously set up porcine anti-PEG ELISA protocols. Hematology tests were prepared on site in the Netherlands.

RESULTS

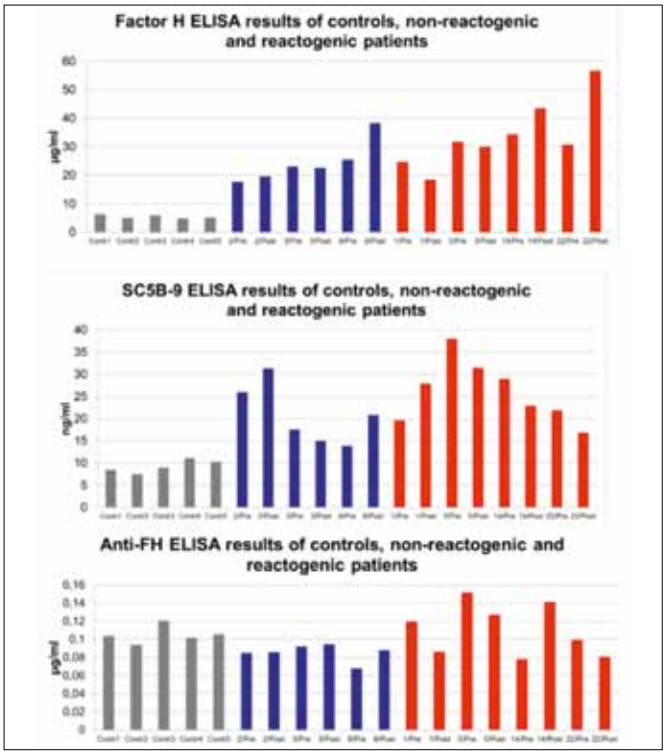

Fig.1.: Results of Factor H, SC5B-9 and Anti-FH ELISA measurements. Controls: five healthy untreated human plasma (grey columns), three non-reacting patients' pre-and post-infusion samples (blue columns) and four patients' plasma samples that had adverse (CARPA) reactions (red columns).

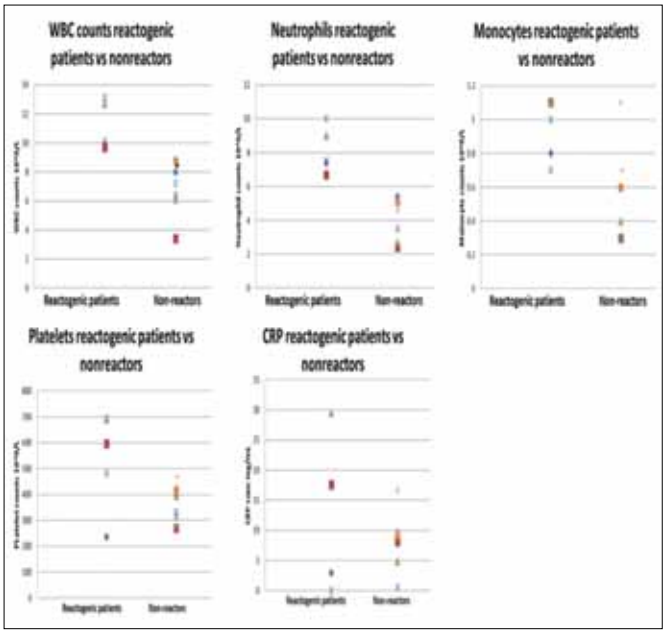

Fig.2.: White blood cell, neutrophil, monocyte, platelet and CRP count of patients with CARPA reaction versus patients that did not have adverse reactions.

There is a visible increase of FH in the plasma of the patients, regardless taken pre-or post treatment. Furthermore, a rise in SC5B-9 can be seen in the patients, though here there are variations in the level of the complex before and after treatment. However, there is no significant difference in the level of anti-FH IgG between treated and non-treated samples. Anti-PEG IgG and IgM ELISAs are being converted and set up according to previously developed porcine Anti-PEG ELISAs by fellow colleague. According to the results of the blood screening made on site from samples taken while the medical treatment, it is important to point out that the white blood cell levels of the reacting patients are significantly higher than patients who got no adverse reaction during treatment. This trend is also seen at neutrophil and monocyte levels. However, there is no significant difference at platelet and CRP levels between these two groups of patients.

CONCLUSIONS

According to results, the hematology tests showed that white blood cell levels (neutrophils, monocytes) of reacting patients are significantly higher than of non-reacting ones. This remarkably suggests possible correlation with CARPA reactions. Furthermore, the presence of complement FH and SC5B-9 is significantly higher in patients' plasma compared to controls, which is not related to the presence of anti-FH IgGs in the blood. These elevations suggest high level of complement activation taking place in the patients giving time for compensatory rise of the natural inhibitor of complement activation: FH and associated proteins. Although, there are no significant changes of the measured proteins between pre- and post-injection, (thus implying connection to CARPA reactions), these findings may suggest new marker of the disease activity.

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TACROLIMUS - LOADED NANOCARRIER HYDROGEL COMPOSITE FORMULATIONS FOR TOPICAL TREATMENT OF PSORIASIS

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We have developed a nanocarrier - hydrogel composite formulation for improved local delivery of Tacrolimus (TAC) to psoriasis lesions. TAC is efficiently solubilized in methoxy poly- (ethylene glycol) hexyl substituted poly- (lactic acid) (mPEGhexPLA) based nanocarriers. Viscosified formulations of TAC nanocarriers are obtained by addition of a gelling agent. Resulting composite formulations are applied topically as hydrogel or viscosified spray.

Three distinct mechanisms enable mPEGhexPLA nanocarriers to achieve efficient loco-regional delivery of TAC: i) incorporation of the drug into the hydrophobic nanocarrier core increases solubility and actual bioavailability ii) particle size in the lower nano-range together with stealth surface properties minimize interactions with the biological matrix and facilitate rapid transport into biological structures iii) dissociation of the carrier leads to the release of the drug cargo inside the biological target site and to the in situ formation of a drug depot[1, 2].

In an Imiquimod (IMQ) - induced psoriasis like inflammation model in mice, approximately twice as high TAC tissue levels were found after repeated application of 0.1% TAC hydrogel compared to the commercially available 0.1% TAC ointment (Protopic®). Topical treatment with TAC nanocarrier – hydrogel composite formulations was well tolerated. Administration of 0.1% TAC hydrogel efficiently prevented symptoms of IMQ induced psoriasis-like skin inflammation *in vivo*. Therapeutic efficacy was comparable to 0.05% clobetasol ointment (Butavate®), which is used as positive control in this animal model.

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EVALUATING LIPOSOMAL NANOPARTICLES FOR CONTROLLED RELEASE OF CHEMOTHERAPEUTICS IN VITRO AND IN VIVO.

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The clinical use of chemotherapeutic drugs is greatly hampered by a combination of severe side effects on healthy organs and low accumulation in the tumor tissue. Although entrapment in stable liposomes has long been known to increase tumor accumulation while protecting the drug in circulation, drug release after accumulation is generally low, leading to poor bioavailability and consequently poor therapeutic effect [1,2]. This essentially underlines the paradoxical problem of how to simultaneously maintain liposome stability in circulation and obtain efficient drug release at the tumor site. One compelling solution is utilizing an endogenous trigger mechanism that relies on a difference between microenvironment of the tumor and the healthy tissue. Secretory phospholipase A2 (sPLA2) is reported to be expressed at an elevated level in many tumor types [3,4]. This enzyme catalyzes the hydrolysis of phospholipids, producing equimolar concentrations of lysolipids and free fatty acids [5]. For liposomes this has a dual effect: rupture of the membrane, causing site specific release of encapsulated drug, and production of potentially lytic agents that can permeabilize the cell membrane mediating more efficient drug uptake [6,7] (Figure 1).

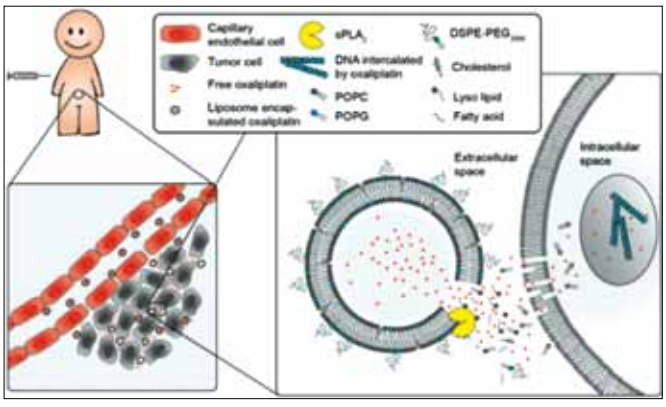

Figure 1: Conceptual illustration. Liposome encapsulated oxaliplatin will circulate until it encounters the fenestrated capillaries in the tumor tissue, where it extravasates. Here it encounters an elevated level of secretory phospholipase A2 (sPLA2), which hydrolyses the phosphoglycerolipids, causing release of the drug. In addition the hydrolysis products, lyso-lipids and free fatty acids, may act as permeability enhancers, thus further contributing to drug transport across the cellular membrane.

The concept of sPLA2 responsive liposomes has been widely studied in cell free systems and *in vitro* [7–10]. Yet very few studies have reported on *in vivo* data [11,12], and only one formulation with this concept has made it to clinical trials [13]. Here we present the rational design of liposomes optimized for secretory phospholipase A2 (sPLA2) triggered drug release, and test their utility *in vitro* and *in vivo*.

Studies with MALDI-TOF MS revealed an sPLA2 dependent hydrolysis of liposomal phospholipids, demonstrating that these nanoparticles are truly enzyme sensitive. Further, *in vitro* release studies with ICP-MS disclosed enzyme dependent release of the liposome encapsulated drug oxaliplatin, signifying their potential for controlled release of cancer drugs.

Treatment of two different cancer cell lines with liposomal oxaliplatin showed efficient growth inhibition compared to that of clinically used stealth liposomes. In the presence of excess sPLA2 the liposomal oxaliplatin was also superior to free oxaliplatin, suggesting a boosting therapeutic effect by the lysis products, possibly due to enhanced cellular uptake over a slightly permeabilized membrane. Empty liposomes induced a small sPLA2 dependent growth inhibition, but did not demonstrate a severe cell death profile, implying that these liposomes should be inactive, and thus safe, in circulation, where the sPLA2 level is low.

Although the *in vitro* results were promising, real clinical potential can only be disclosed by *in vivo* evaluation. For this purpose we utilized the human, sPLA2 secreting, mammary carcinoma cell line MT-3, transplanted onto female nude NMRI mice. Mice received 10 mg/kg oxaliplatin, the liposomal equivalent or isotonic glucose solution by tail vein injection. Three days after the first treatment all mice having received liposomal oxaliplatin were euthanized due to severe systemic toxicity (excessive weight loss, dehydration and subcutaneous bleedings). Mice having received control compounds showed no signs of discomfort.

Although speculated to be related to the phospholipid hydrolysis products, the exact mechanistic cause of the systemic toxicity is not yet known. Preliminary histopathology studies of liver sections displayed acute multifocal necrosis of hepatocytes with a collapse of hepatic sinusoids and hydropical injury to the cell nuclei, which is believed to be the biological cause of the observed toxicity. Consequently, the *in vivo* study was not repeated.

The present study demonstrates that great caution should be implemented when utilizing sPLA2 sensitive liposomes. Even though many have shown potential *in vitro*, the real utility can only be disclosed *in vivo*.

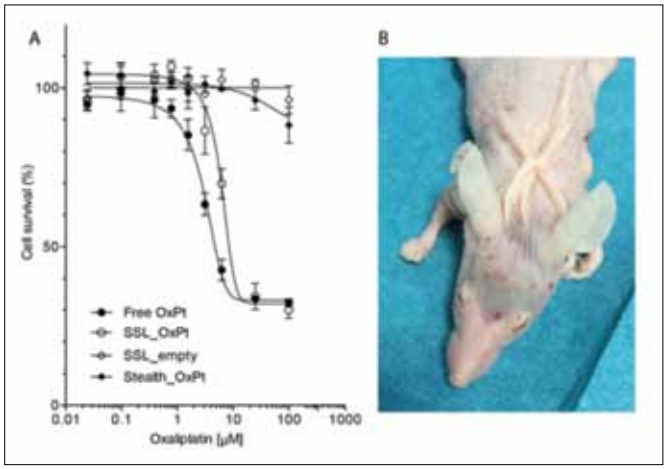

Figure 2: Effect of sPLA2 sensitive liposomes. A) In vitro antiproliferative effect of free oxaliplatin (OxPt) (closed circles), empty (open diamonds) or OxPt loaded (open circles) sPLA2 sensitive liposomes (SSLs) or OxPt loaded Stealth liposomes (closed diamonds). Cell survival was evaluated by MTS staining. Values are mean of triplicates

± SD. All values are normalized to non-treated cells. The data is representative of minimum three separate experiments. B) In vivo evaluation. Mouse treated with oxaliplatin loaded sPLA2 sensitive liposomes was euthanized 3 days after first treatment due to excessive weight loss, dehydration and subcutaneous bleedings.

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INTRODUCTION OF RAMAN TECHNIQUE TO PERIO DENTISTRY. A NON INVASIVE METHOD REGARDING QUALITATIVE AND QUANTITATIVE BONE EVALUATION RELATED TO CALCIUM PHOSPHATES COMPOUNDS

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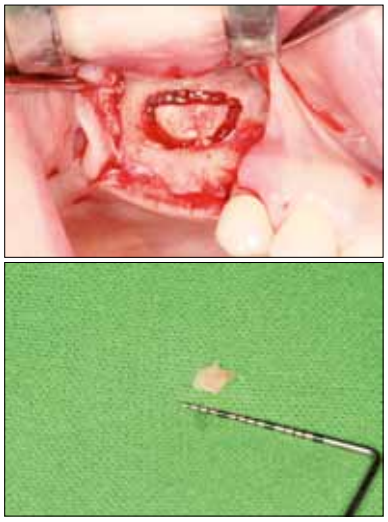
RESUME:

The bone quality has primary influence on treatment planning, implant design, surgical approach, healing time and initial progressive bone loading during prosthetic reconstruction. [1] The term bone quality is commonly used in implant treatment and in reports on implant success and failure. It is emphasized that bone density (Bone Mineral Density, BMD) and bone quality are not synonymous. Bone quality encompasses factors other than bone density such as skeletal size, the architecture and 3-dimensional orientation of the trabecula, and matrix properties. Bone quality is a matter of mineral content and of structure as well.

The HA (hydroxyapatite) crystals in bone have a plate-like habit and are nano sized, with a length of ~20–50 nm and a width of 12–20 nm, depending on age or disease problems (periodontal most). It has been shown that the quality and quantity of bone available at the implant site are very important local patient factors in determining the success of dental implants or evaluation of periodontal diseases. [2, 3]

Outcomes include mineral crystallinity, elemental composition, and collagen crosslink composition. Advantages include the detailed material characterization; disadvantages include the need for a biopsy (histomorphometry) for better results/evaluation. Bone samples were harvested by drilling during the piezo surgery protocol [Fig.1].



(a)

(b)

Fig. 1 Bone harvesting: (a) harvesting area, preparing for sinus augmentation; (b) cortical bone sample

Regarding composition and crystallinity, investigation was performed by RAMAN technique [Fig. 2]. [4, 5, 6] There were evaluated following peaks:

- Carbonated apatite bands, related to PO_4^{3-} at 947 and 957 cm^{-1};
- Carbonate band (CO stretching) of hydroxyapatite at 1070 cm^{-1};
- Carbonate band (v1 mode) at 1107 cm^{-1}.

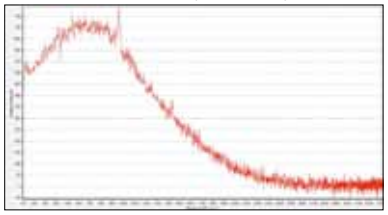


Fig. 2 Raman spectra for one harvested bone sample

The full-width half-height of the ύ1 $PO_{4\ 3-}$ band is inversely proportional to mineral crystallite c-axis length, and it is used as a measure of mineral crystallinity. In bone, cementum and dentin, apa-

tite crystals develop with their long c-axes parallel to the collagen fibril axis. Octacalcium phosphate ($Ca_8(HPO4)_2(PO4)_4 \cdot 5H_2O$, OCP) is considered very important because it is regarded as an *in vivo* precursor of HA[4, 5] and has become an important candidate for use as a biomaterial for bone augmentation. The collagen and associated proteins play an important role in determining nucleation, growth, and proliferation of these crystals – a nanoscale process. Trying to find traces of transformation of OCP to HA, the presence of HA nano rods and plate-like HA particles can be utilized as signs of bone augmentation process and a good quality future bone.
The goal of our future study is that a correlation must be established between RAMAN spectra and bone main organic/inorganic fractions value in order to obtain a one-step complete investigation. Method easily can be adapted for *"in vivo"* bone quality evaluation, being much less invasive method then the well known CT (computer tomography) or CBCT (con beam computer tomography) already used and more accurate.

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REVIEW OF AVAILABLE DATA ON PHYSICOCHEMICAL PROPERTIES, PHARMACOKINETICS, TOXICITY AND SIDE EFFECTS FOR NANOMEDICINAL PRODUCTS ON THE MARKET IN EUROPE

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Increasing numbers of nanomedicinal products (NMPs) are available on the market. They may have distinct physicochemical properties and pharmacokinetics when compared to non-nanomedicinal products. As a consequence, they may present different characteristics with regard to efficacy as well as safety, compared to conventional medicinal products. In a previous study we found that information on the size of NMPs was available in public sources for less than half of the approved products, while data on other physicochemical properties and on pharmacokinetic parameters are generally not available in the public domain at all(1). With access to such data, analyses would become possible to identify nano-specific benefits or risks, stratified in relation to for example size or type of nanostructure. Such analyses might produce highly valuable insights that can reduce uncertainties in the benefit-risk assessment of NMPs. Therefore, we started a follow-up investigation in which public information on the physicochemical properties and pharmacokinetics of NMPs approved in Europe was combined with information in drug registration dossiers available in the Netherlands. The resulting set of data was compared to information needed for a benefit-risk assessment of NMPs based on current scientific insights(2). In addition, we set out to gain insight into any specific toxic effects of NMPs by systematically analyzing the available non-clinical toxicity data on 51 NMPs approved in Europe, and by reviewing clinical side effects for a limited set of products(3). In parallel to these data analyses, we performed a review of the accumulating knowledge on potential immunotoxic effects of NMPs(4). Finally, we considered our findings in relation to existing regulatory guidance and testing requirements for medicinal products.

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SYNAPTOSOMES: NANOVEHICLES OF SYNAPTIC SIGNALING

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Immune molecules mediate a wide range of physiological functions in the brain, and the underlying mechanisms are increasingly recognized nowadays. The classical complement cascade, traditionally known as a part of the innate immune system, has been demonstrated to mediate synaptic refinement during the ontogenesis of the visual system before. Moreover, the expression of C1q, the secreted initiator of the cascade, increases throughout the lifespan in the brain. Selective C1q-tagging potentially leads to the pruning of the synapse by surrounding microglia during development and disease, while the significance of this signal in the healthy adult brain is obscure. Thus, we asked in the current study whether and what extent the protein composition of cerebral cortical synapses tagged by C1q differs from the untagged ones in adult mice. We have purified and extensively characterized synaptosomes. We demonstrated the presence of C1q attached to them with independent techniques. Our data provide evidence for a mostly presynaptic localization of C1q. Fluorescence-activated synaptosome sorting of C1q-tagged synaptosomes and their subsequent proteomic analysis comprising two-dimensional differential gel electrophoresis and tandem mass spectrometry were carried out. The proteomic experiment revealed 18 proteins which were present in different levels between tagged and untagged synaptosomes. Our results high-

lighted several cellular functions which are affected in C1q-tagged synapses, and provided numerous promising guides (e.g., signaling pathways) for further studies on complement-bound synaptic/neuronal preparations. Most notably, we identified neuronal pentraxin 1, which was described before as an important player regulating synaptic plasticity, supposedly interplaying with the local complement system in the brain.

DESIGNING THE BIO-NANO INTERFACE FOR BARRIER TARGETING

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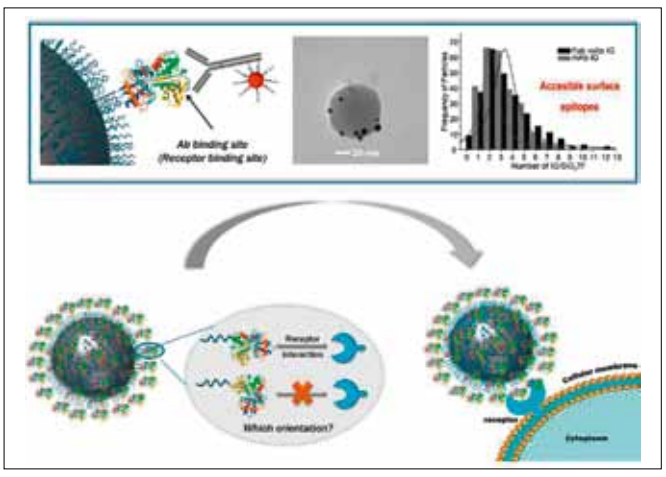
There are many aspects which need to be considered when designing nanomaterials for *in vivo* targeting. The dispersion size and stability, possible non – specific interactions and targeting capabilities all need to be well understood and controlled. One of the key aspects, however, is assessing the availability and accessibility of the active target on the nanoparticle surface. Unlike availability, quantitative assessment of the accessibility of proteins conjugated on particles is a considerable challenge.
In this work we adopt a methodology based on monoclonal antibody conjugated gold nanoparticles (immunogold)[1] to map the accessible transferrin (Tf) epitopes after nonspecific Tf grafting to a silica particle scaffold (SiO2 PEG8 Tf). In previously published work [2] it has been shown that these particles are able to interact with, and specifically enter cells through a receptor mediated pathway only in serum-free conditions. We noticed that using a divalent antibody with SiO2 PEG8 Tf particles leads to aggregation due to low surface density of epitopes available for antibody binding. The problem was addressed by using an antigen binding functional fragment (Fab) instead of the full antibody[3]. Data from the adopted method indicate that on a SiO2 PEG8 Tf particle, an average of only ~3% of the proteins is accessible to interact with the immunogold [4]. This could be due to current random grafting approaches that might not insure optimum presentation of relevant epitopes for receptor recognition and possibly subsequent endocytosis.
Therefore, engineering protein fragments with tags, for site-specific grafting is the next step we take for optimising the biofunctionality of targeted constructs for *in vitro* applications. Specifically, we investigate the change in biofunctionality of two engineered Cys-tagged apolipoprotein E (ApoE) with different orientation grafted onto silica nanoparticles and compare it to a randomly grafted ApoE control. Subsequently, an *in vitro* blood-brain barrier model, developed in-house, is employed to study how controlling the bio-nano interface impacts biological behaviour.



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ULTRA-SMALL, HIGHLY NEGATIVELY CHARGED ARCHAEOLIPID NANOPARTICLES FOR ACTIVE TARGETING TO MACROPHAGES OF THE INFLAMED MUCOSA

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INTRODUCTION

Inflammatory bowel diseases (IBD) such as Crohn's disease and ulcerative colitis, are chronic relapsing disorders of the gastrointestinal tract, characterized by chronic inflammation and epithelial injury induced by the uncontrolled activation of the mucosal immune system. Dendritic cells of lamina propia and macrophages are key antigen-presenting cells in the inflamed mucosa. Following activation, which occurs in response to components of the commensal microbiota and Toll-like receptor signalling, these cells produce large amounts of pro-inflammatory cytokines, such as IL 1β, IL 6, IL 8 and TNF. The imbalance between pro-inflammatory and anti-inflammatory cytokines that occurs in IBD impedes the resolution of inflammation, leading instead to disease perpetuation and tissue destruction.
The current treatment is symptomatic, but the frequent oral intake of anti-inflammatory and immunosuppressant drugs or systemic administration of biological agents such as the anti-TNF antibody infliximab is poorly effective and its serious adverse effects deteriorate the patient's quality of life.
We hypothesize that more efficacious and safer therapies could rely on developing macrophages-targeted drug delivery systems capable of specifically delivering high doses of anti-inflammatory drugs with minimal exposure of healthy or distant tissues.
The inflamed mucosa is characterized by increased of permeability, thinner mucus layer, a reduced pH and accumulation of positive charged proteins such as transferrin. Besides the "holes" at the epithelial line following cells loss, greatly enhance the entry of nanoparticles into mucosa, that once into the tissue, the small size enhances its retention at the target site.
In this work, we report the development of lipid nanoparticles decorated with polar archaeal lipids (archaeolipid nanoparticles, aLN) extracted from the halophilic archaebacteria Halorubrum tebenquichense, for macrophage targeting of the corticosteroid dexamethasone (Dex). Polar archaeal lipids consist of saturated isoprenoid chains linked via ether bonds to the glycerol carbons at the sn2,3 position. We have previously shown that polar archaeal lipids are ligands for macrophages scavenger receptors class A (SRA). Macrophages and bone marrow dendritic cells expressing SRA, avidly uptake vesicles containing archaeal lipids. Besides, vesicles containing archaeal lipids present high superficial negative charge and are more resistant against chemical, physical and enzymatic attacks than classical vesicles made of phospholipids.
Archaeolipid nanoparticles (aLN) made of compritol 888 ATO (glyceryl behenate, compritol) stabilized by archaeolipids, soybean phosphatidylcholine (SPC) and Tween 80 (4; 0.9; 0.3; 3% w/w) were prepared by homogenization-ultrasonication. Dex was incorporated in the core of the nanoparticles by dissolving the drug in compritol. Conventional lipid nanoparticles (cLN) made of compritol, SPC and Tween 80 (4; 1.2; 3% w/w) were prepared as control.
Nanoparticles were characterized in terms of particle size, zeta potential, morphology, crystallinity and colloidal stability upon storage. The toxicity of nanoparticles on macrophages (J774.A1 cells)

and human epithelial colorectal adenocarcinoma cells (Caco-2 cells) was determined by MTT assay in the absence and presence of a mucin layer.
The mucopenetration of nanoparticles was measured as the diffusion of coumarin-6 labeled nanoparticles through a mucus layer using the transwell system. Besides, size and zeta potential of nanoparticles upon incubation with mucins was followed for 4 h. In vitro duodenal lipolysis study was performed by quantification of alkaline compensation in simulated intestinal fluid using pancreatic lipase.
Then, the uptake of coumarin-6 label nanoparticles by J774 and Caco-2 cells was measured by flow cytometry in the absence and presence of a mucin layer. Finally, the anti-inflammatory potential of nanoparticles was measured as the capacity to reduce pro-inflammatory cytokine secretion in lipopolysaccharide (LPS) stimulated macrophages.

RESULTS

Archaeolipid nanoparticles showed a significantly decreased in the mean size from 288 ± 60 nm to 73.05 ± 10.7 nm, in the polydispersity index (PDI) from 0.4 to 0.3 and in the zeta potential from - 8 ± 5 mV to -43.8 ± 7.3 mV, respect to conventional nanoparticles. Dex incorporation into aLN resulted in an 8 fold-increase in drug solubility with an encapsulation efficiency of 60 %. The transmission electron microscopy images showed spherical particles in the nanometer range while cryo-transmission electron microscopy images showed circular, ellipsoidal or elongated edged structures of high contrast (Figure 1). There was no significant change in mean particle size, PDI, zeta potential and drug content upon one-month storage at 4°C.

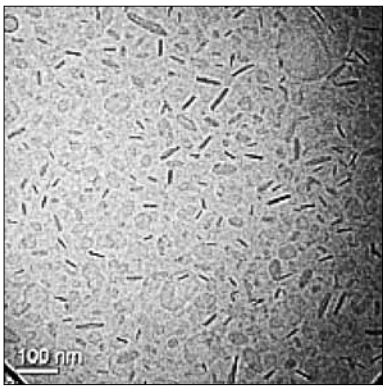


Figure 1. Cryo-TEM images of aLN

The differential scanning calorimetry thermogram of aLN with and without Dex showed a decrease of melting point of compritol from 74.2°C to 66°C. This fact can be explained by the Kelvin effect, where a reduced particle size and increased surface area led to a decrease in the melting enthalpy compared to the bulk lipid. The Dex melting endothermic peak of loaded aLN disappeared, indicating the existence of amorphous Dex or that it has been molecularly dispersed within the compritol matrix.
Mucus permeation studies showed that aLN diffused significantly more than cLN, and upon 4 h incubation no increase in mean size was observed. The combination of small size and negative superficial charge could be responsible of the high mucopenetration of aLN. The lipolysis kinetics expressed as alkaline consumption showed that at the end of 20 minutes, the lipolysis of aLN was 45%, which indicated that aLN possessed a resistance to pancreatic lipase compared to cLN (75%). Viability studies revealed no cytotoxicity of aLN in the range of 40- 400 µg/ml of compritol upon 24 hours of incubation with J774 and Caco-2 cells.
The uptake of aLN by J774 and Caco-2 cell were 6 and 2.4 folds higher than that of cLN upon 5 h incubation at 37 ºC (Figure 2). The uptake was significantly reduced in the presence of a mucin layer for cLN and aLN in both cell types; however, the uptake of aLN by J774 cells was still 3.5 folds higher than that of cLN.

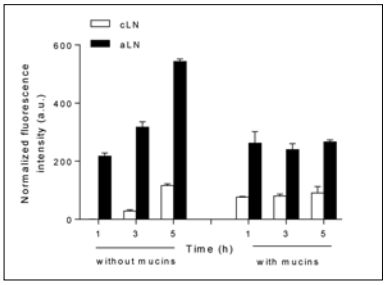
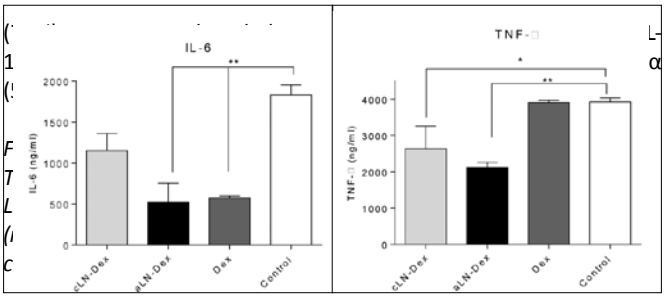
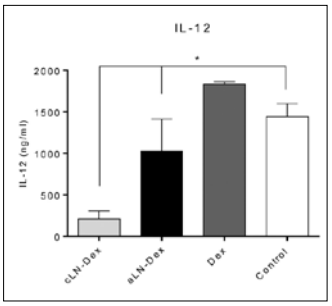


Figure 2. Uptake of cLN and aLNby J774 cells in absence and presence of a mucin layer.

Finally, anti-inflammatory activity of nanoparticles showed that while free Dex significantly reduced the production of IL-6





CONCLUSIONS

The ultra-small, highly negatively charge archaeolipid nanoparticles and, no conventional lipid nanoparticles, resulted highly stable under gastrointestinal conditions, highly up taken by macrophages and reduced the secretion of the pro-inflammatory TNF-α, IL-6 and IL-12 from J774 cells stimulated with LPS. Further studies will be done to test the activity of aLN in a more advanced *in vitro* model based on co-culture of macrophages, dendritic cells and enterocytes that allow simulating the inflammation.

ACKNOWLEDGEMENTS

The authors would like to thank to Laboratório de Microscopia Eletrônica/Laboratorio Nacional de Nanotecnologia for the use of electron microscopy facility and technical support.

NANOSCREEN: RELIABLE AND RAPID IN VITRO SAFETY ASSESSMENT OF NANOMATERIALS

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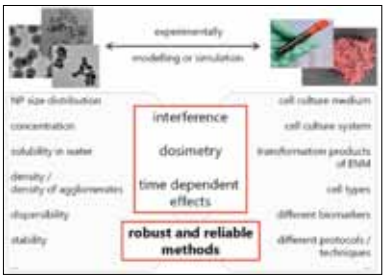
The unique properties of engineered nanomaterials (ENM) render them not only suitable for various consumer and industrial applications but also for medical use. The same properties however, may also change their reactivity towards biological systems. Even though a considerable number of studies on biological effects of ENM are available, standardized and validated test systems are still missing. For instance differences in cell type, assay, dose range, as well as suspension method for ENM testing make it impossible to compare existing studies [1]. Furthermore, interference reactions of nanomaterials with the test systems or assay reagents frequently lead to false results [e.g. 2 – 9]. Hence, standardized, robust and comprehensively validated tools to assess biological effects of ENM are urgently needed.
Here we briefly present the concept of how to understand and quantify the impact of ENM on human health *in vitro*. Using well-characterized ENM libraries we will systematically correlate physico-chemical properties of ENM (such as size, porosity, shape, surface properties) to biological responses in two different exposure scenarios: i) environmental health perspective ii) intended/medical application. With the long-term goal to eventually predict toxicity, efficacy as well as side-effects from material properties. While different biological systems are necessary to address the two different exposure scenarios, basic issues such as interference reactions of ENM with the assay systems, dosimetry considerations, measurement uncertainty, traceability and reliability of test results remain the same. Robust and reliable methods are needed in both scenarios.
An overview on ENM libraries, their interference with selected assays, major difficulties, optimization procedures as well as first tox-

icity results will be presented. In summary our approach not only facilitates reliable and reproducible assessment of ENM toxicity but also offers a guideline how to optimize the *in vitro* assay(s) of interest for ENM suitability.



Correlation of physico-chemical properties of ENM to biological effects.

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STUDYING THE ZETA POTENTIAL OF SURFACES TOWARD ELUCIDATING NANOPARTICLE – SURFACE INTERACTIONS

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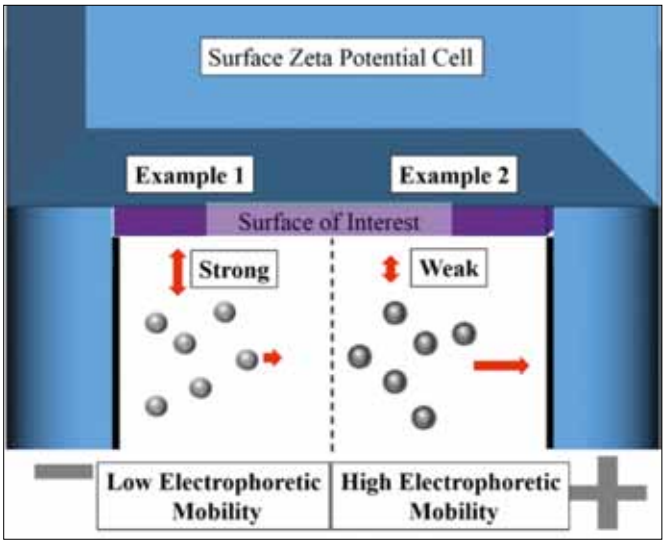
Interactions between nanoparticles and cells, at their most fundamental level, can be viewed as interactions between a sphere and a surface. It is extremely difficult to directly study this phenomena, especially in the context of material evaluation. However, understanding the surface potential of materials prior to application *in vitro* varies depending on the conditions can provide valuable insight into the particle behaviour in biological conditions. For this to be possible both the particle properties and those of the surface need to be well characterized in the appropriate conditions. In this collaboration we are looking into using a benchtop Malvern Zetasizer instrument equipped with a ZEN 1020 cuvette to measure the zeta potential of a Si_3N_4 surface in various conditions and relate that to the observed particle – surface interaction.

Specifically we use a library of tracer particles varying the material, size and surface, and measurement conditions and observe the interaction. The particle behaviour in the experiment is a function of the applied electrical potential and distance from the substrate (figure 1). By using a relatively large variety of particles and conditions we further elucidate the relationship between the tracer particle and the observed surface zeta potential and are able to further develop the methodology.

Ultimately this study is aimed at further sheading light into the particle-surface and specifically particle-cell surface interactions and the effect of size, material and coating chemistry. Additionally it is our belief that this work further sheds light into the technological challenges and limitation of the industrial method of interest.

Figure 1. An illustration of the fundamental principles of operation of the Surface Zeta Potential Cuvette for two example particles, one which exhibits a relatively strong interaction with the surface of interest and one which interacts weakly with the surface.



INVESTIGATION OF NANOPARTICLE INTERACTIONS WITH IN VITRO BLOOD-BRAIN BARRIER CO-CULTURE MODEL

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Nanosized materials have been recognised as potential drug delivery systems, and since they have shown to be able to cross biological barriers, like the blood-brain barrier (BBB), their possible use for the treatment of neurodegenerative diseases has been one of the most researched fields of the past few decades. However, only little is known about the crossing mechanism that nanomaterials use to overcome the BBB.

Therefore, our work focuses on the understanding of the mechanism by which nanomaterials are able to cross the BBB using *in vitro* BBB models and live cell imaging techniques coupled with computational analysis[1]. These techniques enable us to follow dynamic events, such as spatiotemporal movements of nanoparticles inside the barrier, accumulation and localization within the barrier in a quantitative and reproducible manner. We have recently developed an *in vitro* BBB co-culture model using hCMEC/D3 cells as endothelial cells and normal human astrocytes (NHA) (see Figure 1) that allow us to study nanoparticle-BBB and cell-cell interactions, furthermore, this model is also suitable for live cell imaging application. We are currently using Apolipoprotein E grafted silica nanoparticles as a model system and investigating the interactions of these nanoparticles with the BBB using flow cytometry and live cell imaging. The results that have been reached so far will be presented here.

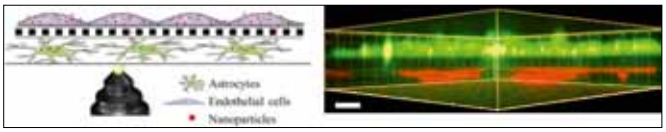

Figure 1. Illustration of in vitro BBB co-culture model (left). The two cell lines can be labelled separately and imaged using live cell imaging (right): green - hCMEC/D3 cells, red - NHA cells; scale bar: 20 µm.

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POLYETHYLENE GLYCOL MEDIATED SYNTHESIS OF IRON OXIDE MAGNETIC NANOPARTICLES: HYPERTHERMIA PROPERTIES AND THEIR INTERACTIONS WITH DIFFERENT TYPES OF CANCER CELLS

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Iron oxide magnetic nanoparticles (IOMNPs), such as magnetite (Fe_3O_4) or its oxidized form, maghemite (Fe_2O_3), have been vastly investigated in recent years due to their various potential biomedical applications such as magnetic hyperthermia [1], magnetic resonance imaging [2], drug delivery [3] or cell tracking [4]. Owing to their biocompatibility, non-toxicity and non-immunogenicity in biological media, they were approved in the 1980s for clinical applications by the Federal Drug Administration (FDA) becoming commercially available such as Feridex© or Resovist©. However, the lack of synthesis methods of water-dispersible IOMNPs with enhanced magnetic properties and better control of their morphology represents one of the major drawbacks for both *in vitro* and *in vivo* applications of IOMNPs. The co-precipitation of iron ions in the presence of the base is the most common method of synthesis of IOMNPs due to the high yields obtained, but the reproducibility of this method remains questionable [5]. The thermal decomposition of iron precursors into organic solvents allows a better control of both size and shape of IOMNPs [6]. This method involves additional post synthesis steps to render the IOMNPs hydrophilic and biocompatible which are difficult to monitor and control precisely in a quantitative manner. The polyol method which involves the thermal decomposition of iron precursors in a polyol solvent, acting as a surfactant as well as reducing agent, have been recently reported to yield water-dispersible IOMNPs displaying high crystallinity [7].

In our study we applied the polyol method to successfully synthesize IOMNPs by thermal decomposition of FeCl3 in the presence of polyethylene glycol (molecular mass of 200) and sodium acetate, in a solvothermal system [8]. This approach allowed the synthesis of poly-dispersed cubic and polyhedral IOMNPs displaying high crystallinity and broad sizes, which could be tuned between 30 nm and 230 nm by varying the reaction time (from 6h to 12h) and the PEG200 volume (figure 1a-d). An amount of 2.5 mmoles of FeCl3 dissolved in sixty milliliters of PEG200 and heated at 240°C gave rises to cubic IOMNPs (figure 1a) and favored oxidative conditions which allowed the formation of a small percent of hematite. An increased volume of PEG200 (90 ml) developed cubic and polyhedral IOMNPS (figure 1c) and prevented the oxidation of magnetite into hematite. The presence of physisorbed PEG200 on the IOMNPs surface was faintly detected through FT-IR spectroscopy. The surface of IOMNPs underwent oxidation into maghemite as proven by RAMAN spectroscopy. The magnetic characterization performed at room temperature on powder revealed that IOMNPs were ferromagnetic with a coercive field of 160–170 Oe. The evolution of magnetization with the temperature in field cooled curves below the blocking temperature (300 K) indicated the occurrence of strong magnetic dipole-dipole interactions between IOMNPs, which varied with both shape and size. Consequently, the specific absorption rate (SAR) values of IOMNPs, obtained in four different magnetic fields at 355 kHz, depended on the concentration of IOMNPs in water (figure 1e-h). The SAR values of IOMNPs significantly increased for applied magnetic field exceeding the coercive field, since larger hysteresis loops were covered. From the fitting of SAR values as a function of applied magnetic field with a sigmoidal function, it was found that SAR values reached saturation when the amplitude of the applied magnetic field was twice the coercive field. At a frequency of 355 kHz, the cubic and polyhedral IOMNPs of lower size prepared in 90 ml of PEG200 released higher amounts of heat (up to 1700 W/g – figure 1g and h) than the large cubic IOMNPs synthesized in 60 ml of PEG200 (1275 W/g – figure 1e and f). By dispersing the IOMNPs in PEG600 (liquid) and PEG1000 (solid), it was found that the SAR values decreased by 50 or 75 %, indicating that the Brownian friction within the solvent was the main contributor to the heating power of IOMNPs.

In order to study the interaction with different cancers cells we selected the best performing IOMNPs in terms of SAR, namely the 30 nm polyhedral IOMNPs. To this aim, four different cells lines: human retinal pigment epithelial cells (D407), human lung carcinoma cells (A549), human melanoma cells (MV 35) and mouse melanoma cells (B16F10), were grown in a 75 cm2 culture flask until confluence was achieved. Then, the culture medium was replaced with a solution of IOMNPs at an administered concentration of 0.2 mg/ml in reference to magnetic nanoparticles. Upon incubation for 4h and 24h at 37°C, the cells did not show any sign of cell suffering; they were still confluent in the culture flask and were not detached, as observed under an inversed optical microscope. After the short incubation time (figure 1i) IOMNPs were found near the cell membranes showing IOMNPs containing endocytosis vesicles closely resembling to pinosomes. A small increment of vesicles containing IOMNPs inside cells was revealed by the TEM images. After a prolonged incubation time (figure 1j), the number of vesicles including large IOMNPs aggregates, as well as membrane-free aggregates increased and they were accumulated in the proximity of nuclei. The IOMNPs aggregates were also seen near cell periphery, in most cases engulfed by cell membrane extensions, indicating the continuation of the macropynocytic IOMNPs uptake process. These results are relevant for the subsequent utilization of IOMNPs for biomedical applications, both for diagnosis by magnetic resonance imaging (MRI) and for targeted therapy of cancer by hyperthermia and releasing anti-cancer molecules. In vitro trials carried out on cancer cells treated with IOMNPS of 30 nm are currently in progress.

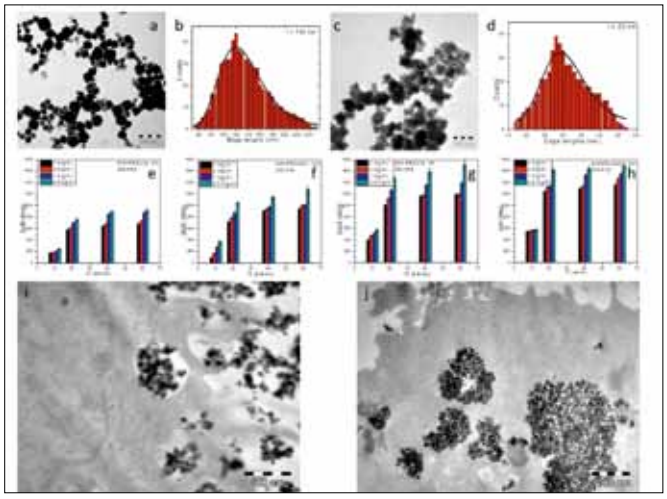

Figure 1: TEM images of IOMNPs synthesized in 60 ml (a) and 90 ml (c) of PEG200 for 6h reaction time and their corresponding size distribution histograms (b and d) fitted to a log-normal distribution (black curves). SAR values of IOMNPs synthesized in 60 ml (e and f) and 90 ml (g and h) of PEG200 for 6 and 12 h corresponding to four concentrations recorded at different applied magnetic fields at 355 kHz. The data are displayed as the mean of 5 measurements ± the standard error of the mean. TEM images of A549 cells incubated with IOMNPs of 30 nm for 4h (i) and 24 h (j).

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TARGETED INDUCTION OF IMMUNOGENIC CANCER CELL DEATH BY MITOXANTRONE-LOADED SPIONS

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Background: There is increasing evidence that activation of the immune system is crucially important for a positive long-term outcome of cancer therapy. Some chemotherapeutics (e.g. the anthracenedione mitoxantrone, MTO) have been previously shown not only to kill tumour cells but also to possess the intrinsic ability of inducing cell death with immunogenic features [1]. Contrarily however, systemic treatment with chemotherapeutics often severely impairs the patients` immune system, potentially precluding effective immune reactions. To induce immunogenic cell death exclusively in the tumour region and preserve immune reactivity at the same time, we developed superparamagnetic iron oxide nanoparticles (SPION) loaded with mitoxantrone (SPIONMTO) for Magnetic Drug Targeting (MDT) in the tumor. SPIONMTO have proven their long-term therapeutic efficacy in tumor bearing rabbits previously [2].
Methods: Multiparameter flow cytometry and fluorescence microscopy of cells treated with free MTO, SPIONMTO and unloaded SPION were performed to investigate efficacy against tumor cells and intracellular localization, respectively. Infiltration of SPIONMTO into three-dimensional cellular structures was investigated in 3D cell culture using HT-29 colon carcinoma spheroids. Supernatants of cells treated with MTO, SPIONMTO and SPION were investigated for release of immunogenic danger signals ATP and HSP70 using luciferase chemiluminescence assay and ELISA, respectively.
Results: Both free MTO and SPIONMTO infiltrated and dose dependently killed tumor cells (HT-29, Jurkat) and also tumor spheroids (HT-29), whereas the drug mainly accumulated in the cellular nuclei. In contrast, unloaded SPION showed excellent biocompatibility. Supernatants from cells treated with MTO and SPIONMTO revealed a dose dependent release of the immune stimulatory factors ATP and HSP70.
Conclusion: As shown by others for free MTO previously, SPIONMTO is also able to induce tumor cell death with immunogenic features. Further experiments are necessary to prove activation of immune cells by SPIONMTO-treated tumor cells. For clinical practice, the targeted induction of immunogenic cell death exclusively in the tumor region mediated by Magnetic Drug Targeting might be a promising possibility to selectively modulate the tumor microenvironment, thus stimulating long-term immune responses against the tumor (Figure 1).

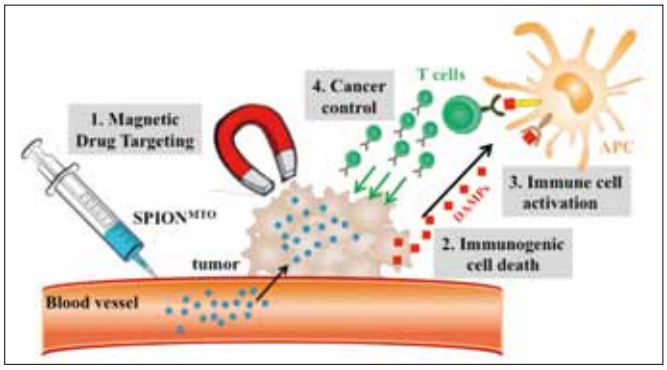

Figure 1: Immunogenic cancer cell death mediated by SPIONMTO in Magnetic Drug Targeting (MDT).

1.For Magnetic Drug Targeting, SPIONMTO are applied intraarterially into the tumor-supplying vascular system. The particles are then specifically accumulated in the tumor region by an external magnetic field.
2.In the tumor, SPIONMTO efficiently induce cell death with immunogenic features characterized by the exposition of calreticulin and heat-shock proteins and the release of HMGB-1 and ATP (damage associated molecular patterns, DAMPs).
3.Altogether, these signals act as endogenous adjuvants and promote the recruitment of Antigen Presenting Cells (APCs) and foster them to take up dead-cell-derived material and to prime an adaptive immune response.
4.Finally, activated T cells infiltrate the tumor and mediate a long-term anti-tumor immune response.

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SELF-ASSEMBLED QUANTUM DOT DNA HYDROGEL FOR DRUG AND SIRNA DELIVERY

SAE RIN JEAN

In recent years, there has been a considerable amount of interest in developing nanomaterials with a high degree of control over their physical features (eg. size, shape, or surface charge) that can be adapted for use in a variety of biological and therapeutic applications.[1] Some nanomaterials have demonstrated great promise in this regard, owing to their ability to overcome low solubility, off-target toxicity, and poor bioavailability. However, there is an unmet need for developing trackable nanomaterials with high biocompatibility that can be easily synthesized in a controllable manner. Using DNA as a building block for synthesizing nanomaterials has become increasingly prevalent.[2] Through sequence-directed hybridization, DNA molecules have the ability to form predictable two- or even three-dimensional nanostructures.[2] DNA hydrogels in particular have attractive features for biological applications such as high solubility, biocompatibility, and versatility (easily modified with bioactive cargo). Most notably, DNA hydrogels can be hybridized to virtually any type of nucleic acids (eg. siRNA, miRNA, or aptamer) without any need for chemical ligation and is amenable to

loading with DNA-binding drugs. DNA-based nanomaterials that can be monitored within an organism with a potential for multiplexing and multimodal imaging are highly desirable for biological studies. There are several examples of fluorescent hydrogels that have been developed to date such as silver nanocluster DNA hydrogels, quantum dot (QD) polymers or DNA hydrogel/polymer hybrids; however, most require complex multi-step synthesis and do not possess many of the aforementioned necessary features for biological applications.[3] We have selected quantum dots as our fluorescent label for their high photostability and quantum yield, spectral tunability, and ease of incorporation into DNA hydrogels. Herein, we describe the one-step synthesis of self-assembled quantum dot DNA hydrogels (QDHs) with precise control over the size and spectral emission and illustrate their versatile functions in biological systems.

Previously, we have shown that QDs can be functionalized with DNA with a phosphorothioate back bone.[4,5] By combining Y-shaped DNA that contain complementary sequence to DNA-templated QDs, we successfully synthesized the QDH entirely through self-assembly of the two designed materials. Furthermore, we have also shown that we can specifically tune the size and fluorescence emission of our QDHs by simply modulating the reaction time or concentration of starting materials. We have shown that QDHs have the ability to accumulate in cells effectively by endocytosis. By coating our QDs with ZnS, we were able to significantly reduce the well-established toxicity associated with QDs, and thereby creating a highly biocompatible material. We have observed through a series of *in vitro* and in cellulo tests that our QDHs are stable over a range of physiologically relevant temperatures and pH and are only subjected to degradation within the cells, possibly by endogenous DNAses. With the optimized ZnS coated QDHs, we delivered an anticancer drug doxorubicin (Dox) to cancer cells and have shown higher accumulation and effectiveness compared to free Dox. Additionally, by modifying the surface of QDHs with nucleic acids, we demonstrated the ability to target certain cell-types using aptamers thereby reducing off-target effects as well as modulation of the specific protein expression level using siRNA negating the use of toxic transfection agents. Taken together, we have synthesized the first self-assembled fluorescent QD DNA hydrogels with highly controllable size and spectral emission. Moreover, we have highlighted the versatility and therapeutic potential of QDHs as delivery vectors in biomedical applications.

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EVALUATION OF FATTY ACID METABOLISM AS TARGET FOR BREAST CANCER THERAPEUTICS

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Dysregulation of fatty acid (FA) metabolism is an early event in tumorigenesis and is a central hallmark of many cancer types, including breast cancer [1, 2], the second leading cause of cancer-related death among women worldwide. Breast cancer cells are known to produce 95 % of their FAs de novo, despite the presence of exogenous FAs, in order to sustain higher proliferation rates and faster growth [3, 4]. De novo FA synthesis is catalysed by fatty acid synthase (FASN), a key metabolic enzyme that is upregulated in 50 % of breast cancer cases [5, 6]. The expression level of FASN has been reported to correlate with poor prognosis conferring a 4-four-fold increased risk for death in breast cancer patients [7]. Thus, targeting of FASN by pharmacological agents may provide a strategy for selective elimination of the breast cancer cells and thereby for breast cancer treatment. One of the well-known FASN inhibitors is Orlistat, which blocks the thioesterase (TE) catalytic site of FASN and is presently in clinical use for obesity treatment [8, 9]. In the current formulation, Orlistat is administrated orally and has several limitations, including its extremely low solubility (180 nM) and low bioavailability (1 %) [9, 10] that hinder its development as a systemic drug. Therefore, a reformulation of Orlistat is required to be used for breast cancer treatment. The low solubility of Orlistat in aqueous culture medium may also be a factor of concern in *in vitro* cytotoxicity studies, since its delivery via solubilizing agents may create false negatives due to the compound`s precipitation in cell culture medium [11]. Therefore, the current study aimed to test in *in vitro* cytotoxicity of the effect of Orlistat dissolved in DMSO, as is conventionally done, and as pre-prepared non-targeted nanoparticles, which are expected to be taken up by the cells via the non-clathrin mediated pathway. We report that Orlistat dissolved in DMSO did not selectively kill the breast cancer cells, with the exception of MCF7 cells. Orlistat dissolved in DMSO precipitated and generated uncontrolled nanoparticles of different sizes upon mixing with cell culture medium. This may influence the nature of the drug uptake by the breast cancer cells and might explain the lack of Orlistat efficacy. However, incorporation of Orlistat into pre-prepared nanoparticles of controlled size improved the IC50 value in ZR-75-1 cells and reduced unspecific toxicity to MCF12A cells. In conclusion, incorporation of the very hydrophobic Orlistat drug into defined nanoparticles might solve the issues of drug precipitation and false negatives. Reformulation of the Orlistat into stable nanoparticle might potentially also solve the issue of using Orlistat for systemic delivery for the treatment of breast cancer.

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GRATING-BASED X-RAY PHASE CONTRAST TOMOGRAPHY FOR VISUALIZATION OF SOFT AND HARD TISSUES

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Grating-based X-ray phase contrast micro computed tomography (µCT) is superior in density contrast for the investigation of soft tissues, enabling simultaneous high-resolution three-dimensional (3D) visualization for soft and hard tissue components of the human body. As synchrotron radiation facilities impose severe restrictions on users, being laboratory systems easier accessible, the advanced conventional µCT-system (nanotom m®, GE, Wunstorf, Germany) was equipped with a grating-based phase contrast interferometer setup. The system consists of a nanopositioning system (PI GmbH & Co, Karlsruhe, Germany), a three-axes positioning stage and a motorized goniometer (Optics Focus Instruments Co. Limited, Hong Kong, China). As a part of this work a quantitative and qualitative comparison of the data acquired by means of the synchrotron radiation based high-performance systems at beamlines ID 19 (ESRF Grenoble, France) and I13-2 (Diamond Light Source, Didcot, UK) and the laboratory system mentioned above is being performed on selected specimens, which include a human knee joint. It was shown that synchrotron radiation-based µCT in the phase-contrast mode yields a true cellular resolution. The question arises to which extend the laboratory-based system can provide a comparable results. Using conventional µCT-system extended towards phase contrast soft and hard tissue samples, such as selected spiders (Hogna radiate and Xysticus erraticus) or human cerebellum block, have been clearly visualized. Phase contrast tomography within the laboratory environment can bring significant benefits for hierarchical 3D investigation of tissue micro-morphology, as it is non-destructive and provides high-resolution data with minimal sample preparation requirements and within a reasonable period of acquisition time.
Keywords: Grating-based phase contrast, micro computed tomography, hard X-ray, 3D visualization, human joint, spider, soft tissue.

INTRINSIC FLUORESCENCE OF PYRROLIDONE-MODIFIED PAMAM DENDRIMERS–CELL STUDIES

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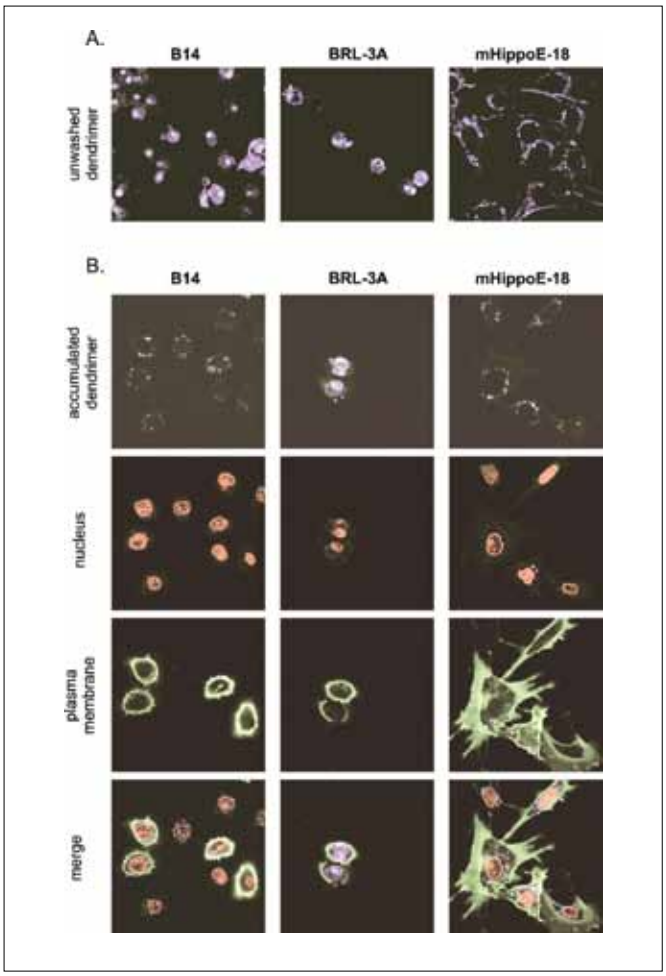
Fluorescence imaging techniques have broad applications in life sciences and clinical research. However, these techniques critically rely on bright and photostable fluorescent probes. Currently available fluorescent probes for biological imaging mainly include organic fluorophores and quantum dots. Small organic dyes suffer from several unwanted properties such as poor solubility, problems with targeting desired cell compartments, rapid irreversible photobleaching, and cell leakage. Inorganic nanoconjugates such as quantum dots are exceptionally bright, photostable, and characterized by narrow emission spectra but they possess important drawbacks. First of all, they are toxic and that can limit their applications *in vivo*. Moreover, their intracellular delivery raises problems that makes it difficult to follow some biological processes. Applying dendrimers – versatile, globular, monodisperse polymers with many surface functional groups – seems to be a solution that may help to overcome limitations of both single organic fluorophores and inorganic nanoprobes. The size of dendrimers places them on the same scale as fluorescent proteins: they are larger than organic dyes and smaller than quantum dots. Dendrimers have been used as scaffolds for fluorophores. However, such a modification creates a risk of decreased dendrimer biocompatibility, and affects its properties. That is why seeking intrinsically fluorescent dendrimers is of paramount importance. Modified G4 PAMAM dendrimers with 4-carbomethoxypyrrolidone surface groups referred to as a PAMAM-pyrrolidone dendrimers are characterized by an unique property. They possess strong intrinsic fluorescence (λexc=370 nm, λmax em=440 nm). Moreover, these dendrimers have been found to be very biocompatible and non-toxic, contrary to amino-terminated PAMAM dendrimers [1, 2]. The intrinsic fluorescence was used to visualize their location in cells (B14, BRL-3A, and mHippoE-18) Cells were incubated with the dendrimer at a concentration of 100 µM. Confocal images showing intrinsic fluorescence of accumulated PAMAM-pyrrolidone dendrimer in three tested cell lines performed after 24 hours of treatment without following washout demonstrated internal localization of the compound (Fig. 1A). Interestingly, some differences in dendrimer localization can be observed between tested cell lines. Although all cells seem to cumulate the dendrimer in cytoplasm, in B-14 and BRL-3A cells nuclear localization can also be observed, whereas in mHippoE-18 cells the fluorescence can be detected in lysosome-like structures, as well as at the cell boundaries (plasma membrane). In order to further confirm internalization of the dendrimer, all cells were washed once with phosphate buffered saline (PBS) and stained to visualize plasma membrane and cell nuclei (Fig. 1B). To detect the blue fluorescence of PAMAM-pyrrolidone dendrimer, before formaldehyde fixation, plasma membranes were stained using NeuroDiO carbocyanine dye and nuclei were stained with RedDot1 nuclear dye. As expected, dendrimer fluorescence was localized internally in all tested cell lines. Only BRL-3A cells retained staining pattern observed before dendrimer washout and cell fixation, confirming cytoplasmic and nuclear localization. In B14 cells fluorescence could only be detected in endosome-like structures, lacking nuclear accumulation, similarly to mHippoE-18 cells, where the part of dendrimer fluorescence at the plasma membrane could no longer be observed. It can also be noted that the RedDot1 staining partially colocalizes with the blue fluorescence signal, probably due to non intended binding of the dye to PAMAM-pyrrolidone dendrimer.
To analyze cellular uptake of the dendrimer by flow cytometry, all tested cells were incubated with the dendrimer at a concentration of 100 µM. Incubation times varied from 5 minutes to 48 hours. All tested cell lines accumulated PAMAM-pyrrolidone dendrimer rapidly, although its largest amount was observed in mHippoE-18 cells (Fig. 2). After 48 hours the intrinsic fluorescence intensity, which is directly proportional to the dendrimer concentration, was almost two times higher for these cells than for B14 cells.

Figure 1. Confocal images of B14, BRL-3A and mHippoE-18 cells treated with 100 µM PAMAM -pyrrolidone dendrimer for 24h. (A) Intrinsic dendrimer fluorescence of unwashed and non-fixed cells. (B) Following dendrimer accumulation (blue channel), cells were rinsed once with PBS and stained to visualize cell nucleus (red channel) and plasma membrane (green channel). Before imaging, cells were fixed with formaldehyde.



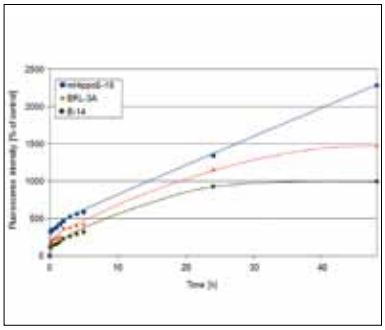


Figure 2. Cellular uptake of G4-PAMAM-pyrrolidone dendrimer at a concentration of 100 µM by mHippoE-18 (blue squares), BRL-3A (red rhombus) and B-14 (green circles) cells after incubation for 5, 15, 30 minutes, 1, 1.5, 2, 3, 4, 5, 6, 24, and 48 hours.

SUMMARY

Due to strong intrinsic blue fluorescence, cellular uptake behavior of PAMAM-pyrrolidone dendrimers could be directly analyzed by confocal microscopy and flow cytometry without additional fluorescence labeling, treatment of dendrimers with chemicals or adjusting pH. This first successful biological experiment opens a broad spectrum of possible PAMAM-pyrrolidone dendrimer applications as gene vectors, and drug delivery platforms that combine two functions: transporting and bioimaging at the same time.

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AUTOFLUORESCENCE OF PAMAM DENDRIMERS WITH 4-CARBOMETHOXY-PYRROLIDONE SURFACE

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Dendrimers are repetitively branched nanoparticles that have gained great popularity in recent years owing to their numerous biomedical, diagnostic and therapeutic applications. They are characterized by a highly ordered and regular structure. An interesting property of some groups of dendrimers is the fact, that they emit fluorescence despite having no known fluorophores in their structure. This phenomenon is called non-traditional intrinsic fluorescence (NTIF). It was observed in poly(amido amine) (PAMAM), poly(propylene imine) (PPI), poly(ether imide) (PEI) and poly(ethylene imine) (PETIM) dendrimers[1-3]. It has been proven, that the critical factor in the occurrence of fluorescence is the presence of tertiary amines in the dendrimer structure, whereas the fluorescence intensity is affected by such factors as generation and concentration of the dendrimer, pH, and oxidation[4-6].

The phenomenon of fluorescence might find applications in cell imaging by providing visibility of its constituent parts and processes that occur within the cell. Up to now, this is usually achieved by means of fluorophores (fluorescent dyes or quantum dots). These approaches have numerous disadvantages, which include e.g. limited resolution, low specificity, uncontrollable cellular efflux, as well as issues related to solubility and toxicity[7]. Autofluorescent dendrimers seem an interesting alternative for fluorophores that have been applied so far. To meet the expectations, dendrimers should be characterized by high intensity of fluorescence. Recently, it has turned out that PAMAM dendrimers possesing 4-carbomethoxypyrrolidone groups on the surface (4CMP-PAMAM) (Fig. 1) emit much stronger fluorescence than non-modified PAMAM dendrimers. To detect fluorescence of PAMAM dendrimers by spectrofluorimetry approx. 1 mM concentration is needed, whereas for higher generations of 4CMP-PAMAM dendrimers it is enough to apply 20 µM concentration. Apart from the core molecule (etylenodiamine versus diaminobutan) the interior of 4CMP-PAMAM and PAMAM dendrimers is identical, therefore this characteristic must be related to the functional pyrrolidone groups situated on the surface. Fluorescence intensity of 4CMP-PAMAM dendrimers was found to be dependent on the generation (Figure 2).

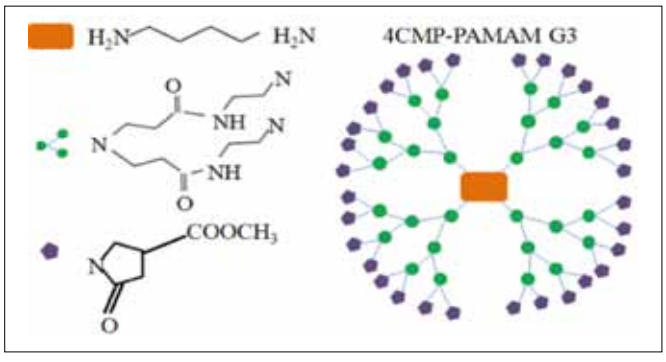

Figure 1. The structure of 4CMP-PAMAM G3 dendrimer

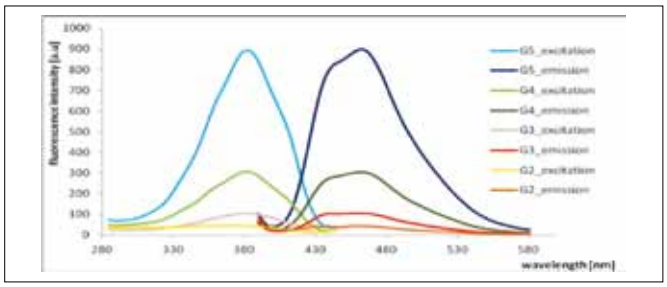

Figure 2. Excitation and emission spectra of G2, G3, G4, G5 4CMP-PAMAM dendrimers. c=20 µM, pH 7.4.

Up to now, the phenomenon of intrinsic fluorescence in 4CMP-PAMAM dendrimers has not been sufficiently investigated and still raises a number of concerns. For this very reason, our research aimed to examine spectral properties of these dendrimers by applying three quenchers: cesium chloride, potassium iodide and acrylamide. The above quenchers vary in terms of charge and size. CsCl did not quench fluorescence of 4CMP-PAMAM dendrimers. Acrylamide and potassium iodide were proven to be efficient quenchers.

The correlations between the concentration of the quenching substance and the decrease in fluorescence intensity are presented by means of the Stern-Volmer plots (Fig. 3).

A B

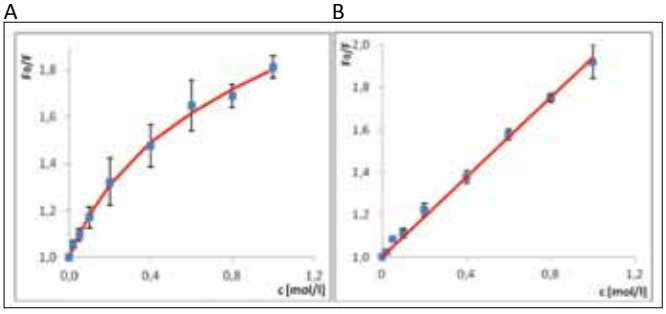

Figure 3. Examples of Stern-Volmer plots. Quenching fluorescence of 4CMP-PAMAM G2 (A) and 4CMP-PAMAM G5 (B) by acrylamide.

The obtained curves allowed to estimate Stern-Volmer parameters using (depending on the shape of the curve) either linear or non-linear least-squares analysis based on equations:

$$\frac{F_0}{F} = 1 + K_{sv}\ [Q] \quad (1)$$

$$\frac{F}{F_0} = \sum_{i=1}^{2} \frac{f_i}{1 + K_{SVi}\ [Q]} \quad (2)$$

where:

F_0 – fluorescence intensity in the absence of the quencher

F – fluorescence intensity in the presence of the quencher at a concentration [Q]

K_{SV} – Stern-Volmer constant

f_i – fractional intensity of component i

The results are summarized in Table 1

Table 1. Estimated Stern-Volmer parameters

	K1 [M^{-1}]	f1	K2 [M^{-1}]	f2	R^2
acrylamide					
G2	0,122±0,125	0,526±0,089	4,504±1,067	0,474±0,089	0,997
G3	0,950±0,495	-	-	-	0,991
G4	0,853±0,247	-	-	-	0,994
G5	0,939±0,031	-	-	-	0,996
potassium iodide					
G2	0,953±0,059	0,841±0,022	32,805±9,68	0,160±0,022	0,999
G3	0,5649±0,031	0,714±0,016	13,206±1,161	0,286±0,016	1,000
G4	0,610±0,134	0,709±0,078	8,834±3,125	0,291±0,078	0,997
G5	1,411±0,131	-	-	-	0,982

In some cases data modeling indicates the presence of two independent fluorescence-emitting sites in the structure of the dendrimers and their varying accessibility to the quencher. It can be attributed to the fluorescence properties of both the pyrrolidone surface and the dendrimer interior.

ACKNOWLEDGEMENTS

This study was funded by the National Science Centre (project "Intrinsically fluorescent dendrimers - spectrofluorimetric and cell biology studies" UMO-2014/14/M/NZ3/00498).

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EVALUATION OF NUCLEOLIN AND SOMATOSTATIN RECEPTOR TARGETING FOR THERAPEUTIC DELIVERY TO LUNG CANCER STEM CELLS

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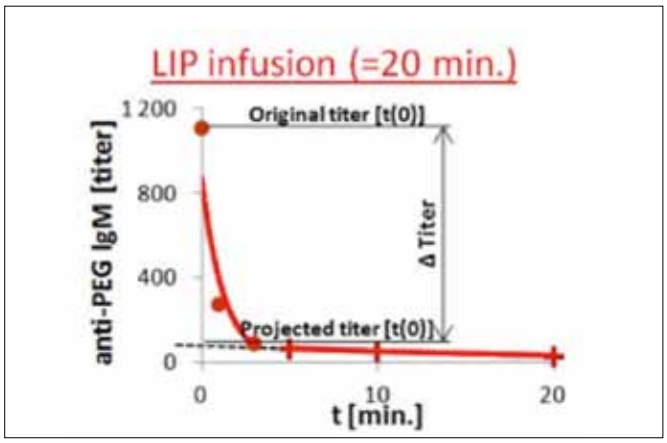
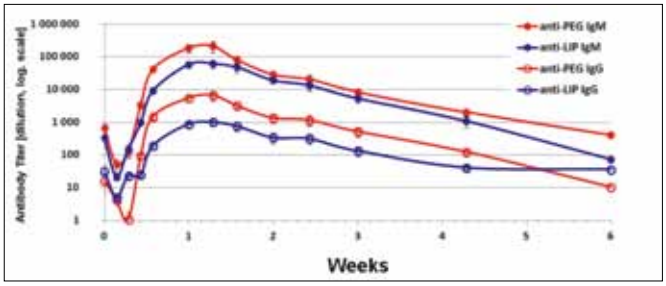


Intravenous injection of pigs with PEGylated liposomes mimicking doxorubicin-free Doxil®/Caelyx (Doxebo) was previously shown to prevent complement activation-related pseudoallergy (CARPA) to liposomal drug carrier, in a model of human hypersensitivity reactions to nano-drugs. To explore the time window of Doxebo's anaphylaxis-prophylactic (tachyphylactic) effect, we measured the pigs' cardiovascular reactivity to Doxebo and Doxil up to 6 weeks following Doxebo prophylaxis, along with the blood levels of anti-PEG and anti-liposome (anti-LIP) IgM and IgG antibodies (Abs) by own developed ELISA tests. Doxebo led within minutes to depression of preexisting anti-PEG/LIP Abs, explaining its tachyphylactic effect. However, from day 3, massive rises of these Abs were



seen, peaking at day 8 and returning to near baseline after 6 weeks. The rise was particularly massive with anti-PEG IgM, whose titer reached peak at over 200 000.During this time the *in vivo* reactions to liposomes strengthened converting the protective effect of drug carrier to dangerous poison, leading to anaphylactic shock in 5 out of 6 cases within minutes after the repeated injection of Doxebo.

THE UNLIKE TWINS: UPTAKE OF GRAPHENE OXIDE BY CACO-2 CELLS IS DEPENDENT ON CELL PHENOTYPE AND DIFFERENTIATION

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Due to the steadily increasing production of graphene-related materials (GRM) concerns regarding possible adverse effects on human and environmental health have been raised(1). The determination of a safety profile is urgently needed, not only because some GRM, especially graphene oxide (GO), are considered for potential biomedical application. In a recent study we have investigated the interaction of different GO with human intestinal cells *in vitro*. We have shown that exposure of non-confluent Caco-2 cells to four different GOs for up to 48 hours in a concentration range of 5-80 µg/ml GO did not lead to acute toxicity(2). Close interaction of GO with the cell surface of non-confluent Caco-2 cells gave hints towards a possible cellular uptake of GO. Other research groups have previously shown that different cell types are able to take up GO *in vitro*(3). Nevertheless, an evidence for uptake of GO by human intestinal cells has not been given to date. Aim of the here presented study was to explore the uptake of two GOs with different lateral size distributions by Caco-2 cells. To increase the physiological relevance Caco-2 cells were differentiated to an enterocyte-like phenotype forming tight monolayers of polarized cells with brush border and tight junction complexes. Uptake of GO was assessed in non-confluent as well as differentiated Caco-2 cells. Transmission electron microscopy (TEM) analysis revealed considerable amounts of internalized GO in non-confluent Caco-2 cells. Not only GO sheets of lateral dimensions below 1 µm were taken up by the cells. Even uptake of large GO sheets with lateral dimensions around 10 µm could be found. The formation of wave-like membrane structures associated with the GO sheets could be observed by scanning electron microscopy (SEM) analysis, giving hints towards an active uptake mechanism. Similar observations were made with graphene nanoplatelet (GNP) aggregates. In contrast, no uptake of the same applied GO samples could be found for differentiated Caco-2 cells. The obtained results clearly show that polarization and differentiation of Caco-2 cells have a dramatic influence on the graphene oxide uptake behaviour.

Figure 1: Scanning electron microscopy (SEM) analysis showed attempts towards uptake of GO, as well as of other graphene-related materials (GRM) such as graphene nanoplatelets (GNPs). The image shows the surface of non-confluent undifferentiated Caco-2 cells after exposure to GNP for 24 hours. Aggregated GNP of aggregate sizes up to 5 µm and more were partly enveloped by membrane protrusions giving hints towards active uptake attempts by the cells. The GNP aggregate is displayed in purple to enhance visibility.

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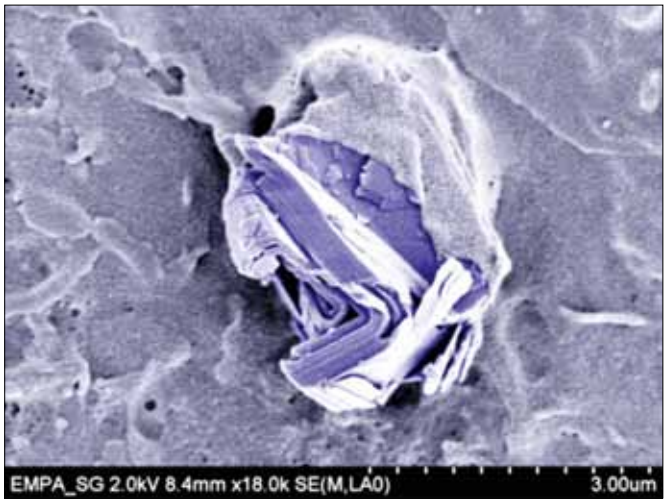

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ON-CHIP ELUCIDATION OF THE ROLE OF MYELOID CELLS IN T CELL IMMUNOTHERAPY

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Immune therapies hold tremendous promise as the next breakthrough in cancer treatment. The concept builds on harnessing the intrinsic properties of a patient's own immune cells to therapeutically override immune tolerance that cancer cells exploit for their aggressive outgrowth and eventual metastasis[1]. One salient example of T cell immunotherapy is CD8+ T cells engineered to express a T cell Receptor (TCR) directed against Hepatitis B Virus (HBV)-associated Hepatocellular Carcinoma (HCC). Their killing efficacy has been characterized *in vitro* [2-4] and has formed the basis of a recent clinical trial[5].

There is increasing evidence, however, that tumour-associated myeloid cells may undermine the anti-tumour effects of CD8+ T

cells. Of particular interest are tumour-associated monocytes/macrophages that have been identified for promoting the exhaustion and suppression of CD8+ T cells[6, 7]. However, the underlying mechanisms of the interaction between myeloid cells and T cells have yet to be clearly elucidated in the context of engineered T cells. With a clearer understanding of the role of myeloid cells, cancer immunotherapy can be optimally designed for patients to benefit from its full therapeutic potential.

In this scenario, microfluidic technologies could serve as an elegant tool for pre-clinical testing to elucidate the role of myeloid cells in T cell cancer immunotherapy[8, 9]. Such platforms can mimic important physiological cues in the cellular environment through spatial and temporal control over gradients of soluble factors and cell-cell interactions in a 3D extracellular matrix-like hydrogel[8, 9]. Most importantly, the combination of multiple relevant cell types in a controlled system bridges the gap between simplified 2D assays and complex *in vivo* models. Whereas 2D assays lack the structural architecture of body tissues, *in vivo* animal models, in addition to their complexity, might not effectively replicate the features of human tumors[10].

Recently, there have been collective indications that 3D platforms provide a more realistic setting to investigate a cell's sensitivity to biological factors[11]. Microfluidic platforms thus play an important role in probing for signalling mechanisms that underlie cell-cell interactions. For T cell immunotherapy, interfering with inhibitory signals, particularly programmed death-ligand 1 (PD-L1), could augment the effector function of tumour antigen-specific CD8+ T cells to mediate sustained cancer regressions[12]. Moreover, evidence from human tissue samples indicates a correlation between PD-L1 expression by tumour-associated myeloid cells and the suppression of CD8+ T cell activities[13]. However, the mechanism of PD-L1-mediated cellular interactions remains unclear and might be efficiently unravelled through an appropriately designed microfluidic platform.

In this work, the impact of autologous monocytes on HBV-HCC-specific T cell anti-tumour efficacy is investigated in a 3D microfluidic system (Figure 1a-c). With the advantage of high-resolution real-time imaging, the goal of this study is to elucidate specifically this role of monocytes in an environment that closely mimics the intra-hepatic carcinoma environment. Preliminary data indicates that autologous monocytes suppress the killing efficacy of engineered T cells. Ongoing investigations focus on the role of PD-L1 as a potential mediating factor. In turn, the insight obtained through this work may be used to improve the design of these engineered T cells for more robust cancer immunotherapy.

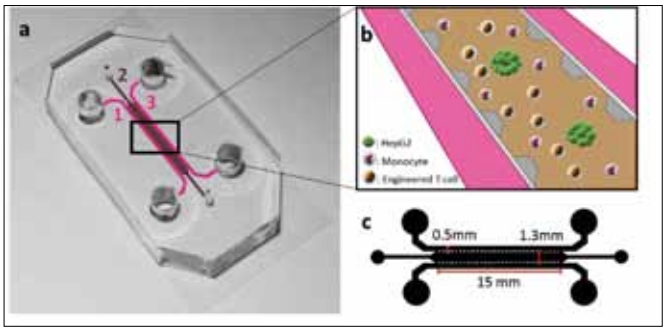

Figure 1 (a-c): (a) Microfluidic device with middle gel channel ('2') flanked by media channels ('1', '3'). Scale bar = 5mm. (b) Illustrative diagram of tumour microenvironment generated in microfluidic device. (c) Microfluidic chip design and dimensions. Channel height = 150µm, post width = 300µm, spacing between posts = 150µm.

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THE IMPACT OF NANOPARTICLES ON THE RELEASE OF NEUTROPHIL EXTRACELLULAR TRAPS (NETS) FROM PRIMARY HUMAN NEUTROPHILS

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A number of nanomedicines are currently licensed for use clinically with many more in development[1]. However, in order to translate these nanoparticles through to use clinically, careful consideration must be given to their compatibility with immunological and haematological systems[2]. Nanoparticles have been shown to both stimulate and suppress the immune system via a number of mechanisms[3] which may be of use therapeutically. However, the relationship between particle characteristics and biological effect must be carefully defined. The uptake of nanoparticles by cells of the immune system can affect their biodistribution as well as their biocompatibility[4, 5]. Neutrophils are part of the first line of defence against pathogens and foreign material[6] and, as phagocytic

cells, capable of internalising nanoparticles[7, 8].

Previous reports have shown that uptake of nanoparticles into phagocytic cells may cause oxidative stress[9]. Uptake of nanoparticles into neutrophils has been shown to generate a respiratory burst, characterised by the presence of reactive oxygen species (ROS)[10]. The generation of oxidative stress has also been shown to be linked to the generation of Neutrophil Extracellular Traps (NETs) [11, 12], and many nanoparticles are known to generate ROS upon accumulation within cells[13, 14], providing a putative mechanism for their toxicity and/or immunogenic effects[9]. Recent evidence also suggests that increased NET formation or insufficient dismantling of NETs can lead to breaking of tolerance and the promotion of autoimmunity[15]. Previous reports within the literature have shown that titanium dioxide (TiO2) nanoparticles can induce the release of NETs from a piscine model as well as human neutrophils[16, 17]. The aim of the current study was to investigate the possible generation of NETs from human neutrophils by nanoparticles known to generate oxidative stress within the cells in which they accumulate.

In order to determine the potential for silver nanoparticles (Ag-NP) to generate oxidative stress THP1 cells were treated with Ag-NP using a range of concentrations (0.01, 0.1, 1 and 10µg/mL) for 24 hours. Camptothecin (10mM) was included as a positive control and following incubation; cells were assessed via flow cytometry using the CellROX green reagent for the presence of ROS. Neutrophils from adult healthy controls were isolated using HetaSep and seeded onto coverslips at a concentration of 2x105 cells per condition. The neutrophils were seeded for 1hr and then incubated for 2hrs with either phorbol myristate acetate (PMA) previously used as a positive control for NETosis[18], titanium dioxide nanoparticles previously demonstrated to induce NETosis (TiO2; 1µg/mL) or a range of concentrations of Ag-NP (0.5, 1, 5, 7.5, 10 or 50µg/mL). To visualise any NETs formed the cells were stained with DAPI, Neutrophil Elastase and Myeloperoxidase and images we taken using a confocal microscope. Data are presented as mean ± Standard deviation of, at least, n = 4 experiments conducted in triplicate. Statistical analysis was conducted using unpaired t-test using Stats Direct software (version 2.7.9).

Ag-NP were shown to cause significant oxidative stress as evidenced by greater glutathione content in cells treated with Ag-NP than that of untreated cells (figure 1a) and a reduced level of ROS (figure 1b). Camptothecin treatment resulted in a significantly higher level of glutathione in treated cells (32% greater; P=0.034) with a subsequently lower level of ROS (56% lower; P=0.023); indicative of an antioxidant response. Similar effects were also observed with Ag-NP as 10µg/mL, which resulted in greater glutathione content than untreated cells (35% greater; P=0.035) and lower ROS levels (41% lower, P=0.02).

NET generation (figure 2) was observed with PMA treatment (60% of cells generated NETs) and camptothecin (12.5%) both of which are known to induce oxidative stress. At higher concentrations Ag-NP were able to induce NETosis in primary human neutrophils (7.5, 10 and 50µg/mL, 50%, 81% and 100% respectively) likely as a consequence of the generation of oxidative stress.

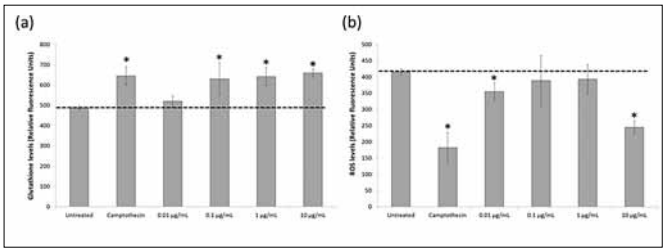

*Figure 1. Level of reactive oxygen species (a) and glutathione (b) in THP1 in response to treatment with silver nanoparticles (Ag-NP) for 24 hours. Camptothecin included as a positive control. Data expressed as mean ± SD, N=4, dashed bar represents levels in untreated cells. *=P<0.05, **=P<0.01, ***=P<0.001*

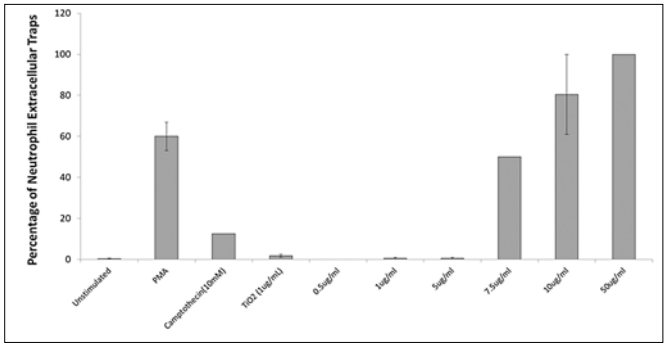

Figure 2. Percentage of neutrophil extracellular traps (NETs)in response to treatment with silver nanoparticles (Ag-NP) for 2 hours. PMA was included as a positive control for NET generation Camptothecin included as a positive control. Data expressed as mean ± SD, of at least 2 experiments.

In summary the generation of oxidative stress by nanoparticles within neutrophils may contribute to the induction of NETosis. The generation of NETs has been linked to inflammation and autoimmunity, which may serve to impact the biocompatibility of nanomaterials via an indirect mechanism. Further study is now warranted to determine the impact of organic based nanomaterials that may be used as drug delivery systems on NETosis and if this may serve as a useful marker of nanoparticle biocompatibility. Additionally the consequences of nanoparticle induced NETosis must be determined *in vivo* to fully understand its consequences for translation of novel, engineered, nanoparticles.

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RGD-TARGETED MICROBUBBLES LOADED WITH ACTIVE AGENTS FOR SONOPORATION ENHANCEMENT

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INTRODUCTION

Efficient drug delivery to and into tumours can be limited due to differences in vascular permeability, interstitial fluid pressure and extracellular matrix in the tumour microenvironment. The application of ultrasound (US) pulses in combination with i.v. administered microbubbles (MB) has been shown to induce vascular permeability (also referred as sonoporation) and to be improve drug delivery across biological barriers[1-3]. In this study, we aimed to enhance sonoporation efficiency by encapsulating vascular disrupting (combretastatin) and anti-cancer (doxorubicin) agents into the shell of RGDfK-targeted polymeric MB.

METHODS

The loading process for encapsulation of drugs was performed using established protocol[1]. The loading capacity of combretastatin and doxorubicin was optimized by investigating different amounts of drug and solvent. In order to enhance the binding and retention of PBCA-based MB to activated endothelial cells in tumor blood vessels, RGDfK peptide was coupled on the surface of the MB. The binding efficiency of RGD-targeted MB was evaluated in-vitro by flow chamber experiments using TNFa-stimulated HUVEC[4].

Results: PBCA-MB have a diameter in 2.5 µm, a shell-thickness of approx. 50 nm, and they can be efficiently loaded with doxorubicin (Figure 1, A-C). By varying drug concentrations and solvents, we could improve the drug loading efficiency of combretastatin and doxorubicin (Figure 1, D-F). In the *in vitro* flow chamber model, RGDfK-targeted MB showed significantly higher to the stimulated HUVECs than non-targeted MBs (Figure 2). Specific binding was confirmed by blocking experiments.

CONCLUSION

Combretastatin and doxorubicin could be efficiently loaded into the shell of PBCA-MB by varying different parameters such as drug concentration and solvent. Also, RGDfK targeted MBs showed a better binding efficacy than non-targeted MBs. These findings may provide a starting point for making drug-loaded MB which can be employed to enhance sonoporation-mediated drug delivery.

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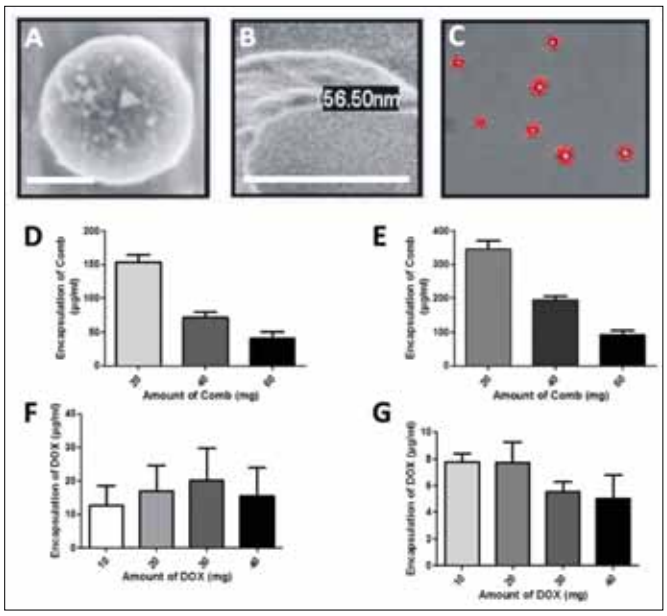
Figure 1: MB synthesis and drug loading efficacy. Panel A-C: SEM and fluorescence microscopy images of PBCA-MB with a diameter of ~2.5 µm (A) and a shell-thickness of ~50 nm (B). Efficient loading of DOX into MB shell (C). Panel D-E: Loading capacities of combretastatin into MB with varying concentrations of drug in 1:1 of DMSO and ethanol (D) and only DMSO (E) as a solvent. Panel F-G: Loading capacities of doxorubicin into MB with varying concentrations of drug in HEPES (F) and HEPES (free) Triton-100 solution (G) as a solvent.



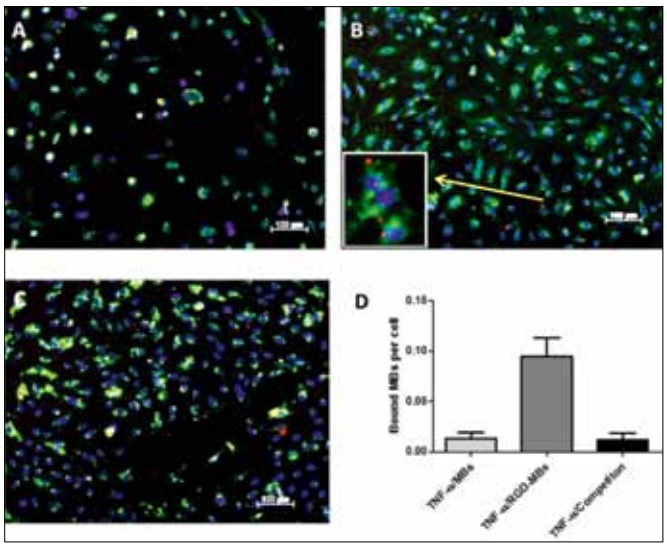


Figure 2: In vitro binding experiment of targeted and non-targeted MB using Tumor Necrosis Factor-α (TNFα)–stimulated Human Umbilical Vein Endothelial Cells (HUVEC). Panel A-C: Fluorescence microscopy analysis of RGDfK targeted MB to HUVECs. The cell membrane was labeled with wheat germ agglutinin Alexa Fluor 488 (green), the nucleus with Hoechst (blue). Significantly lower binding was observed for non-targeted MBs (A), a higher binding efficacy

was observed in the case of RGDfK targeted MB (B) and competition experiment proved the specific binding of RGDfK-MB (C). Quantitative analysis confirming significantly higher binding of RGDfK targeted MB to HUVECs as compared to other groups (D).

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PRESENTATION AND MAPPING OF MULTIVALENT RECOGNITION SITES ON BI-FUNCTIONAL NANOPARTICLE-PROTEIN CONJUGATES

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Grafting of biomolecules onto NPs offers the opportunity of linking biologically active sequences to artificial and "biologically inert or non-specific" materials. This can potentially impart a biological function to nanomaterials, by increasing the NP's biological recognition, enabling selective targeting, and responding to multiple stimuli in biological milieus[1,2]. The nanoparticle (NP) surface provides a unique space for the grafting of different biological moieties. This enables the generation of multivalent, for example, bifunctional or bispecific NP-protein conjugates which will create new prospects in cellular targeting by stimulating diverse pathways with one object, therefore implementing the selectivity of the targeting[3].
Here, we show the optimization of the synthesis and characterization strategies for bifunctionalized NP-protein conjugates containing two recognizable proteins (Figure 1).

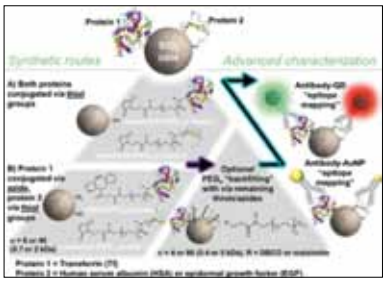

Figure 1 Schematic illustration of the synthetic and the epitope mapping strategies utilized.

As a model system we used combinations of transferrin (Tf), human serum albumin (HSA), and epidermal growth factor (EGF) on fluorescent silica NPs. By quantification of the proteins and mapping of exposed recognition sites with gold nanoparticle (AuNP) and quantum dot (QD) nanoprobes, data on the composition, distribution and numbers of recognizable sequences exposed on bispecific NP-protein conjugates are obtained (Figure 2). Our results show that the exposed and accessible protein sequences can be one to two orders of magnitude lower than overall conjugated proteins while still possessing specific cellular recognition[4]. Methods presented here are crucial for a careful design of future bispecific and multivalent NPs and are an initial step towards successful exploitation of multivalent surfaces in bionanotechnology and nanomedicine.

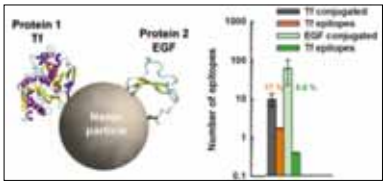

Figure 2 Comparison between the total amount of Tf and EGF protein detected on the NP surface and the amount of corresponding exposed epitopes.

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CHARACTERIZATION OF NANOPARTICLE-SURFACE INTERACTIONS BY NEAR-FIELD LIGHT SCATTERING

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Measuring and understanding the interactions between nanoparticles (NPs) and cell surfaces, are of paramount interest for the developing specific targeting drug delivery complexes based on NPs. Experimentally, the direct measurement of the forces between nanoparticles (NPs) and surfaces by traditional methods (e.g. AFM) in realistic exposure conditions is challenging due to that these interaction occur in complex environments (usually under fluid flow conditions, at high salt concentrations, at room temperature, etc). As an alternative, in this work we present a novel technique to characterize NP-surface interaction in a microfluidic chamber. The proposed method is based on generating an optical potential to trap NPs close to a surface. A schematic representation of the experimental setup is shown in Fig. 1a. The main element of the device is a waveguide (WG) that transports light from a laser source through the experimental chamber. The propagated light generates an exponentially decaying field that extends above the waveguide. This decaying field is referred to as evanescent field and when a NP passes close to the waveguide it is trapped by an optical force that is a result of the sharp gradient in the light intensity. The NP will then go under Brownian motion and will scatter light. The closer to the WG the more light it will scatter (as the light intensity decays from the WG to the medium) and this effect is used to measure the fluctuations around the minimum of the potential well (Fig. 1c). The NP will also absorb and scatter photons from the evanescent field which results in a momentum transfer to the particle in the direction of light propagation. Using a video camera, the position of the NP is recorded and a the full 3D trajectory is reconstructed as shown in Fig. 1b. The obtained trajectory is then analyzed by statistical mechanic methods which enables to quantify the interation of the NP with the surface of the WG. Also, averages over a set of NPs can be used to characterize the size distribution of a sample of NPs. As a proof of concept, we study the interaction of Silica NPs coated with covalently grafted polyethylene glycol (PEG) and a Si3N4 WG. Additionaly, the mesuarements are performed in a set of phosphate buffer saline (PBS) solutions of different concentration to study the effect of the salt concentration on the NP-WG interaction.

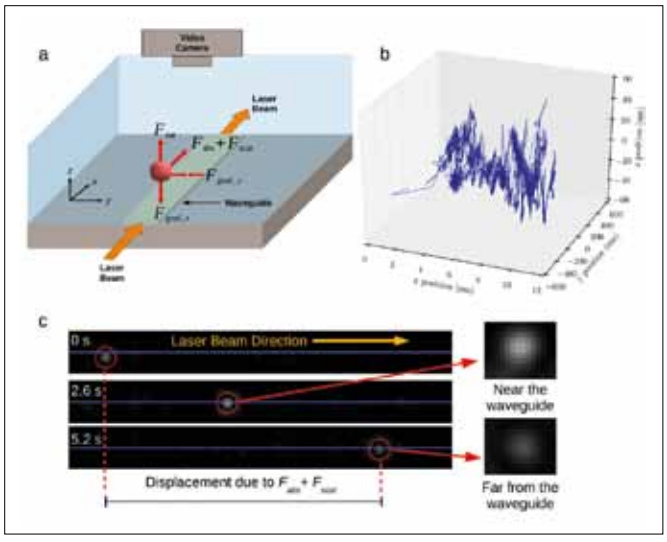

FIG. 1. (a) Schematic representation of the experimental setup. (b) Full 3D trajectory of one NP. (c) Images of a NP traveling over the WG at different times. Due the exponential decay of the evanescent field, NPs closer to the surface scatter more light and are detected brighter. The direction of the light in the WG is from left to right and so is the movement of the NP.

MAGNETIC AND PLASMONIC NANOPARTICLES CHARGED LIPOSOMES FOR DRUG DELIVERY APPLICATIONS

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Liposomes represent a main drug delivery carrier system, intensively tested for improvement therapeutic efficacy, many liposomal drugs being currently approved for cancer therapy. The liposomal platform has been the object of many recent researches aiming at a continuous optimization for improved stability *in vivo*, high drug loading, targeted delivery at the tumor site, stimuli responsivness for efficient uptake by cancer cells[1].

We report herein two strategies developed in our group for the creation two types of nanoobject, namely plasmonic and magnetoliposomes.

Firstly, plasmonic liposomal nanocarriers have been prepared by taking advantage of the electrostatic interactions between small unilamelar cationic liposomes and negatively charged biocompatible gold nanoparticles, synthesized using an original method developed in our laboratory[2]. The cationic liposomes were decorated on their outer surface with anionic gold nanoparticles (figure 1.).

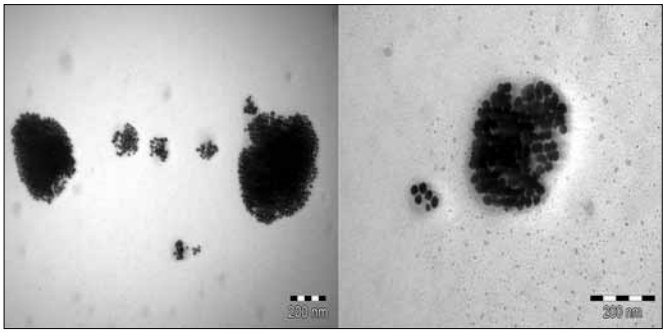

Fig.1. Transmission Electron Microscopic images of Au nanoparticles decorated cationic liposomes.

The decorated liposomes have been characterized by UV-Vis absorption spectroscopy, TEM and DLS. The aggregation of the Au nanoparticles at the outer surfacde of the lipid vesicles lead to a red shift in their plasmonic resonance. The attachement of the anionic nanoparticles to the cationic liposomes reduces the zeta potential of the latter (figure 2 d), however the residual charge on the nano hybrids is large enough to overcome liposome aggregation, as revealed by large scale TEM and no significant change in the size distribution obtained by DLS. The plasmonic liposomes were tested for their Surface Enhanced Raman Spectroscopy (SERS) capabilities. In the case of PEGylated Au nanoparticle decorated liposomes we were able to record SERS spectra of lipids within the liposomal membrane due to the strong attachement of the nanoparticles to the lipid vesicles[3].

In a different type of approach magnetic liposomes were created by embbeding hydrophoblic superparamagnetic iron oxide nanoparticles (SPION) within the bilayer of neutral lipids liposomes. The magnetoliposomes have been synthesized using the dry film hydration method by mixing, neutral phospholipids with hydrophobic SPIONs produced by thermal decomposition of magnetic precursors. The organic solvent of the lipid-SPIONs mixture has been removed using a rotary evaporator. The as obtained lipid film has been hydrated with Tris-buffered saline solution (TBS) containing hydrophilic chemotherapeutical agents, followed by sonication. Several classes of phospholipids and the effect of the addition of cholesterol have been tested.

The TEM images confirmed the intercalation of hydrophobic SPIONs with an average diameter of 7 nanometers into liposomal lipid bilayer membrane. The drug release capacity upon interaction with an external alternating magnetic field of different amplitudes and frequencies has also been evaluated. The magnetoliposomes have been characterized by UV-Vis absorption spectroscopy, Dynamic Light Scattering (DLS), Zeta Potential Measurements and Transmission Electron Microscopy (TEM). Their magnetic induced hyperthermia properties have been assessed in external magnetic fields with intensities ranging between 5 and 60 kA/m and frequencies between 100-400 kHz. The toxicity of the nanohybrids have been assessed in-vitro on different cell lines using the standard MTT assay.

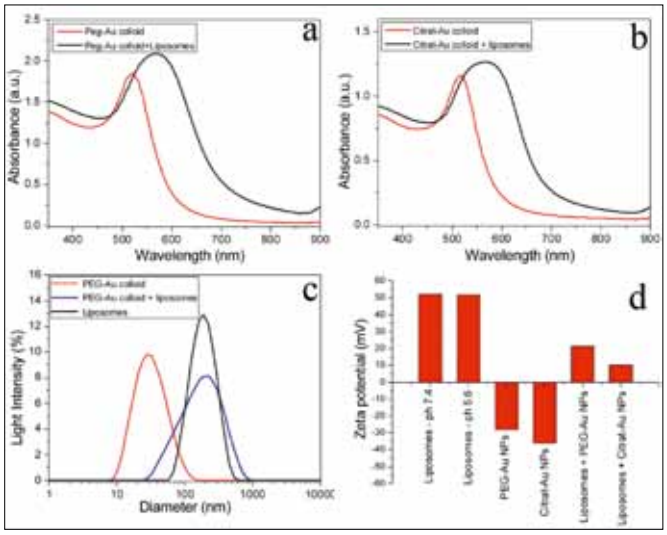

Fig.2. UV-Vis absorption spectra of Au nanoparticles and Au nanoparticles decorated liposomes, for PEGylated (a) and citrate capped (b) Au baboparticles. DLS (c) and zeta potential data for base and Au nanoparticles decorated liposomes.

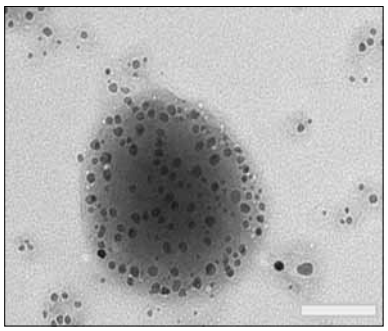

Fig.3. Transmission Electron Microscopic image of magnetoliposomes.

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EVALUATION OF NUCLEOLIN AND SOMATOSTATIN RECEPTOR TARGETING FOR THERAPEUTIC DELIVERY TO LUNG CANCER STEM CELLS

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Cancer stem cells represent the putative tumor-driving subpopulation thought to account for drug resistance, relapse, and metastatic spread of epithelial and other cancer types. Accordingly, cell surface markers for therapeutic delivery to cancer stem cells are subject of intense searches. Somatostatin receptor 2 and nucleolin are known to be overexpressed by various cancer types, which elicited comprehensive efforts to explore their therapeutic utilization. Here, we evaluated somatostatin receptor 2- and nucleolin-targeting for therapeutic delivery to cancer stem cells from lung cancer. We report that nucleolin is expressed highly, but not selectively, while somatostatin receptor 2 is expressed selectively, but not highly, by cancer cells. The non-small cell lung cancer cell lines A549 and H1299 displayed average levels for both surface molecules as judged based on analysis of a larger cell line panel. H1299 compared to A549 cells showed significantly elevated sphere-forming capacity, indicating higher cancer stem cell content and, thus, qualifying as a suitable test system. Uptake studies with radiolabeled somatostatin receptor 2-targeting ^{57}Co-DOTATATE revealed that somatostatin receptor 2 expression levels are not sufficiently high in H1299 cells to confer efficient uptake by either non-cancer stem cells or cancer stem cells. By contrast, nucleolin-targeting ^{57}Co-DOTA-AS1411 aptamer resulted in efficient internalization by non-cancer stem cells and remarkably at even higher efficiency by cancer stem cells. These data provide indication that the nucleolin-targeting AS1411 aptamer might be repurposed for therapeutic delivery to non-small cell cancer stem cells.

DENSITY OF NLS PEPTIDES ON THE SURFACE OF QUANTUM DOTS CORRELATES WITH THEIR IN VITRO ENDOCYTOSIS AND NUCLEAR TARGETING EFFICIENCY

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Organelle-targeted drug delivery can enhance the efficiency of drugs acting on intracellular targets and reduce their toxicity. We generated core-shell type fluorescent CdSe-ZnS quantum dots (QDs) decorated with varying densities of nuclear localization sequence (NLS) peptidic targeting residues and investigated their endocytosis and nuclear targeting efficiencies in HeLa cells. Increased number of surface NLS groups induced more efficient endocytosis of QDs and enhanced their targeting to the cell nuclei. We continue to investigate the QDs-NLS nuclear targeting, its limiting factors (mechanisms and efficiencies of endocytosis, endosomal escape, and intracellular trafficking), and their dependence on the formulation properties (QDs size and charge, type and varying density of NLS residues, etc.). These findings will contribute to development of subcellularly-targeted drug delivery systems (DDSs) that will deliver specific drugs (such as anti-cancer agents and DNA drugs) to the nuclei of the target cells and will enhance efficacy and reduce toxicity of these drugs.

INTRODUCTION

Nucleus is site of action of numerous drugs, including some anticancer and immunosuppressive agents, steroids, DNA drugs, etc. Inefficient penetration of these drugs to the site of their desired action in the nucleus of target cells limits their efficiency and enhances their toxicity due to pharmacological activities at other locations. Therefore, encapsulation of drugs into specialized drug delivery systems (DDSs) targeted to the nucleus has been suggested as an approach that can enhance the desired drug effects and limit their toxicity. For this purpose, nanoparticles, liposomes, and other types of DDSs can be used, and decoration with specific residues, such as nuclear localization sequences (NLS) or cell penetration peptides, can be applied to enhance nuclear targeting of DDSs (i.e., preferential accumulation of the DDSs in the nucleus, as compared to the other organelles).[1]

Dozens of studies reported endocytosis and accumulation of DDSs in the nucleus in different experimental settings (usually, in *in vitro* experiments with specific cell lines), and efficient targeting of DDSs to the nucleus has been claimed in some of these studies. Unfortunately, in many cases these claims were based solely on qualitative data. Limited volume of quantitative data on efficiency of nuclear DDSs targeting hampers the investigation of the mechanisms that govern the DDSs intracellular biofate and the factors that limit their efficiency. For instance, without quantitative characterization of the DDSs formulation properties and their intracellular disposition (distribution and elimination), conclusions can't be reached regarding the preferred size of the DDSs, type/number/density of targeting residues, and other formulation properties that are associated with preferential DDSs accumulation in the nucleus.

Previously, we developed experimental techniques for dense decoration of DDSs with NLS, tools for quantification of the decoration efficiency and imaging-based techniques for quantification of DDSs intracellular disposition and targeting efficiency.[2,3] The main objective of this work is to analyze quantitatively the nuclear targeting efficiency of a model nano-DDS (based on quantum dots, QDs) decorated with varying densities of nuclear localization sequences and to identify the limiting factors for nuclear targeting of nano-DDSs.

EXPERIMENTAL METHODS

Core-shell type fluorescent CdSe-ZnS QDs were prepared using high temperature organometallic approach.[4] Then, hydropho-

bic CdSe-ZnS QDs (QD-octadecyl) were transformed into water-dispersed hydrophilic PEGylated QDs (QD-COOH) using reverse micelle-based polyacrylate coating chemistry. In the next stage, linker (cysteamine) was conjugated to the carboxylic acid groups on the QDs surface using carbodiimide reaction. Finally, nuclear localization signal (NLS) peptide was conjugated to the QDs using maleimide-thiol coupling reaction at room temperature. At each decoration stage, the unreacted reagents were thoroughly removed (by precipitation-redispersion, dialysis, etc.) to produce the formulations (QD-COOH, QD-thiol, and QD-NLS) that were investigated in subsequent experiments.

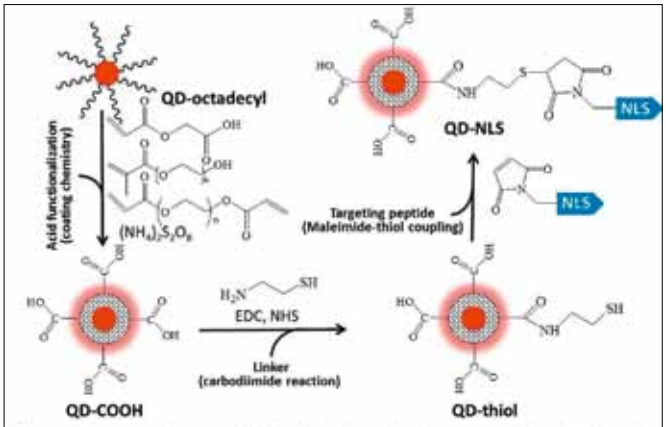

Figure 1: The consecutive stages of QDs surface decoration that were carried out in this study.

Morphology of QDs was studied using transmission electron microscopy (CM120 Super Twin TEM, Philips, operating at 120 kV). Size distribution of QDs and their zeta-potential at pH 7.4 and 4.8 was measured using ZetaSizer Nano ZS, (Malvern Instruments, Malvern, UK). Fourier transform infrared spectra of solid and dry samples were measured using Nicolet FTIR spectrometer 6700 (Thermo Fisher Scientific, Inc.) Number of NLS peptides per QD was calculated based on HPLC-based analysis of unreacted peptides in the reaction mix.[4]

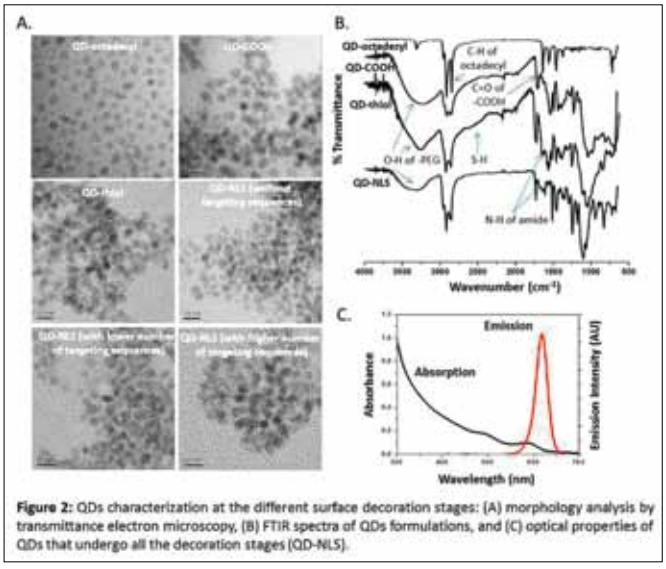

Figure 2: QDs characterization at the different surface decoration stages: (A) morphology analysis by transmittance electron microscopy, (B) FTIR spectra of QDs formulations, and (C) optical properties of QDs that undergo all the decoration stages (QD-NLS).

HeLa cells were cultured in DMEM medium with 5% fetal calf serum, 4.5 g/L D-glucose, 2 mM L-Glutamine, 100 IU/mL penicillin and 100 µg/mL streptomycin. The cells were maintained in an incubator at 37°C in a humidified atmosphere with 5% CO_2. HeLa cells (100,000 per well of a 24-well plate) were grown on coverslips and were incubated with the investigated QDs (~1015 QDs/well) for 14 hours. After that, the cells were extensively washed with PBS, stained with DiO membrane dye, fixed with 2.5% formaldehyde solution, washed with PBS, and mounted on slides using DAPI Fluoromount-G solution. Confocal images of the HeLa cells incubated with the investigated QDs were acquired using Olympus FV100-IX81 confocal microscope (Tokyo, Japan) equipped with 60x oil objective. Intracellular localization of QDs in the individual cells was quantitatively analyzed using a custom-written IntraCell plugin for ImageJ software.

RESULTS AND DISCUSSION

We prepared CdSe-ZnS QDs (QD-octadecyl) and then used a 3-stage approach to change their surface chemistry and to decorate them with the PEG, linker and NLS peptide molecules. The QDs had spherical shape (Figure 2A) and increased in size following each decoration stage. Coating and individual decoration steps induced characteristic changes in the FTIR spectra of QDs (e.g., 1720 cm^{-1} and 3250 cm^{-1} peaks characteristic to C=O and –OH stretching, respectively, in the spectrum of QD-COOH; 1630 cm^{-1} peak characteristic to N-H bending of amide functional groups in the spectrum of QD-NLS; see Figure 2B). Analysis of the spectral properties (Figure 2C) of the fully decorated formulation (QD-NLS) revealed that it has a broad absorption window with substantial absorbance in the 300-600 nm range, but with a narrow emission spectrum centered at 620 nm.

Surface decoration of QDs with different numbers of NLS has profound influence of their interaction with HeLa cells (Figure 3). Specifically, decoration with NLS groups significantly affected the endocytosis of QDs and enhanced their accumulation in the nucleus.

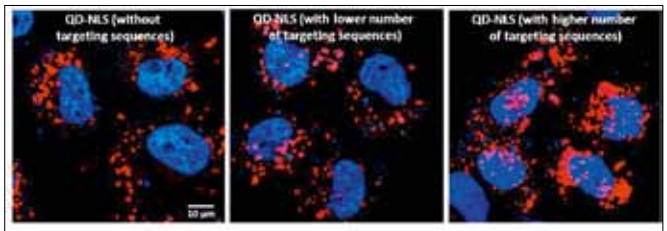

Figure 3: Relation between surface density of NLS targeting residues and intracellular localization of QD-NLS particles in HeLa cells. PEGylated QDs were decorated with different amounts of NLS targeting sequences (to generate formulations with similar size and surface charge), and were incubated *in vitro* with HeLa cells for 14 hours. Representative confocal images are shown (red – QDs, blue – DAPI nuclear stain).

CONCLUSION

We generated QDs decorated to a different extent with NLS peptidic targeting residues. Increased number of surface NLS groups induced more efficient endocytosis of QDs and enhanced their targeting to the cell nuclei. The experimental system that is reported in this study is suitable for quantitative analysis of the mechanisms that govern the QDs nuclear targeting and their limiting factors (mechanisms and efficiencies of endocytosis, endosomal escape, and intracellular trafficking), and their dependence on the formulation properties (QDs size and charge, type and density of NLS residues, etc.). These findings will contribute to development of subcellularly-targeted DDSs that will deliver specific drugs to the nuclei of the target cells and will enhance efficacy and reduce toxicity of these drugs.

ACKNOWLEDGMENTS

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PREPARATION AND CHARACTERIZATION OF WOUND DRESSING USING SURFACE MODIFIED LIGNIN NANOFIBERS BY ARGININE

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INTRODUCTION

Wound healing is a complex process including different stages. A suitable dressing could protect the injury and contributes to the recovery of damaged tissues. In comparison with conventional wound dressings, nanofiber-based wound dressings have different advantages such as haemostasis induction, good absorption of wound exudates and facilitation of cell growth due to their nanofibrous structure. In addition of using suitable wound dressing, the use of wound healing agents such as growth factors could improve the wound healing process rate[1]. Nitric oxide (NO) and its precursors such as arginine are promising wound-healing agents that could regulate collagen formation and wound contraction. Delivery of wound healing agents in controlled release manner could promote wound healing process efficiently. Among different polymer, lignin is widely used in the preparation of hydrocolloid wound dressings[2]. In current study we prepared surface modified lignin nanofibers by arginine as an effective product with easy application for the treatment of wounds.

METHODS

Lignin nanofiber gel (2.5%) was prepared by chemical and mechanical method. 200 mg arginine was added to 1 mL deionized water. The prepared arginine solution was added to 1 g of lignin nanofiber gel and was stirred for 24 h until the arginine was conjugated to lignin nanofiber by electrostatic interaction. The percent of attached arginine to lignin nanofibers was determined. The effect of pH on the amount of attached arginine was evaluated in three different pH; 5, 6 and 7. The viscosity of surface modified lignin nanofibers by arginine were evaluated. To determine the spreadability of formulation, 0.5 g of surface modified nanofibers gels was placed within a circle of 1 cm diameter pre-marked on a glass plate of 20 × 20 cm, over which a second glass plate was placed. A weight of 500 g was allowed to rest on the upper glass plate for 5 min. The increase in the diameter due to gel spreading was noted. The release of arginine from surface modified lignin nanofibers was evaluated for 24 h at 32 ° C at phosphate buffer pH 7. The wound healing effect of prepared surface modified lignin nanofibers was evaluated *in vivo* by wound excision method on Wistar rats (275 ± 25 g) in comparison with mixture of non-modified lignin nanofibers, arginine solution and normal saline solution for 9 days. Skin tissue (1.5 cm × 2.5 cm) was surgically removed using sterile surgical tools to create full thickness wound on the back of the animal. Materials was locally applied to the full thickness skin wounds, from day 0, once a day for 9 days. The images of wounds were captured using a digital camera. The images of wounds were analyzed for the wound area using Photoshop (versus CS5) software (day 0, 3 and 9).

RESULTS AND DISCUSION

The results showed that arginine could sufficiently conjugated to lignin nanofibers by electrostatic interaction. 90.16±1.1%, 94.1±2.2% and 97.9±1.9% of arginine could attached to lignin nanofibers in pH 5, 6 and 7. The viscosity of gel was reported to be significantly had no significantly change by decreasing of the pH of the gels. The spreadability of the surface modified lignin nanofibers was 7.3±0.8, 7.2±0.4 and 7.1±0.5 for surface modified lignin nanofibers with pH 5, 6 and 7, which was proved the ease of applicability of gels on skin. Moreover about 82.95±2.5%, 82.62±2.1 and 83.2±3.4 of arginine was released from surface modified lignin nanofibers with pH 5, 6 and 7 after 24 h in a controlled release manner (Figure 1).

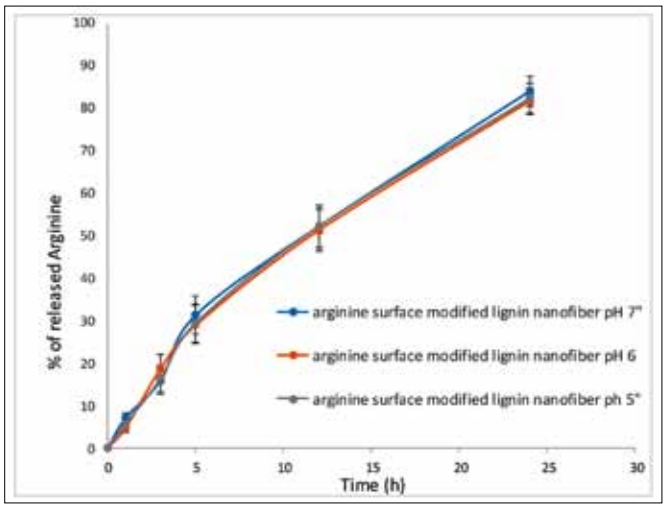

Figure 1. Effect of pH of lignin nanofiber on the % of released arginine from lignin nanofibers

In vivo wound healing effect of surface modified lignin nanofiber was performed using arginine pH 7. The results clearly substantiate the beneficial effects of the topical application of surface modified lignin nanofiber with pH 7 containing arginine in the acceleration of healthy wound healing process with less scarring in comparison with non-modified lignin nanofibers, arginine solution and normal saline solution (Figure 2).

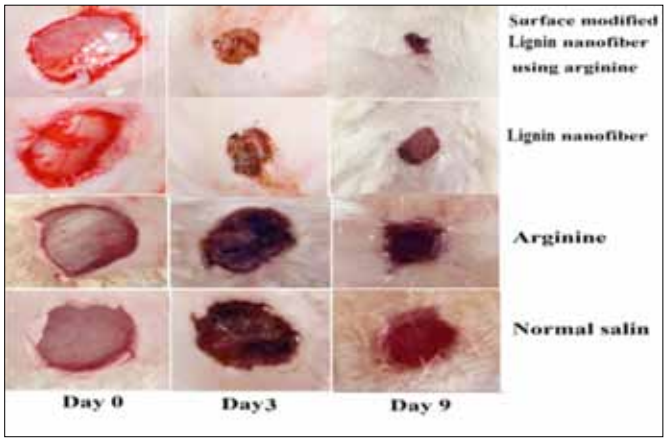

Figure 2. In vivo wound healing effect of surface modified lignin nanofiber, non-modified lignin nanofibers, arginine solution and normal saline solution

CONCLUSION

Nanofibers from natural polymers such as lignin are promising materials for preparation of wound dressing. These results suggest that this arginine-releasing nanofibers have the potential to use as a novel topical wound-healing therapy in acute wounds.

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UNDERSTANDING THE IMPACTS OF COMPLEX BIOLOGICAL FLUIDS AND THEIR ROLE ON THE UPTAKE OF NANOPARTICLES IN A LIVER CELL MODEL IN VITRO

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Nanoparticles in contact with a complex biological milieu (e.g. human serum) form a layer of long-lived biomolecules known as the 'biomolecular corona' that provides a new biological identity to the nanoparticles[1-3]. Nanoparticle-biomolecular complexes interact with biological barriers via single cell interactions and gain access to specific pathways via these molecules that are presented on the surface of the nanoparticles. Biomolecular motifs presented on the nanoparticle surface will determine how a nanoparticle interacts with a wide range of receptors on specific cells. This may define many key interactions and impacts which can mediate the interactions with nanoparticles and the immune system, in particular in, *in vivo* bio-distributions, nanomedicine targeting strategies and other biological outcomes[4].
We present here, the impact of the presence of the apolipoprotein ApoB-100, on the surface of silica nanoparticles in a competitive media with the presence of the free ligand[5]. This fact leads to low density lipoprotein receptor (LDLR) recognition, mostly expressed in the liver. These results suggest that the 'labelling' of nanoparticles by biomolecular adsorption processes allows for nanoparticle multi-pathway involvement in biological processes, in which nanoparticles may be misinterpreted to be an endogenous object, in this specific case a lipoprotein, and be recognized and taken up by LDLR.

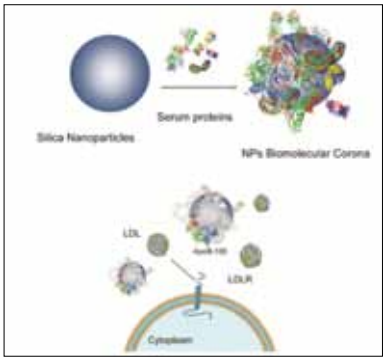

Figure 1. Schematic for the formation of the biomolecular corona on the surface of nanoparticles when they are in complex biological milieu like human serum, and the competitive binding between motifs on the corona (e.g. ApoB-100) and the free ligands present in the human serum (e.g. LDL) to bind LDLR.

We connect the nanoparticle-biomolecular corona complexes with specific cell receptor interactions using a model of functional receptor library hosted in HEK-293T cells. This work is focused on the identification of specific receptors that can recognize nanoparticles in a complex biological milieu. Therefore, providing an understanding of how specific cell receptors interact with nanoparticle-biomolecular corona in complex biological milieu, can be valuable to better predict the bio-distribution of nanoparticles and understand how they interaction with the immune system.

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GLASS-LASER MULTIPLEXED BIOSENSOR-BETTER GENITOURINARY CANCER DIAGNOSIS

MASA MARC

Glass-Laser Multiplexed Biosensor (GLAM) project develops a device to monitor and diagnose genitourinary cancers in a personalised way, rapidly, and at low cost. Additionally, it is done in a less invasive and unpleasant way.
Currently, differential cancer diagnosis takes place daily in clinical settings for both patient stratification and monitoring patient responses to existing treatments. However, the outcome of this diagnosis today is still poor, with many deficiencies and false positives and negatives due to the low sensitivity and specificity of available methodologies. Moreover, as new targeted therapies are available to patients and to oncologists there is a huge need to improve personalised diagnosis and therapy. The objective of GLAM is to develop a new diagnostic tool to detect biomarkers from biofluids, obtained in a "non invasive" manner, specifically in urine and focusing on genitourinary cancers, enabling oncologists to take better treatment decisions. To this end, GLAM project will develop an integrated device based on novel label-free photonic biosensors with ultra-sensitivity, simplicity of use, portability, multiplexing and low cost by simply applying a drop of urine and reading 10 biomarker levels. The GLAM unique technology will make the device also usable with other biofluids aside of urine and might also be used to help physicians in personalised medicine in many other biomarker driven diseases, aside of cancer.

DIAGNOSIS AND THERAPY MONITORING

GLAM develops an innovative device for personalized diagnosis and therapy monitoring for genitourinary cancers.

PHOTONIC BIOSENSORS

GLAM develops an integrated device based on novel label free photonic biosensors with ultra-sensitivity, simplicity of use, portability, multiplexing and low cost.

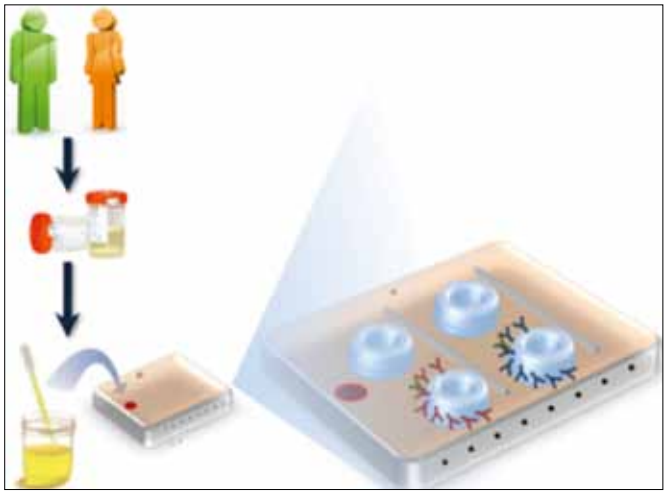
LASER MICRORING

GLAM capitalizes on the unprecedented sensitivity achieved using laser microring resonators to detect key biomarkers in tumor development and treatment.

THE DEVICE

GLAM device is based on an innovative concept for the detection of soluble biomarker levels. It combines the ultrasensitive photonic detection capabilities of microring lasers with the selectivity of biomarker antibodies. Altogether, the use of these two aspects will result in a novel, miniaturised, ultra-sensitive, robust, reliable, fast, and cost-effective device, capable of multiplexed biomarker level determination.

THE GLAM SYSTEM



GLAM takes advantage of the use of urine as the biological material with no need of invasive and aggressive sampling of the patients. A second main feature of GLAM is that it uses cheap, environmentally friendly, disposable cartridges that require only simple handling with no need of further processing, washing, or manipulation by expert technicians. Every step is rapid, intuitive and "user friendly". Additionally, a final performance of GLAM lays its rapidity to read the samples through the laser application. The potent data processing software and tools herein used will be translated and adopted to clinical professional and non-professional use.

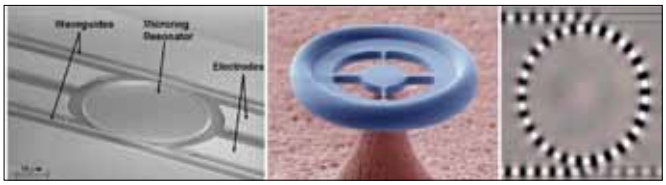
THE OBJECTIVES

Photonic biosensors

To develop novel photonic biosensors based on microring lasers functionalised with biomarker-detection antibodies. This new, label-free, ultra-sensitive photonic detection system will allow physicians to monitor soluble key biomarkers without labelling, washing, or amplification steps.

Validate biosensor

To experimentally validate the final biosensor with preclinical and clinical samples in order to prove its personalised medicine focus within the frame of a more sustainable health system. Integrate components To integrate all the components in a small, easy-to-use, robust, fast, cheap, and single step diagnostic device to be used in point-of-care settings.



Multiplexed platform

To set up a tuned multiplexed platform to allow the detection of up to 10 different biomarkers that will enable and guide clinical personalised treatment decisions from just a single small biological sample.

TECHNICAL DOCUMENTATIONS

To set-up technical documentations for the GLAM Prototype compliant with the applicable EC Directives. Such technical documentations shall serve as the regulatory basis for the CE Certification and ISO 13485 Certification of the finished In-Vitro Diagnostics medical device.

COMPLEMENT ACTIVATION OF ARTIFICIAL, NON-SPHERICAL LIPOSOMES

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Atherosclerotic cardiovascular disease, that is the usual cause of heart attacks, strokes, and peripheral vascular diseases, is the leading cause of morbidity and mortality in the world. Atherosclerosis results in the hardening and narrowing of the arterial blood vessels, due to the plaque formation associated with wall thickening. This disease can block arteries, compromising the blood flow. This occlusion leads to significant increase in the arterial pressure, creating a shear stress difference between normal and constricted arteries. In fact, one can take advantage of such a phenomenon, using it as a mechanically based trigger for the targeted drug delivery. A drug delivery system specifically targeting constricted arteries is required to restore the blood flow at critically constricted sites of the arteries and prevent further myocardial ischemia and death. Liposomes are well-established drug delivery systems[1] and their ability of controlled drug release makes them especially attractive for targeting stenosed arteries. The recently discovered mechanosensitive liposomes, formulated from the artificial Pad-PC-Pad (1,3-palmitoylamido- 1,3-deoxy-sn-glycero-2-phosphatidyl-choline)[2], use shear force differences between healthy and diseased blood vessels that result in the local release of their payload. Nowadays, methods used in the ambulatory treatment provides the intravenous administration of vasodilators, which, in turn, leads to the rapid systemic drop down of the blood pressure and, as a result, reduced blood perfusion[3]. Such nanocarriers can essentially improve the efficiency of the specific drug targeting, improving distribution, absorption and reducing the toxicity of the encapsulated therapeutic agent[4]. Despite such evident benefits, even some FDA-approved lipid-based vesicles have been recognized by the immune system as foreign antigens, inducing immediate hypersensitivity reactions[5]. The activation of the complement system, as a part of the innate immune system, enhances the ability of the phagocytic cells to clean the environment from the foreign substances. This can give rise to the severe adverse reactions, like anaphylaxis or even risk of lethal effects.

In order to progress this nanotherapeutic drug delivery platform to the bedside, the *in vitro* hypersensitivity induced by the artificial Pad-PC-Pad liposomes has been tested.

Keywords: constricted arteries; mechanosensitive liposomes; nanotherapeutic drug delivery system; Pad-PC-Pad; complement activation; ELISA.

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NANOMEDICINE FOR CANCER IMMUNOTHERAPY: TRACKING CANCER-SPECIFIC T-CELLS IN VIVO WITH GOLD NANOPARTICLES AND CT IMAGING

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Cancer immunotherapy is treatment that uses the power of the immune system to fight cancer. Specifically, an important treatment is direct injection of killer T-cells that target tumor antigens and kill the cancer cells. Studies have shown that these cells could mediate a complete tumor regression. However, application of immune cell-based therapy in routine clinical practice is challenging, due to the poorly-understood mechanisms underlying success or failure of treatment. Development of accurate and quantitative imaging techniques for non-invasive cell tracking can provide essential knowledge for elucidating these mechanisms. We designed a novel method for longitudinal and quantitative *in vivo* cell tracking, based on the superior visualization abilities of classical X-ray computed tomography (CT), combined with state-of-the-art nanotechnology.

Herein, T-cells were transduced to express a melanoma-specific T-cell receptor and then labeled with gold nanoparticles (GNPs) as a CT contrast agent. The GNP-labeled T-cells were injected intravenously to mice bearing human melanoma xenografts, and whole-body CT imaging allowed examination of the distribution, migration and kinetics of T-cells. Using CT, we found that transduced T-cells accumulated at the tumor site, as opposed to non-transduced cells. Labeling with gold nanoparticles did not affect T-cell function, as demonstrated both *in vitro*, by cytokine release and proliferation assays, and *in vivo*, as tumor regression was observed. Moreover, to validate the accuracy and reliability of the proposed cell tracking technique, T-cells were labeled both with green fluorescent protein for fluorescence imaging, and with GNPs for CT imaging. A remarkable correlation in signal intensity at the tumor site was observed between the two imaging modalities, at all time points examined, providing evidence for the accuracy of our CT cell tracking abilities. This new method for cell tracking with CT offers a valuable tool for research, and more importantly for clinical applications, to study the fate of immune cells in cancer immunotherapy.

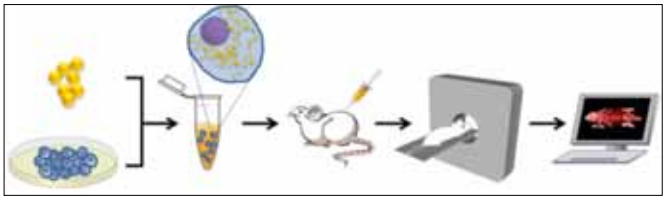
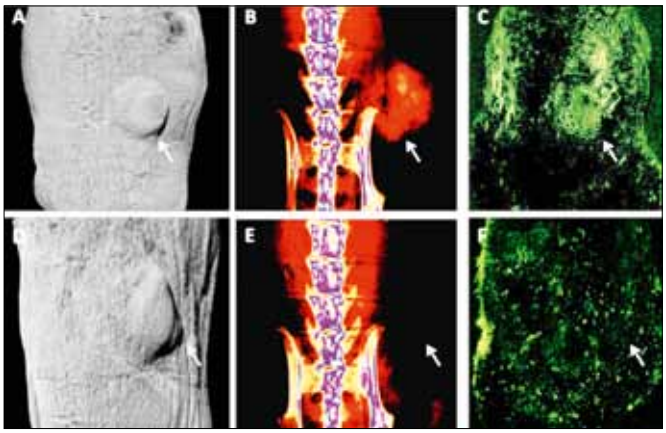


Figure 1: T-cell tracking process: T-cells were labeled with GNPs in vitro; the cells were then injected to mice, and tracked in vivo using CT imaging.

Figure 2: 3D volume-rendering images of T-cell accumulation in the tumor, over time. A. Four hrs post injection, minor accumulation of T-cells can be observed. B. 48 hrs post injection, substantial accumulation of T-cells can be seen (yellow).

Figure 3: Comparison of CT to FLI for targeted T-cells (A-C) and non-targeted T-cells (D-F). A, D: 3D CT images indicating location of tumor. B, E: MPI CT images of the tumor area. C, F: GFP florescence imaging. The images demonstrate a direct correlation between FLI and CT imaging of the tracked T-cells. The presented images were obtained 48hrs post injection, at maximal accumulation of the targeted-T-cells at the tumor site.



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ANAPHYLACTIC REACTIVITY AND IMMUNOGENICITY OF PEGYLATED LIPOSOMES IN PIGS PART II – INHIBITION VIA IMMUNE SUPRESSION BY DOXIL®

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Intravenous injection of pigs with PEGylated liposomes mimicking doxorubicin-free Doxil®/Caelyx (Doxebo) was previously shown to prevent complement activation-related pseudoallergy (CARPA) to Doxil® in a porcine model of human hypersensitivity reactions to nano-drugs. In the course of Doxebo's anaphylaxis-prophylactic (tachyphylactic) effect, levels of anti-PEG and anti-liposome (anti-LIP) antibodies showed major rises and anaphylactic shock was observed (see Part I – Inhibition via antibody scavenging). Astonishingly, both antibody production and the anaphylactic reactions were abolished by co-administration with Doxebo of the human equivalent dose of Doxil®, suggesting that an immune suppressive effect of Doxil® prevented the immunogenicity of liposomes.

Fig. 1 shows the changes of pulmonary and systemic arterial pressure (PAP, SAP) in pigs caused by sequential injections of 0.1 and 1 mg/kg PEGylated liposomes (LIP) and 0.1 mg/kg Zymosan 1 week after pretreatment of pigs with PBS ("nothing"), 0.1 mg/kg Doxebo ("Doxebo only") or Doxebo + the human equivalent dose (HED) of Doxil® ("Doxebo + 6.4 mg/kg Doxil"). The changes were expressed as area under the blood pressure curves normalized as % change relative to baseline. It is seen that the huge LIP-induced rises of PAP and SAP in Doxebo pretreated pigs was near entirely suppressed in Doxebo + Doxil® HED-treated animals, indicating that Doxil® provided significant suppression of Doxebo-induced immunogenicity. These data explain why Doxil® is not immunogenic in cancer patients, and suggest that Doxil® can be used for the prevention of CARPA

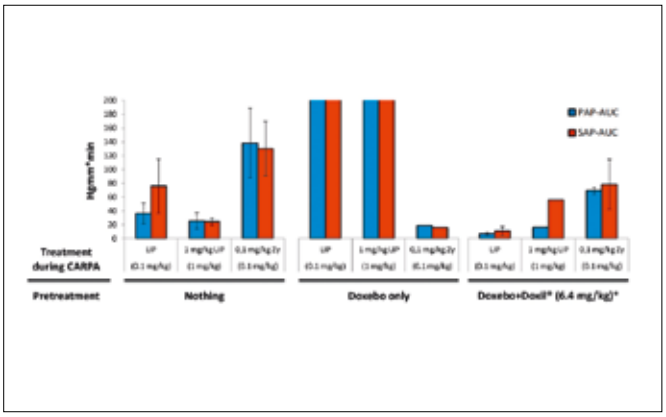

Figure 1. Blood pressure changes in pigs caused by PEGylated liposomes and zymosan 1 week after pretreatment of animals with 0.1 mg/kg Doxebo with or without 6.4 mg/kg Doxil pigs. „Nothing" means no pretreatment. AUC, area under the PAP or SAP curve.

EVALUATION OF COMPLEMENT REACTOGENICITY BY PEG-FREE ABA BLOCK-COPOLYMERS

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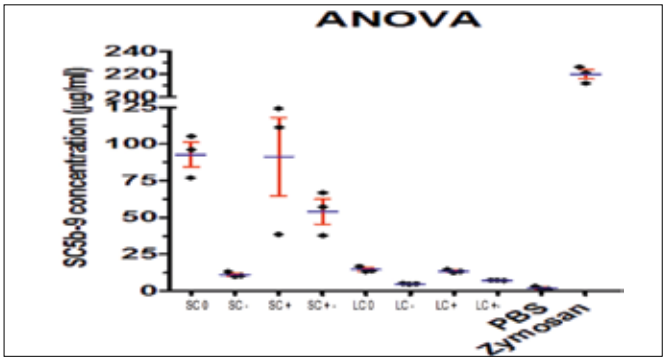
Polymer based PEG-free nanocarriers provide alternative to PE-Gylated liposomes as a long circulating drug carrier systems. As part of the R&D of an ABA block-polymer-based micellar system, we examined the complement (C) activating capabilities of different copolymers and correlated C activation with the size and size distribution of particles. We evaluated the effects of structural modificatitons, such as chain length and charge of the copolymers on C activation through measuring SC5b9 production in human serum after incubation with with the micelles. The results showed that longer chain lengths polymers activated C complement significantly more than short chain polymers. Surprisingly, non-charged polymers were the most reactogenic, positively charged and zwitterioninc polymers showed lower level of activation, while negatively charged polymers were the least prone to activate C. Particle size and size distribution had no, or minor impact on C activation. It is concluded that physico-chemical, rather than nano-structural factors determine C activation, and that polymer chain lengths and charge are key variables in this aspect.



THE KEY ROLE OF NEW CARBOSILANE DENDRIMERS AND DENDRONS WITH DUAL-PREVENTION AGAINST THE HSV-2/HIV-1 CO-INFECTION AS TOPICAL VAGINAL MICROBICIDES: CHARACTERIZATION AND PROOF-OF-CONCEPT

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BACKGROUND

Despite the continuous progress in understanding the epidemiology, pathogenesis, and transmission HSV-2/HIV-1, currently, a safe and effective prophylactic vaccine for both viruses remains elusive. Among main modes of the HSV-2/HIV-1transmission, sexual transfer is the responsible for the majority of infections, resulting in the transmission of HSV-2/HIV-1 due to infected semen or cervico-vaginal secretions containing infected lymphocytes. The HSV-2 infection is associated with a 3-to 4-fold increased probability of HIV-1, and establishes a reservoir in neurons of the sensory sacral ganglia. The majority of HIV infections worldwide occur as a consequence of unprotected heterosexual encounters with an HIV-seropositive person, with the highest percentage of women infected than men. Several mechanisms are proposed to explain the HSV-2 mediated facilitation of HIV-1, such as damages and ulceration caused by HSV-2 in the genital mucosa. Moreover, under certain situations, such as immunodeficient states, the virus can reactivate, causing recurrent lesions at the site of primary infection.
Although physical barriers such as condoms have been demonstrated to have a high efficacy in the prevention of the HSV-2/HIV-1 transmission during the sexual intercourse, it is a method of prevention that remains almost exclusively under the control of the male partner. The vulnerability of women to the HSV-2/HIV-1 co-infection due to cultural/social aspects (religion or polygamy) does not provide women power to negotiate the use of a condom, discuss fidelity with their partners or leave risky relationships. Therefore, the development of new prevention strategies aimed at halting the spread of HSV-2/HIV-1 in regions such as sub-Saharan Africa are clearly needed. One of these strategies includes the development of safe, effective, and low-priced topical microbicides. The development of topical vaginal microbicides should prevent the HSV-2/HIV-1 entry and maintain the integrity of the vaginal epithelial barrier. Thus, the emerging field of nanotechnology, specifically dendrimers and dendrons, play an important role in addressing this challenge. Despite the urgency to develop novel approaches to prevent the HSV-2/HIV-1 transmission, this process has been hindered by the lack of adequate small animal models for preclinical efficacy and safety testing. In this context, BALB/c and humanized (h)-BLT (bone marrow-liver-thymus) mice are susceptible to intravaginal infections and makes these systems excellent candidates for preclinical evaluation of microbicides.
Several generations of polyanionic carbosilane dendrimers with silicon atom core, G1-S4 (generation zero, 4 sulphate end-groups), G2-S16 (first-generation, 16 sulfonate end-groups), and G3-S16 (second-generation, 16 sulphate end-groups) have demonstrated a potent and broad-spectrum anti-HIV-1/HSV-2 activity *in vitro*. However, a proof-of-concept for evaluating its antiviral activity in BALB/c (HSV-2) and h-BLT (HIV-1) mice, and its mode of action has not been probed. Moreover, a group of polyanionic carbosilane dendrons synthesized via thiol-ene from first to third generation with palmitic or hexanoic fatty acids as a core and capped with sulfonate groups (2-8 depending on generations) were evaluated for their anti-HSV-2/HIV-1 activity *in vitro*.

METHODS

Six-to-eight-week-old female BALB/c (or h-BLT) mice were housed in a specific-pathogen-free animal facility for at least 1 week before experiments were conducted. Mice were subcutaneous injected with 2 mg of medroxyprogesterone acetate 5 days before gel application to minimize the effect of menstrual change in final results. For vaginal irritation assays, 40 µL of 2.0% HEC containing different controls was carefully applied to the vagina vault of mice for 7 consecutive days. Mice were anesthetized by isoflurane inhalation during procedures and placed in an inverted position for 15 min post-inoculation to allow for free flow and to prevent immediate discharge of the vehicle out the vagina. On day 7, mice were euthanized and vaginal tissues were excised and fixed in 4% formaldehyde solution for histology. Formalin-fixed excised vaginal tissues were embedded in paraffin, and sectioned transversely with a microtome. Sections were mounted on slides and were subjected to a blind evaluation for epithelial exfoliation, leukocyte infiltration, edema and epithelial vascular congestion. Irritation scores were assigned by a semiquantitative system for inflammation: 0 (absent adverse effects), 1 (minimal), 2 (mild), 3 (moderate) and 4 (severe irritation). The cumulative score were correlated to human vaginal

irritation potential as follows: 1-4 (minimal), 5-8 (mild), 9-11 (borderline), and 12-16 (unacceptable). Formulations with vaginal irritation ranging from 1 and 8 are considered acceptable for vaginal application.

For *in vivo* HIV-1 experiments, H-BLT mice were prepared using NOD/SCID/γc-/- (NSG) mice. Briefly, female NSG mice were surgically implanted with human fetal thymus and liver fragments, and injected intravenously with human hematopoietic stem cells (CD34+). Anesthetized h-BLT mice were treated with 20 µL of vehicle (HEC alone) or 3% G2-S16 in 2.0% HEC gel applied intravaginally. H-BLT mice were maintained in a supine position with slight elevation of the pelvis for 30 min post-application to ensure the correct absorption of the active compound. After 2 h pre-treatment with vehicle or 2.0% HEC gel containing 3% of G2-S16, 2x104 TCID50 of R5-HIV-$1_{JR\text{-}CSF}$ diluted in PBS to a final volume of 10 µL was applied atraumatically to the vaginal mucosa of h-BLT mice. H-BLT mice were maintained in a supine position with slight elevation of the pelvis until they woke up to ensure an even spread of the gel and to allow the gel to be mixed with R5-HIV-$1_{JR\text{-}CSF}$ for the subsequent safety and efficacy evaluation. Human reconstitution of BLT mice assessed by flow cytometry, and plasma viral loads following HIV-1 challenge were performed on peripheral blood leukocytes through puncture of submandibular vein (Figure A).For *in vivo* HSV-2 experiments, BALB/c mice were treated with 30 µL of vehicle (HEC alone) or 3% G2-S16 or 3% G1-S4 in 2.0% HEC gel applied intravaginally. After 1 h pre-treatment with vehicle or 2.0% HEC gel containing 3% of G2-S16 or G1-S4, 10^5 PFU of HSV-2_{333} diluted in PBS to a final volume of 20 µL was applied atraumatically to the vaginal mucosa of BALB/c mice and maintained in a supine position for 15 minutes post-application. Mice were examined daily for body weight and genital pathology over 16 days. Disease score was graded according to a 4-point scale: 0 (no apparent infection), 1 (genital erythema), 2 (moderate genital infection), 3 (purulent genital ulceration and hair loss, generally poor condition), and 4 (severe ulceration of genital and surrounding tissue, and hind limb paralysis). We also assessed the mode of antiviral of action on the inhibition of the HSV-2/HIV-1 infection through a panel of different *in vitro* antiviral assays in TZM.bl cells or PBMCs (in the case of HIV-1) and in Vero cells (in the case of HSV-2): time-of-addition, binding and internalization, virucidal activity, cell-based fusion, and cell-to-cell transmission.

RESULTS

When applied intravaginally to h-BLT mice, 3% G2-S16 protected against the R5-HIV-$1_{JR\text{-}CSF}$ vaginal transmission in 84% without irritation or vaginal lesions. Topical 3% G2-S16 and 3% G1-S4 proved capable to prevent the HSV-2 infection vaginally at 100% and 90% in female BALB/c mice, respectively, upon exposure to a lethal dose of HSV-2. No irritation or inflammation processes were detected in female mice after seven consecutive doses vaginally. Moreover, both dendrimers G2-S16 and G1-S4 prevented the rectal HSV-2 transmission over 90% in female BALB/c mice. These results suggest that G1-S4, G2-S16, G3-S16 and polyanionic carbosilane dendrons from third generation with palmitic or hexanoic fatty acids (BDCG048 and BDCG054) exert anti-HSV-2/HIV-1 activity at early stages of the viral replication inactivating the virus, blocking the adsorption, and the HSV-2/HIV-1 entry. We demonstrated that dendrimers and dendrons are active against semen-exposed HIV-1 particles which their infectivity has been enhanced by the presence of amyloid fibrils of semen. Besides, we show the dendrimer's capability to provide a barrier to infection for long periods and to inhibit the cell-to-cell transmission, confirming its multifactorial and non-specific ability.

CONCLUSIONS

This study represents the first demonstration indicating that HIV-1 vaginally infects humanized BLT mice and that transmission of the virus can be efficiently blocked by vaginally applied G2-S16. Our results indicate that polyanionic carbosilane dendrimers and dendrons have an excellent potential to prevent the vaginal transmission of HSV-2/HIV-1. These results provide strong experimental evidence in the development of dendrimers (and dendrons)-based topical microbicides to prevent vaginal the HSV-2/HIV-1 transmission in humans.

Key Words: BALB/c mice, polyanionic carbosilane dendrimers/dendrons, HSV-2/HIV-1 transmission, humanized BLT mice, nanotechnology, topical vaginal microbicide

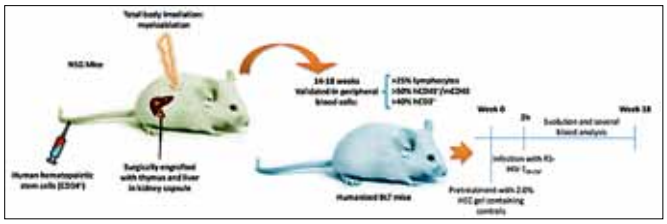

Figure A. Scheme of BLT mice humanization process and experimental design for vaginal challenge of humanized (h)-BLT mice with R5-HIV-$1_{JR\text{-}CSF}$

NANOPARTICLE TRANSPORT THROUGH HUMAN UNDILUTED TRACHEAL MUCUS

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INTRODUCTION

Pulmonary mucus is a complex hydrogel that lubricates the epithelium and provides first-line protection to the conducting airways of the lung. A type of glycoproteins, the mucins, plays a key-role in the mucus architecture. Mucins are continuously secreted to the airways and incorporated into the mucus layer, forming an entangled mesh with a very strict pore size(1). Inhaled particulates, including pollutants and pathogens, are entrapped in the mucus blanket, which in turn is continuously propelled out of the lungs by the ciliary beating of the epithelial cells. Thus, this efficient protective barrier represents an extraordinary challenge for drug delivery, including the delivery of nanopharmaceuticals.

In addition, in several pulmonary conditions such as Asthma, Chronic Obstructive Pulmonary Disease (COPD), or Cystic Fibrosis (CF) the composition and the mechanical properties of mucus might be significantly altered. For instance, in COPD patients, mucus hypersecretion is common whereas the mucus of CF patients is highly elastic, adding another layer of complexity in terms of therapeutic delivery. The expectation that nanomedicine may improve the available treatments for bronchial diseases is high. Nanomedicine covers a wide spectrum of possibilities ranging from gene-therapy to the delivery of poorly soluble anti-infective molecules by means of nanoparticle (NP)-based drug delivery systems. Unfortunately, the mucus barrier remains often unnoticed in the initial development stages of many nanopharmaceuticals, which ultimately leads to the entrapment of the NPs within the mucus mesh, away from the primarily intended target. Although the mechanical structure and the complex chemical interactions that take place within the pulmonary mucus are not yet completely understood, fine-tuning the NP size and modifying the surface chemistry increase the mobility of NPs through mucus(2). In the present study we compared the mobility of NPs with different sizes (diameters: 500 nm, 200 nm and 100 nm) through human undiluted tracheal mucus and we evaluated the efficacy of PEGylation as a strategy to improve the transport of NPs through mucus.

METHODS

Human undiluted tracheal mucus was extracted from the endotracheal tube of patients undergoing elective surgery not related to pulmonary diseases. 1) The characterization of the bulk rheological properties of mucus was performed with an Anton-Paar MCR 102 rheometer equipped with cone-plate geometry. Frequency dependency of the storage modulus G' and the loss modulus G'' was measured in the range between 0.02 and 40 rad/sec at strain amplitudes of γ = 1%. 2) The mobility of 100 nm, 200 nm and 500 nm COOH-functionalized red-fluorescent NPs (Fluospheres, Invitrogen) was determined by Fluorescence Recovery after Photobleaching (FRAP) using a LSM 710 Axio Observer confocal laser scanning microscope (Zeiss, Germany). Briefly, regions of interest were defined and pre-bleaching images were recorded at 2% laser transmission, immediately followed by bleaching with the laser transmission set at 100%. A post-bleaching recovery step followed, for a duration of 180 s at a frame rate of 30 frames/min. 3) 100 nm COOH-functionalized NPs were PEGylated by covalent modifying of the carboxylic groups on the surface of the NPs with 3 kDa methoxy-PEG-amine (Sigma Aldrich). The transport of COOH and PEG-functionalized NPs through human tracheal mucus over 24 hours was compared using modified transwell® supports. A 150µl volume of NPs (0.02% w/v) was placed on the apical compartment (above the mucus layer, 120 mg of mucus) and the permeation over time was determined by sampling 100 µl volumes from the basolateral compartment. The amount of permeated NPs was determined with a fluorimeter.

RESULTS

1) The amplitude sweep showed that in the amplitude (γ) range from 0.1% to 10% pulmonary mucus was within the viscoelastic linear region. The frequency sweep was conducted at a γ of 1% and G' dominated over G'' in all tested frequencies, as expected. These characteristics are typical of cross-linked gels. 2) FRAP experiments highlighted the strict diffusion barrier that mucus represents for NPs; almost 100% of 500 nm particles were immobilized by the mucus mesh, whereas the mean mobile NP fraction of 200 nm and 100 nm NPs was increased with decreasing particle size (Figure 1). Still, the mean mobile fraction of 100 nm particles was rather low, below 15%.

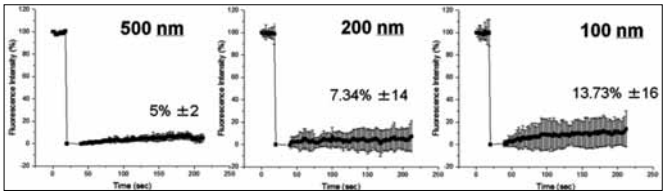

Figure 1. Fluorescence intensity recovery over time determined from FRAP experiments using 100 nm, 200 nm, and 500 nm particles. n = 10, from three independent samples.

3) The transport of COOH- or PEG-functionalized particles through mucus was compared over a time-scale of 24h. NP PEGylation increased both the size (from 112 ± 0.5 nm to 220 ± 4 nm) and the z-potential (from -45 ± 1 to -19 ± 0.5mV). We found a slight trend towards a higher transport of PEGylated particles through mucus but irrespective of the surface functionalization, the mean percentage of permeated NPs remained below 10% (Figure 2), confirming the strict mesh pore-size of the undiluted human tracheal mucus.

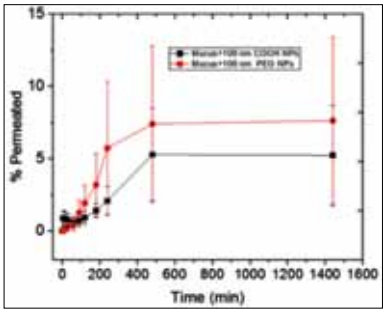

Figure 2. Percentage of permeated COOH or PEG-functionalized nanoparticles through mucus over 24 hours.

CONCLUSION AND OUTLOOK

Pulmonary mucus possesses extraordinary barrier properties. This hydrogel was able to immobilize roughly 100%, 90% and 85% of the 500 nm, 200 nm, and 100 nm NPs, respectively. Moreover, NP PEGylation could not significantly increase the transport of NPs through mucus, although it must be acknowledged that the process of surface functionalization with PEG markedly increased the size of the NPs. The pulmonary mucus, as a non-cellular barrier of the lung, should be thoroughly considered for the design and during delivery of nanomedicines intended to treat bronchial diseases. In particular the size and the surface chemistry of the particles must be properly adapted. Considering the results presented here, the use of mucolytic agents in combination with nanomedicines may also represent an interesting approach.

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PRIMING TUMOR BLOOD VESSELS AND THE TUMOR MICROENVIRONMENT WITH LIPOSOMAL CORTICOSTEROIDS TO IMPROVE DRUG DELIVERY

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INTRODUCTION:

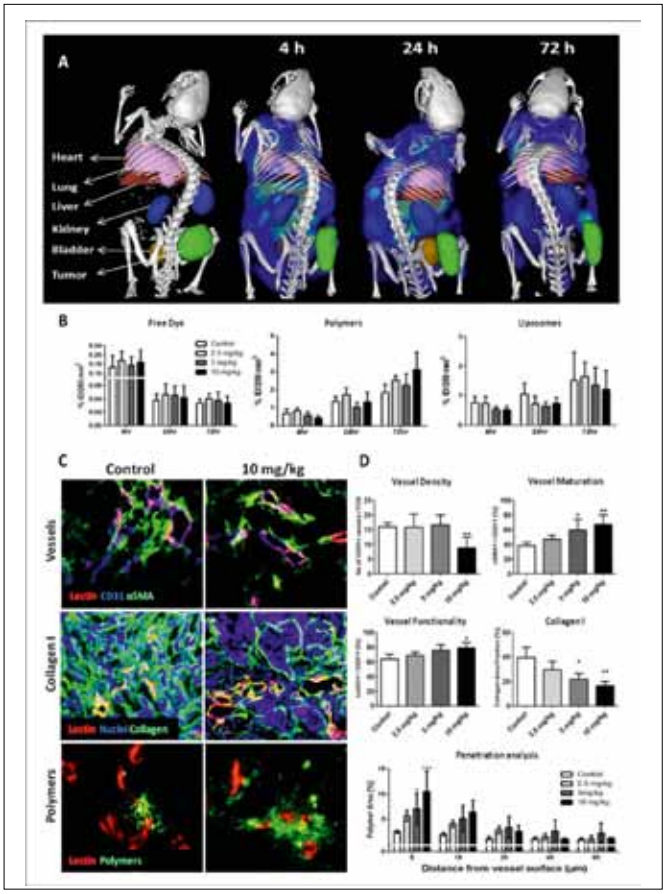
Tumors typically possess defective blood vessels, and a dense and disorganized microenvironment. Together, these two features result in inefficient and heterogeneous drug delivery. By employing clinically relevant corticosteroid-loaded liposomes, and by targeting them to tumor-associated macrophages (TAM), we aimed to inhibit macrophage-mediated angiogenesis and inflammatory signaling. Via mechanisms such as vascular normalization, stroma remodeling and collagen breakdown, the goal of such neo-adjuvant nano-treatments is to improve the accumulation, penetration and intratumoral distribution of subsequently administered drugs and drug delivery systems.

METHODS:

Mice bearing subcutaneous MLS ovarian cancer xenografts, which were previously shown to be highly angiogenic and stromal (Ehling et al, Am J Pathol 2014), were treated with three different doses of liposomal dexamethasone (LipoDex: 2.5, 5 and 10 mg/kg), once weekly for three weeks (n=7-8 per group). The effect of LipoDex pretreatment on drug delivery was evaluated by *in vivo* and ex vivo optical imaging of the target site accumulation of fluorophore-labeled model drugs (1 nm), polymers (10 nm) and liposomes (100 nm). Techniques employed include computed tomography - fluorescence molecular tomography (CT-FMT; Kunjachan et al, ACS Nano 2013), fluorescence reflectance imaging (FRI), standard fluorescence microscopy (FM) and two-photon laser scanning microscopy (TPLSM).

Figure 1: Effect of tumor priming on drug delivery and tumor microenvironment. A: Representative in vivo CT-FMT images showing segmented tumors and organs, as well as probe (polymer) biodistribution. B: Quantification of the effect of tumor priming on the accumulation of model drugs, polymers and liposomes, exemplifying somewhat higher overall accumulation for small model drug and polymers, and no change in the overall accumulation for liposomes. C: Immunohistological analysis of CD31 vessel density, the maturation (aSMA/CD31) and functionality (lectin/CD31) of tumor blood vessels, analysis of collagen content and analysis of polymer penetration out of blood vessels into the tumor interstitium in control

and pretreated tumors. D: Quantification of the images in panels C (n=5 images per tumor, n=3-4 tumors per group). For penetration analysis, the mean polymer area percentage in concentric rings surrounding blood vessels is shown, indicating improved penetration.



RESULTS:

Mice pre-treated with LipoDex showed a 20-40% increase in the tumor accumulation of the 1 nm-sized free dye and of the 10 nm-sized polymeric drug delivery system (Fig. 1A-B). Liposome concentrations were not affected. These findings were validated ex vivo using FRI, FM and TPLSM. Microscopy showed that LipoDex pretreatment resulted in vascular normalization, as exemplified by an increase in vessel maturation (aSMA/CD31 ratio), in an increase in vessel functionality (lectin/CD31 ratio), as well as a significant reduction in collagen content, which together resulted in a significantly improved penetration and intratumoral distribution of both the 10 nm-sized polymeric drug carriers, as well as of 100 nm-sized liposomes (exemplarily shown for polymers Fig. 1C-D).

Conclusion: Our findings demonstrate that pretreating tumors with liposomal corticosteroids affects tumor blood vessels and the tumor microenvironment, and that it beneficially affects the accumulation, penetration and intratumoral distribution of subsequently administered drugs and drug delivery systems.

ACKNOWLEDGEMENT:

This work is supported by the European Research Council (ERC starting grant 309495: NeoNaNo) and by the EU (7th Framework Program: Marie Curie grant no. 642028).

CHARACTERISTICS OF COMPLEMENT ACTIVATION-RELATED PSEUDOALLERGY OF LIPOSOMAL NANODRUGS IN NMRI MICE

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INTRODUCTION

Complement activation related pseudoallergy (CARPA) is a phenomenon triggered by various nanomedicines. Intravenous drugs containing nanoparticles are often recognized by the immune system as foreign. This type of hypersensitivity reaction is initiated by the complement system, which then triggers a cascade of events leading to serious, sometimes lethal side effects including hemodynamic and cardiopulmonary changes, rashes on the skin, arrhythmia, and changes in blood count. CARPA can be best modeled in pigs as pigs are similarly sensitive to CARPA as hypersensitive humans. The generated side effects can be frequently observed after intravenous therapy. Although routine *in vitro* tests are able to indicate complement activation caused by nanomedicines, the side effect profile and the severity of symptoms can only be tested in whole animals.

In our experiments we were aiming to develop a cost effective and reliable animal model that recognizes the changes related to CARPA.

MATERIALS AND METHODS:

Male NMRI mice weighing 28-35g were anesthetized with pentobarbital (60 mg/kg i.p.). The right carotid artery and the left jugular vein were cannulated to measure the blood pressure (BP) and for drug administration. Systemic arterial pressure (SAP) and heart rate (HR) were continuously registered (PowerLab and LabChart, ADInstruments, Budapest, Hungary). Separate groups of awake animals were treated via the tail vein, and before or at different times after treatment (1-3-5-10-20-30 min) were exsanguinated from the transected vena cava under deep isoflurane anesthesia. One part of the blood samples were collected in an Eppendorf tube containing EDTA for hematological analysis. Blood count was measured by Abacus cell counter (Budapest, Hungary). The other part was centrifuged at 1500 rpm for 10 min at 4°C, and then the plasma was stored at -80°C until further analysis. The complement (C) activation was evaluated by a complement hemolysis assay (CHA) on antigen-sensitized sheep red blood cells, measuring OD_{541} by a photometer.

From many modern medicines like liposomal drugs and monoclonal antibodies that are carpagenic, we tested the Abelcet. Abelcet is a sterile, pyrogen-free suspension for intravenous infusion. It consists of amphotericin B complexed with two phospholipids in a 1:1 drug-to-lipid molar ratio. The two phospholipids, L-_-dimyristoylphosphatidylcholine (DMPC) and L-_-dimyristoylphosphatidylglycerol (DMPG), are present in a 7:3 molar ratio. Abelcet is yellow and opaque in appearance, with a pH of 5 - 7. The active component of Abelcet, amphotericin B, acts by binding to sterols in the cell membrane of susceptible fungi, with a resultant change in the permeability of the membrane. Mammalian cell membranes also contain sterols, and damage to human cells is believed to occur through the same mechanism of action.

Zymosan was used as positive control (an active ingredients of a yeast). Zymosan is a glucan molecule, formed by glucose units connected with β-1,3-glycoside bonds.

IN VITRO COMPLEMENT ACTIVATION:

The total complement activation was determined using the classical C hemolytic (CH50) assay. A fixed volume of optimally sensitized SRBCs was added to serum with appropriate dilution. After incubation, the mixture was centrifuged, and hemolysis was quantified by measuring the absorbance of the hemoglobin released into the supernatant at 541 nm. The amount of complement activity was

determined by examining the capacity of test serum to lyse antibody coated SRBCs.

RESULTS:

The following figure (Figure 1) shows that NMRI mice (n= 5) were sensitive for zymosan (30 mg/kg), SAP approximately 15% increased after 3-4 min. Trombocitopenia has also expressed. Treatment with Abelcet (n=4, 30mg/kg) (Figure2) resulted 30% SAP increase. *In vitro* studies are still in progress, the SRBC assay confirmed significant activation of the complement system by the above treatments.

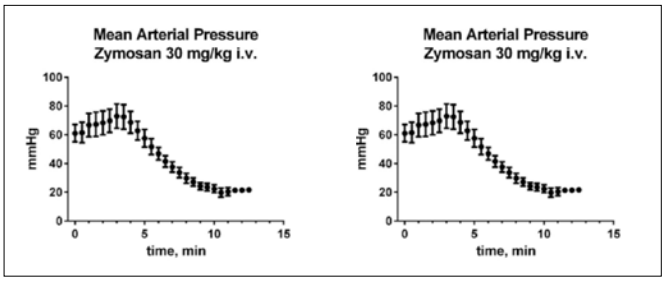

Figure 1: Mean arterial pressure (=SAP) changes after zymosan 30mg/kg
Figure 2: Mean arterial pressure (=SAP) changes after giving Abelcet 30mg/kg

CONCLUSION:

Cardiovascular changes typical of CARPA can be induced in NMRI mice with Abelcet and zymosan at 30mg/kg. Thus, the phenomenon can be studied in this model, although the animals' sensitivity to these reaction inducers is at least 100-fold lower compared to pigs.

METABOLISM-BLOCKING NANO-CONSTRUCTS: A POSSIBLE SOLUTION FOR DRUGS WITH NARROW THERAPEUTIC WINDOW

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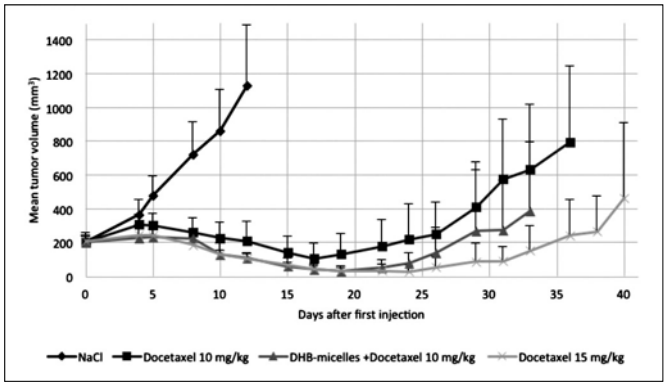
Differences in drug response among patients are common, often leading to challenges in optimizing dosage regimen for an individual patient. Major drugs are effective in only 25% to 60% of patients, and more than 2 million cases of adverse drug reactions occur annually in the United-States [Wilkinson 2005]. Therefore, there is a need to enhance the useful dose while reducing the useless dose of drugs administered in patients. We wondered if we may do so by sequential administration of natural compounds inhibiting hepatic CYP450 metabolism, and the drug, specifically addressing these natural compounds to hepatocytes using a nanocarrier.
Here, DHB-micelles, a nano-object encapsulating the natural furanocoumarin 6,7-dihydroxybergamottin, administered 24 hours prior to the drug docetaxel enhanced antitumor efficacy in HT-29 and MDA-MB-231 tumor models when compared to docetaxel alone (Figure 1). The optimum percent test versus control (%T/C) were multiplied respectively by 1.2 and 2. In the MDA-MB-231 study, at day 50, 50% or more of the animal were still alive in the two groups, docetaxel 15 mg/kg and DHB-micelle in association with docetaxel 10 mg/kg, versus 12.5% in the group docetaxel 10 mg/kg. Good tolerance to treatments were observed.

Figure 1. Antitumor efficacy studies comparing the association DHB-micelles and docetaxel versus docetaxel alone on MDA-MB-231 tumor model (A) Mean tumor volume (+SD) (B) Kaplan-Meier diagram. Filled black diamonds: NaCl 0.9% IV injected on days 0, 1, 4, 5, 8 and 9 (6 mice); filled grey squares: Docetaxel 10 mg/kg IV injected on days 1, 5, 9 (8 mice); filled grey triangles: DHB-micelles 13.3 mg/kg IV injected on days 0, 4, and 8 and docetaxel 10 mg/kg IV injected on day 1, 5 and 9 (8 mice); grey cross: docetaxel 15 mg/kg IV injected on days 1, 5, 9 (8 mice). Arrows: injections (clear arrow DHB micelles injection and dark arrow docetaxel injection).
In vitro, the DHB-micelles proved to inhibit hepatic CYP3A4 metabolism using HepaRG cells and murine hepatocytes (Figure 2).

A



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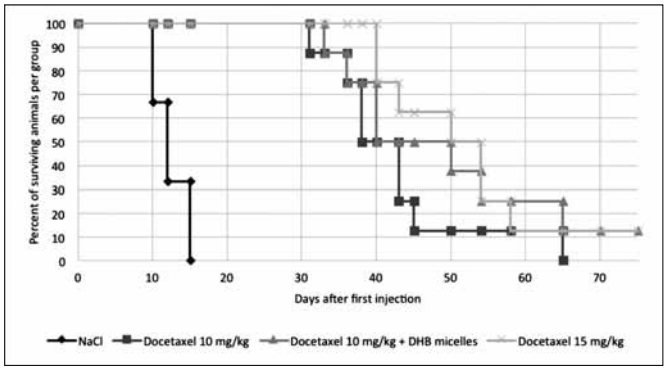
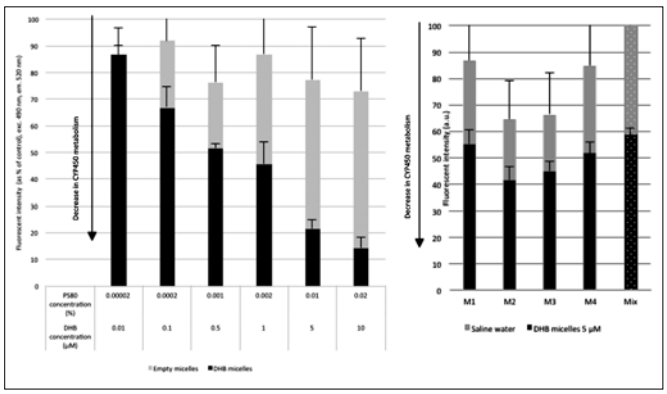


Figure 2. Metabolic activity measured with DBOMF as a fluorogenic CYP3A4 substrate (A) on HepaRG cells, co-incubated with DHB micelles (dark grey bars) or formulation alone (light grey bars) Each point is the mean + SD from three independent experiments, each one with three replicate per condition (B) on freshly isolated hepatocytes from 4 mice, following in vitro incubation of 5 µM DHB-micelles (dark grey bars) or saline water (light grey bars). Each bar is a mean of three replicated wells +SD. "Mix" is the mix of hepatocytes from the 4 mice (M1, M2, M3 and M4).

A B



Besides, an *in vivo-in vitro* study was implemented, allowing to take biodistribution of DHB-micelles into account. Mice were injected with DHB micelles and the CYP3A4 metabolism was evaluated *in vitro* 24 hours later. Marked inhibition of CYP3A4 was observed with pre-treatment with DHB-micelles, confirming the mechanism of action (Figure 3).

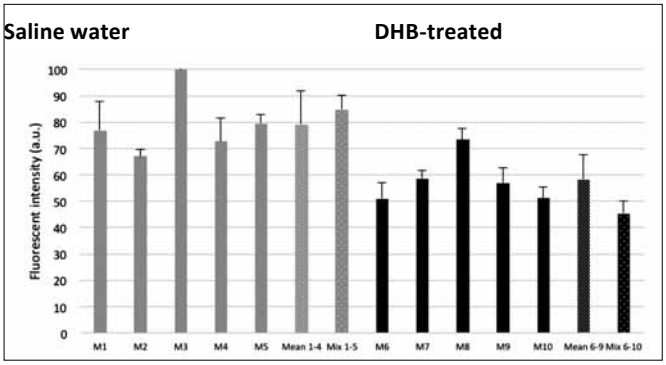


Figure 3. In vitro metabolism assay on freshly isolated murine hepatocytes performed 24 hours after intravenous injection of saline water (Light grey bars) or 3.6 mM (13.3 mg/kg) DHB-micelles (dark grey bars). Each bar is a mean of eight replicates Mix is a mix of hepatocytes from the 5 mice that received the same treatment (M1, M2, M3, M4 and M5, or M6, M7, M8, M9 and M10), seeded as if it was from one mice, in eight replicate wells. Data are represented + SD.

These DHB-micelles represent the first generation of nanosized metabolism-blocking agent, designed to enhanced the useful dose of a drug and/or reduce its useless dose. Future works concentrate on the design of nanoconstructs with high CYP450 inhibition ability addressing only the hepatocytes.

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PRETREATING METASTATIC TUMORS WITH ANTI-INFLAMMATORY DRUGS AS A STRATEGY TO IMPROVE DRUG DELIVERY

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INTRODUCTION

Chaotic and non-functional blood vessels, dense stroma and tumor-associated macrophages are among the biggest challenges for drug delivery to tumors. Metastatic lesions, because of their widespread location, small size and vascularization stage, are even more difficult to treat from a drug delivery point of view. Multi-targeted delivery approaches that aim to reduce/repair tumor abnormalities alongside killing the tumor cells may offer more efficient ways to target and treat metastatic cancers. In this study, we aimed to evaluate if pretreating tumors with the routinely used anti-inflammatory drug dexamethasone encapsulated in liposomes can be used as strategy to improve drug delivery to different types of breast cancer derived metastatic lesions

MATERIALS AND METHODS

Fluorescently labeled dexamethasone-loaded liposomes (lipdex) were injected into mice bearing 4T1 metastatic mammary breast carcinoma at doses of 2.5, 5 and 10 mg/kg, once weekly for 4 weeks, alongside with free drug. The accumulation of lipdex in tumors and metastases was analyzed using hybrid computed tomography and fluorescence molecular tomography (CT-FMT) and validated with ex vivo fluorescence reflectance imaging (FRI) scans and histological stainings. Further immunohistochemistry was employed to evaluate the potential of liposomal dexamethasone in normalizing the tumor microenvironment of primary tumors as well as metastatic lesions.

RESULTS

Dexamethasone-loaded liposomes accumulate well in tumors as well as in metastatic lesions located at different organs (lungs, lymph node, bone, ovary, etc) (Fig1). Treatment resulted in more mature vessel networks with increased pericyte coverage in dose-dependent manner compared to the control group in primary tumors (Fig 2A, C, D). Collagen stainings revealed that the tumor microenvironment becomes less dense with reduced collagen network compared to the control and the free drug treated group (Fig 2B, E). Analog studies in different types, origin and sizes of metastatic lesions are being performed, providing us insights on the (histopathological) microenvironment differences between primary tumor and secondary sites, as well as potential therapeutic strategies to better tackle lesions at distinct colonization sites. Systematic microenvironment temporal and morphological characterization at these metastases can potentially lead to more efficient therapy regimen and windowing for lesions at distant sizes.

CONCLUSION

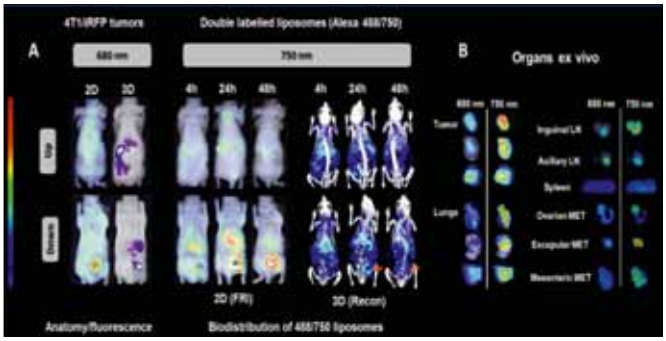
Our study reveals that liposomal delivery of glucocorticoid dexamethasone repairs abnormal tumor vasculature and microenvironment of primary tumors as well as metastatic lesions as exemplified by the presence of more mature vessels and reduced collagen networks. Such neoadjuvant treatment strategies can have significant impact on enhancing drug delivery to metastatic tumors and on optimizing the dose regimen of combination therapies.

ACKNOWLEDGEMENTS

This work is supported by the European Research Council (ERC starting grant 309495: NeoNaNo) and by DAAD/CNPq (290084/2011-3).

Fig 1. Representative CT-FMT in vivo images showing tumor and metastatic lesion in 680 nm channel and biodistribution of liposomes in 750 nm channel (A). Representative Ex-vivo FRI images

showing tumor and other metastatic lesion in different organ and liposome accumulation in the same (B).



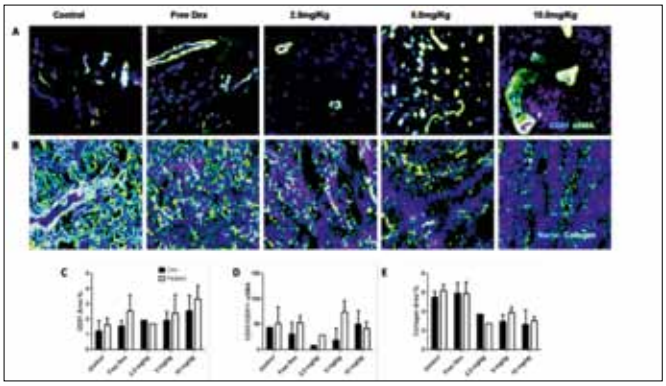


Fig 2. Representative images of tumor vessel characterization showing more vessel maturity in a dose dependent manner (A). Representative images of collagen showing decrease in dense collagen network in a dose dependent manner compared to control and free drug group (B). The mean area fraction of CD31 positive vessels (%) (C). The ratio of CD31/CD31+αSMA positve vessels (D). Mean collagen area fraction (%) (E).

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POLYELECTROLYTE NANOCOMPLEXES BASED ON CHITOSAN DERIVATIVES FOR WOUND HEALING PROMOTION

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Background: Dermal wound healing is a complex process, which includes four overlapping steps: inflammation, migration, proliferation and maturation[1]. In most cases, tissue repair can occur spontaneously, but depends on the size and depth of the wound. Therefore there is a growing need for developing biomaterials promoting wound healing to improve tissue regeneration in non-healing, chronic wounds. Wound regeneration needs to be guided by biological cues, such as Arg-Gly-Asp (RGD), a peptide known to induce cell adhesion and migration[2]. Nanocomplexes based on polyelectrolyte self-assembly are suitable carriers for these cues.

Aim of the study: Our focus is to develop different formulation of polyelectolyte nanocomplexes for topical wound application: a sprayable suspension of nanocomplexes, nano-structured hydrogels and freeze-dried foams, which would hydrate upon exudate absorption. Formulations are based on the chitosan derivative O-carboxymethyl-N,N,N-trimethyl-chitosan (CMTMC) grafted with RGDC peptide (Fig. 1).

Materials and methods: CMTMC was functionalized with RGDC through a 6-carbon spacer (1,6-diaminohexane, DAH), leading to RGDC-DAH-CMTC. Nano-sized polyelectrolyte particles were prepared by complexation of the cationic chitosan derivative with anionic chondroitin sulfate. Hydrogels were obtained by mixing RGDC-functionalized chitosan with hyaluronic acid (HA) at a 1:1 volume ratio. Foams were produced by lyophilization of the previously prepared hydrogels. Both nanocomplexe suspensions and hydrogels were formulated and tested for their potential to induce human dermal fibroblast (HDF) adhesion, migration and subsequent wound healing.

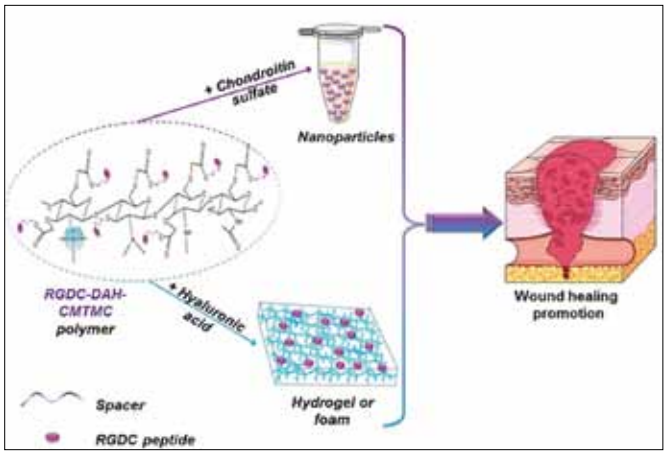

Fig. 1. Schematic representation for chitosan derivatives application.

RESULTS:

The synthesis process allowed controlled covalent binding of RGDC with high peptide substitution degree (15.3 µg of peptide per mg of chitosan). Nano-sized polyelectrolyte particles were obtained with a size of about 200 nm as confirmed by differential light scattering (DLS) and scanning electron microscopy (SEM).

Hyaluronic acid gels embedding RGDC-DAH-CMTMC nano-gels were fabricated, with viscosities adapted for topical patient application. Upon lyophilization, dry foam bandages were also obtained.

In vitro bio-adhesion assay demonstrated that HDF treated with formulations based on RGDC-derivatized chitosan showed a spread phenotype (instead of round cells) and increased motility compared to CMTMC treated control cells. Moreover, these formulations showed *in vitro* to promote wound closure after 24 h. These results were attributed to the presence of the adhesion peptide.

Conclusion: Overall, adhesion peptide-bearing nano-formulations promoted HDF survival, motility and migration. They have the potential to accelerate cell migration *in vivo* and promote healing of chronic wounds.

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TWO-PHOTON, NIR LIGHT-TRIGGERED DRUG RELEASE FROM ORGANIC NANOCARRIERS

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INTRODUCTION

By selectively targeting pathological tissue and controlling local drug concentrations, nanomedicine has the potential to improve cancer treatment efficacy and minimize deleterious side effects in patients. One of the major challenges in nanomedicine is the spatial and temporal control of drug release. To improve selective delivery of cytotoxic drugs to cancerous tissues, core-shell NanoCarriers (NC) that are capable of accumulating in solid tumors via the EPR effect have been well studied and developed.[1,2] NCs alter the biodistribution of chemotherapeutics and have the ability for specific tissue targeting, minimizing systemic delivery of cytotoxic drugs.[1,2] These vehicles add spatial specificity, but do not adequately address temporal control of the drug release. Several research groups are developing macromolecular vehicles with triggered release.[3] Most use simple triggers, ie. endogenous triggers such as pH shifts and changes in temperature.[3] However, the gold standard is a release mechanism that relies on activation by a spatially controllable, exogenous trigger, such as light.[4] Examples of light-triggered release can be found in the literature, but most require high-energy, UV light to initiate release, which has poor tissue penetration and causes cell damage.[4] The ideal trigger is near infrared (NIR) light, which has the advantage of deep tissue penetration and minimal cell damage.[4] Herein we will describe a novel NC capable of two-photon NIR light-triggered drug release. The triggered release is afforded by a NIR light-responsive prodrug platform to which a variety of anti-cancer drugs can be covalently linked. This prodrug is encapsulated within the core of an organic NC. Upon NIR illumination, the drug is released from the NC, while the prodrug platform simultaneously forms a fluorescent reporter. The fluorescent reporter serves both as a confirmation of successful drug release and as a quantification tool for drug release. The concept is depicted schematically in Figure 1. As an example, we will demonstrate the light-triggered release of the anti-cancer drug N-deacetylcolchicine, a tubulin polymerization inhibitor, from NCs.[5]

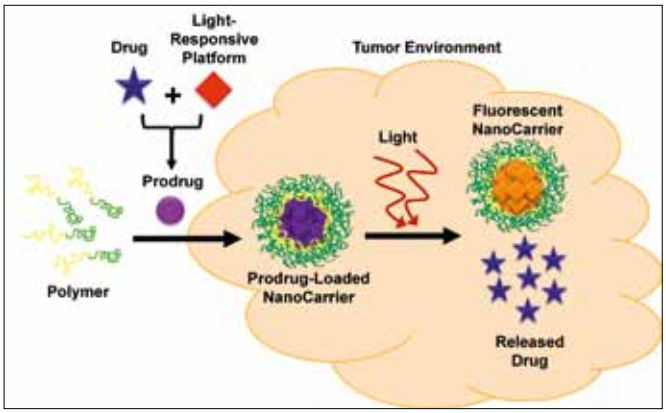

Figure 1: Our drug release approach - A NIR light-responsive prodrug is encapsulated into a tumor targeting, PEGylated nanocarrier (NC). Upon two-photon NIR excitation, drug is released from the NC and a fluorescent reporter is formed.

Approach

The light-responsive platform is based on the ortho-hydroxycinnamyl skeleton 1 (oHC).[6,7] Upon NIR light-activation, the double-bond in the oHC construct is known to isomerize (1'), which allows an intramolecular trans-esterification process (1'') to occur, which is mainly driven by a gain in entropy.[6,7] This results in the formation of the fluorescent dye coumarin 2 and the liberation of a molecule XH in a 1:1 molar ratio as shown in Figure 2.[6,7] This enables not only the triggered release of a molecule, but also a confirmation of the release, creating a theranostic platform.

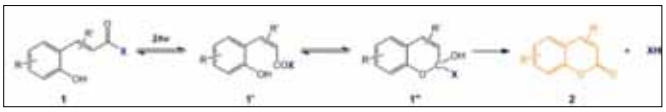

Figure 2. When illuminated by two NIR photons, the ortho-hydroxycinnamyl-based structure 1 undergoes an isomerization 1' followed by an intramolecular reaction 1'' to form a fluorescent coumarin 2 and release a molecule HX.

The prodrug-loaded NCs are formed via Flash NanoPrecipitation (FNP), a simple, one-step precipitation process that forms NCs with a hydrophobic core and a stabilizing polymeric corona.[8,9] FNP enables the control of prodrug loading, particle size and surface functionality.[8,9] Hydrophobic active pharmaceutical ingredients, fluorophores and polymers have been easily encapsulated into NPs via FNP.[8,9] By using PEGylated, stabilizing block copolymers, a dense PEG corona is formed, conferring colloidal stability in physiological conditions to the NCs.

RESULTS

The N-deacetylcolchicine prodrug 3 was successfully synthesized in four steps. Upon irradiation with 315 nm light (200 mW/cm2), the prodrug in solution successfully released the N-deacetylcolchicine 4 and formed the coumarin reporter 5 confirmed via HPLC and fluorescence measurements (Figure 3a). With a logP of 8.1, the prodrug was successfully encapsulated into 90 nm PEGylated NCs via FNP (Figure 3b). The particles had a 3 wt% prodrug loading with an encapsulation efficiency of 100%. The light-triggered release of N-deacetylcolchicine from the NC and the formation of a coumarin reporter was confirmed via HPLC (315 nm; 200 mW/cm2). The NC stability was not affected by the irradiation and maintained the same particle distribution (Figure 3b). As shown in figure 3c, the prodrug conversion rate was similar for the prodrug in solution and nanoparticle form. This is an unexpected and intriguing result, which suggests that the isomerization of the prodrug occurs at the same rate in solution and in an amorphous matrix (the polymeric NC core). In both cases, 80% of the prodrug is converted after 10 minutes of irradiation. The NIR release characterization and *in vitro* efficacy tests are currently on going.

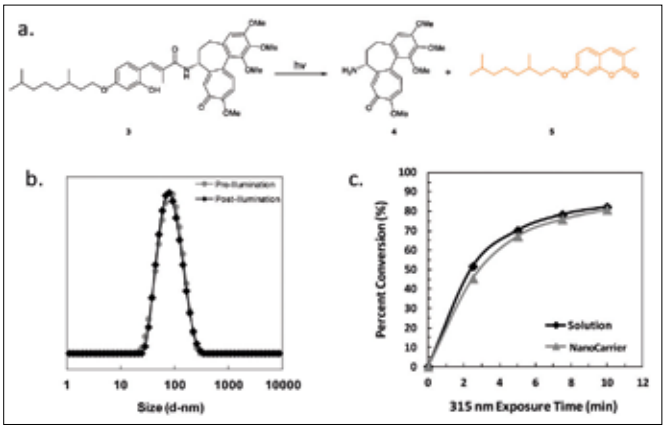

Figure 3. (a) Upon irradiation with light, the N-deacetylcolchicine prodrug 3 releases N-deacetylcolchicine 4 and forms the fluorescent coumarin reporter 5. (b) The prodrug was successfully encapsulated into monodisperse, 90 nm nanocarriers (NCs). The NCs distribution did not change after 10 minutes of irradiation at 315 nm. (c) The conversion of the prodrug as a function of irradiation time in solution and NC form.

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TARGETING MELANOMA BY IN VIVO SURFACE-ENHANCED RAMAN SCATTERING (SERS): MOLECULAR RECOGNITION ON THE NANOSCALE TOWARDS NOVEL MOLECULAR DIAGNOSTIC

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Keywords: SERS, early melanoma, noble metal nanoparticles, confocal micro-Raman spectroscopy, skin, *in vivo*, molecular recognition.

Melanoma is a tumor whose expansion is reflected in the melanocytes, the cells responsible for skin pigmentation. The incidence of malignant cutaneous melanoma (MCM) in humans is increasing worldwide, from 21.1 to 49.8 per 100,000 people, faster each year than any other type of cancer [1]. Furthermore, the mechanism involved in melanoma initiation and development is not fully elucidated, but the histopathological stages are well defined: the presence of common and dysplastic nevi, radial growth phase melanoma (RGP), vertical growth phase melanoma (VGP) and the last stage and the most critical one, is metastatic malignant melanoma [2].

Due to its high incidence and aggressiveness it is imperatively necessary to find methods for rapid diagnosis of skin tumor *in vivo*. In a preliminary study, Raman spectroscopy and surface enhanced Raman scattering (SERS) was employed to describe melanoma tissue at molecular level using skin tissue from ex vivo mice with induced melanoma [3, 4]. Unlike Raman spectroscopy, based on the inelastic light scattering in tissue, the ultrasensitive SERS technique uses nanoparticle-based multiplexed platform that has the potential for simultaneous read-out of large numbers of biomolecules. We showed that both labelled and unlabeled silver or gold nanoparticles could be used as SERS reporters buried in tissue and labelled nanoparticles introduced systematic differences in tissue response compared with unlabeled ones, suggesting that the label functional groups tag specific tissue components revealed by proteins or nucleic acids bands [5].

However, skin cancer as well as the benign and malignant skin lesions could provide an extended number of pathological groups, whose specificity is further related to the genetic, geographic, or living conditions of individuals. The latest development in laser-based Raman spectroscopy techniques and in particular SERS, can be considered a significant step forward in the analysis of biological samples, since Raman spectra could provide biochemical information *in vivo* at molecular level, while SERS can enhance the signal and provide valuable localized and sensitive information on the involved molecular species and their identity. In addition, considering the strong enhancement of the Raman signal in the presence of noble metallic nanoparticles, the typical problem of low signal usually encountered in poor light scattering media like biological tissue can be surpassed. Due to the easy optical access to the skin, numerous studies involving Raman spectroscopy for in-vivo diagnosis of skin cancers are available [6], however, SERS technique, in spite of its enhancement benefit was less involved. One of the main reasons could be the lack in understanding the wide dependencies concerning the plasmonic properties of nanoparticles in tissue as well as the molecular species associated with the malignancy and SERS signaling. SERS prospects for clinical diagnostic are currently in an early stage [6-8] and we are confident that the immense potential of this technique for health care units is a matter of additional experimental data collection and adaptive nanotechnology in answer to the specific need for translation to end-users.

Recently, Lui et al. (2012) [8] established that real-time Raman spectroscopy can be used to distinguish malignant from benign skin lesions with good diagnostic accuracy, comparable with the clinical examination and other optical based methods. Multivariate principal component with general discriminant analysis and partial least-squares analyses show that Raman spectroscopy can distinguish (a) malignant and premalignant lesions from benign lesions, (b) melanomas from benign pigmented skin lesions, and (c) melanomas from seborrheic keratoses. Such experimental approach is however user-unfriendly and large data processing steps are required.

We report here the proof of concept in employing SERS techniques with silver plasmonic platform to sensitively characterize the *in vivo* skin tissue from mice models with induced melanoma. A schematic four steps algorithm was designed as showed in the Fig. 1. Each of the steps supports advantages and drawbacks and these aspects will be discussed in details. Such algorithm could be easily adapted for compact SERS screening systems.Balb/c nude mice were inoculated subcutaneously, on the dorsal side with a suspension of A375 cells human melanoma cells ($1x10^7$ A375 cells/100 µl/mouse). The weight of mice and tumor dimensions were measured/determined daily until the end of experiment. To determine whether SERS spectra can be acquired from nanoparticles buried in animal tissues, small dosages of silver nanoparticles were injected subcutaneously in live rodents and the signal was acquired from the respective points. Raman and the SERS signal was acquired from a spot where nanoparticles were injected. Employing the confocal function of the Raman instrument, the laser was focused on the surface of the skin and 3 measurements were selected at the same x-y position but at different sample depths (on the z axis). The distance between the individual measurements was 1000 um. Using the 50 x 1000 um confocal aperture with the 50 x objective, a depth (axial) resolution of 3.2 mm could be obtained. Although the preliminary results suggests SERS specificity for *in vivo* skin monitoring based on the specific spectral signal, additional experiments would be needed with compact, portable, adaptive Raman-plasmonic devices to deeply understand the dependencies in the characteristic SERS signal associated with a specific melanoma stage *in vivo*. The inherent auto-fluorescence of the tissue is even more intense in the case of enhanced melanocytes activity. Melanin exhibits strong fluorescence when excited with the visible laser lines or even NIR. Its adsorption on the Ag nanoparticles is expected to quench its fluorescence and its characteristic signal acquired from skin layer with different surrounding species should be spectrally recognized. The correct assignment of the SERS signal is highlighted taking into the specific pigment contribution as well as the characteristic nucleic acids damage in malignant tissue. Thus, understanding SERS in tissue is of crucial importance for extracting pertinent conclusions on the SERS diagnostic protocol. Several specific SERS biomarkers associated with the early malignancy were identified. The experimental results clearly suggest that direct spectral differences are visible for different stages of malignancy.

The discrimination of the benign melanotic lesions (nevi) from malignant melanoma is essential for early diagnostic and efficient cure. Sensitive, non-invasive and real-time measurement techniques that can diagnose or directly monitor the applied treatment or disease evolution are highly desirable in health care units. The great appeal of SERS spectroscopy technique based on the NPs-enhancement of inelastic laser light scattering lies in its potential for *in vivo* prompt assessment of molecular changes along the disease evolution. Current medical diagnostic is in great need of *in vivo* evaluation tools in order to avoid long delays caused by laboratory-based biochemical analyses or to replace the current invasive methods by non-invasive ones, roles which could be fulfilled by the SERS techniques. Additionally, continuous patient monitoring, guidance of surgical interventions or monitoring the effects of therapies as well as using the feedback for personalized medicine are all areas

where adapted vibrational Raman techniques could be successfully implemented. SERS spectroscopy study was assisted by the toxicology evaluation. The SERS spectral changes were directly correlated with the skin pathology without additional statistical methods for discrimination.

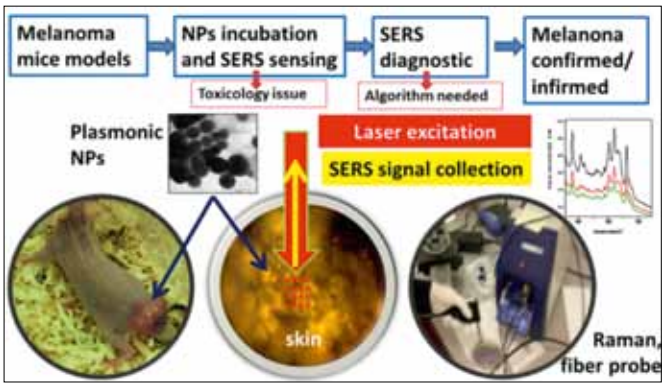

Fig. 1. Graphical sketch describing the experimental steps for SERS diagnostic of suspected melanoma tissue. Balb/c nude mice with induced melanoma were investigated by exploiting the SERS effect in skin in the presence of noble metal nanoparticles. Typical SERS signals from melanoma tissue histologically confirmed, are shown in the top right.

Optic fiber probes designed for Raman spectroscopy combined with the SERS effect, spectral data pre-processing, feature extraction and classification between normal/benign and malignant tissues were correlated, concluding the great potential of the technique toward clinical applications.
Concluding, based on the in-vivo SERS measurements as well as the histopathology evaluation (ex-vivo), the ability of technique to provide sensitive evidence of the molecular changes associated with the malignancy was proved. These results create future perspectives in personalized nanomedicine and cancer therapy assisted by Raman-derived techniques, as non-invasive, rapid and highly sensitive alternative for *in vivo* diagnostic and pathology monitoring.

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IN SITU CHARACTERIZATION OF PROTEIN CORONA COMPOSITION

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The interaction of NPs with biomolecules (such as proteins, lipids, etc.) presented on such biological fluids results in the formation of a biomolecular corona on the NPs surface, influencing their subsequent interaction with the biological machinery.[1] Recently, evidences in the literature shown a strong correlation between the nature of the biomolecular corona and the cellular uptake of nanoparticles *in vitro* and *in vivo*.[2,4] Therefore, understanding and controlling this complex and highly dynamic multilayer of biomolecules that ultimately mediates the interactions of nanoparticles with cells and organisms, remains a central question in nanomedicine and nanotoxicology.
Lot of efforts have been made to link the biological behaviour of nanoparticles with the nature of their biomolecular corona and several methods to determine the composition of the corona have now been established. Unfortunately, the average compositional information obtained with the current techniques does not fully account for the complexity of the nanoparticle-corona-cellular receptor interactions. In this respect a key role is played by the organization and mutual orientation of the molecules on the nanoparticle surface. The exposure of certain protein domains, for instance, can potentially trigger specific cellular recognition pathways, resulting in the activation of determined biological processes.
In this work we develop a novel platform for the in-situ biologically relevant structure characterization of the composition and organization of biomolecules presented on the nanoparticle surface that allows predicting the biological impact of engineered nanomaterials.3,5 By using fluorescence probes and flow cytometry we can monitor the in situ characterization of the nanomateriales under relevant biological scenarios (see Figure 1), providing information on specific motifs or epitopes presented at the nanoparticle surface, and therefore defining how the nanoparticle first interacts with and is recognized by cells.

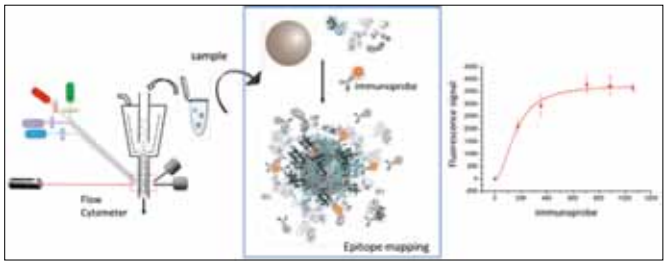

Figure 1. Schematic representation of protein-nanoparticle complexes characterization by flow cytometry analysis: immuno-Quantum Dots are used as labels for epitope mapping of a protein presented on the corona formed on nanoparticle's surface. The fluorescence signal is recorded for increasing concentrations of the immunoprobe till the total number of epitopes are detected.

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ULTRASMALL ZWITTERIONIC GOLD NANO-PARTICLES AS MULTIMODAL AGENTS FOR TARGETED CANCER IMAGING

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Surface functionalization of inorganic ultra-small gold nanoparticles (AuNPs) is an essential prerequisite to their development as multimodal imaging agents for biomedical applications that include computed tomography (CT), optical imaging (OI) and positron emission tomography (PET) [1]. In the presence of biological fluids, however, the non-specific adsorption of proteins and other biomolecules to nanoparticles dramatically influences their surface properties and their targeting efficacy [2]. Coating of nanoparticles (NPs) with zwitterionic ligands represents a promising strategy to reduce nonspecific protein absorption in biological environments, potentially limiting uptake by macrophages *in vivo* and helping to maintain an active targeting capability in appropriately engineered systems. Advanced nanoprobes can be used to target the epidermal growth factor receptor (EGFR) over-expressed in many cell types such as; epidermoid carcinoma cells (A431) [3].
Here, we present a novel nanoparticle platform in which AuNPs with a core of ca. 5 nm were coated with a variety of zwitterionic surface ligands to allow the AuNPs to evade phagocytosis, target the EGFR, and subsequently permit multimodal molecular imaging of the cancer cells. The majority of surface sites of the NPs are cover by a compact zwitterionic coating which reduces the surface zeta potential while not significantly increasing the hydrodynamic diameter. Reducing the surface charge and size is one way to evade phagocytosis and increase the chance of success of binding to tumor receptors. In order to target the EGFR, a single domain antibody (sdAb) capable to bind to the extra-cellular pocket of the receptor was conjugated to the surface of the NPs. Imaging functionalities such as PET using a radiochelator capable of chelating 64Cu as well as a zwitterionic NIR-fluorescent dye were also attached to probes to track the position via confocal microscopy and OI (Figure 1A). In addition to these imaging functionalities, Au NPs themselves have SPR effects which allows increased contrast in CT scans.

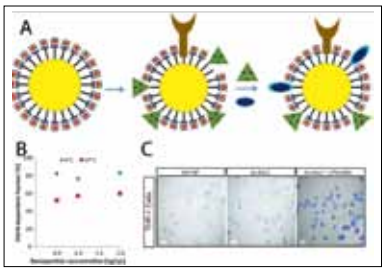

Figure 1. (A) Scheme of the funtionalization of AuNPs with zwitterionic ligands and succesive 64Cu labeling (B) Specific EGFR binding of fully funtionalized sdAb-Zw-AuNPs (C) Macrophages (THP-1) uptake of bare zwitterionic AuNPs and sdAb-Zw-AuNPs.

Specific targeting and uptake of the EGFR by fully functionalized sdAb-AuNPs was confirmed *in vitro* in A431 cells by receptor blocking with endogenous EGF (Figure 1B). In vitro studies in A431 mice models were also performed via PET. The success of zwitterionic ligand conjugation and monodispersity was confirmed by high resolution electron microscopy, dynamic light scattering, UV-vis spectroscopy, fluorometry, and inductively coupled plasma optical emission spectrometry. Purification of the nanoparaticles after 64Cu labeling was performed using gel chromatography. Furthermore, ultrasmall AuNPs were not uptaken by macrophages in presence of complete human serum leading to a improvement of target-off effects and pharmacokinetics (Figure 1C). This nanoprobe possess great capabilities to be used as multimodal imaging agent for theranostic applications as well as opens up new avenues for probing the zwitterionic ligands at the nano-bio interface.

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PDMS-PMOXA POLYMER VESICLES FOR TARGETED DELIVERY TO LECTIN RECEPTORS

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Polymer vesicles, or so called polymersomes, are attracting much attention as alternative nanodelivery system to implement drug targeting strategies. Polymersomes have several interesting features. For instance, ease of chemical modification of the polymer chains can be used to modulate their tissue specificity and organ distribution. Furthermore, they can adopt a vesicular structure and are, therefore, and attractive alternative to liposomes. A wide variety of polymers is available, however a good candidate for pharmaceutical formulations is the di-block copolymer poly(dimethylsiloxane)-b-poly(2-methyloxazoline) (PDMS-PMOXA). This polymer is formed by two subunits which are FDA approved for use in human and for pharmaceutical applications.
In this work we present an innovative polymer vesicle formulation with PDMS-PMOXA di-block copolymer able to specifically target hepatocytes. PDMS-PMOXA polymersomes have been chemically modified with asialofetuin (AF), a desiaylated glycoprotein whose uptake is mediated by the hepatocyte asialoglycoprotein receptor. AF was conjugated on the surface of PDMS-PMOXA polymer vesicles (Figure 1). The protein retained the initial functionality upon chemical modification, allowing a successful uptake of polymersomes in human liver carcinoma cells (HepG2). Active uptake of modified PDMS-PMOXA polymersomes was successfully demonstrated using fluorescence activating cell sorting (FACS) analysis. Biocompatibility of PDMS-PMOXA polymer vesicles has been investigated using an alternative animal model as the zebrafish. Danio rerio has a great advantage compared to classical animal models as its transparency allows the direct inspection of nanoparticles in the blood circulation. PDMS-PMOXA polymer vesicles have shown similar properties compared to long circulating nanoparticles; moreover they uniformly disperse in the blood circulation, and no protein aggregation was observed.
In conclusion, active targeting of HepG2 cells using AF modified PDMS-PMOXA polymersomes was successfully achieved. We envision that PDMS-PMOXA polymersomes can act as a platform to develop innovative drug delivery systems with tunable features for clinical applications.

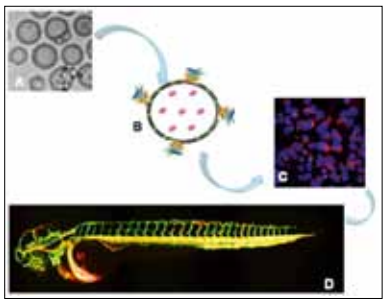

Figure 1: PDMS-PMOXA polymer chains self-assemble in water in stable vesicular structures (A), which can be covalently modified using targeting moieties (B). In vitro receptor mediated uptake has been demonstrated using hepatic cancer cell lines (C), moreover in vivo biocompatibility has been investigated using zebrafish as animal model (D).

TISSUE ENGINEERING USING VOCAL FOLD FIBROBLASTS TREATED WITH SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES FOR VOICE REHABILITATION

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INTRODUCTION

The voice is the most important instrument of communication. It originates in the larynx by motion and vibration of the vocal folds. Tissue defects in this area, occurring commonly after tumor surgery, lead to serious aggravation in quality of life. Apart from their severe diseases, the patients are faced with social problems, caused by their inability to communicate. Hitherto, no satisfactory vocal fold transplant or implant exists together with a test system that meets demands for biomechanical investigations. Using nanotechnology we aim for an establishment of functional vocal fold implant in a rabbit model by magnetic tissue engineering with superparamagnetic iron oxide nanoparticles (SPION).

MATERIAL AND METHODS

Rabbit vocal fold fibroblasts were incubated for 24 h with different concentrations of SPIONs (5, 20, 40, 60 and 80 µg/cm2). Vocal fold cell behavior under SPION treatment was tested extensively for adhesion, spreading and migration, which are important for formation of 3D structures. Migration was measured with a wound healing assay using Incucyte live cell analysis. For adhesion and spreading cells were washed, fixed and stained with crystal violet after different time points. Spreading was analyzed via microscopically investigation of cell area and for adhesion crystal violet was dissolved from cell and measured photometrical at 560 nm. The possibility of magnetic guidance of SPION-loaded cells was tested in 2D as well as in 3D by placing a magnet either under or above the cell tissue plate.

RESULTS

The effects of SPIONs on cells behavior were dose-dependent for adhesion, with good tolerability observed up to the nanoparticle concentration of 20 µg/cm^2, migration and spreading were not significantly influenced by SPION uptake to up to 80 µg/cm^2. Magnetically guidance of cells loaded with SPIONs was demonstrated in 2D with 20 and 40 and 80 µg/cm^2 (Figure 1, 2D), with cells only growing in areas where a magnet is present. To establish a 3D structure of vocal fold fibroblasts a magnet (0.7 T) was placed above the cell culture plate and cells loaded with only 5 µg/cm^2 were already able to form three-dimensional structures only after 48h (Figure 1, 3D).

CONCLUSION AND DISCUSSION

Here, we present first results of magnetic tissue engineering for voice rehabilitation. To develop 3D structures cell behavior must not be affected by SPION uptake. Therefore, cell features including adhesion, spreading and migration were proven to be intact after SPION treatment. As a proof of principle for magnetic cell guidance SPION loaded vocal fold cells were allowed to "choose" either to grow on the side were a magnet or none was placed. Interestingly, 5 to 20 µg/cm^2 are sufficient to induce cell growth solitary at site of the magnet in 2D. Furthermore, magnetic cell guidance as only initiator for 3D cell formation was proven to work with very low amount of SPIONs (5 µg/cm^2). Next steps include the isolation of epithelial cell and establishment of 3D co-cultures, as well as the proof of functionality in a flow channel model of the rabbit larynx. Our results will constitute a solid basis for a successful transfer of this technique into humans, in order to provide an individual and personalized vocal fold implant. This is particularly important for patients, who suffer from dysphonia or even aphonia as a consequence of a vocal fold tissue defect, and will help to improve their quality of life.

ACKNOWLEDGEMENT

This work is supported by the Deutsche Krebshilfe e. V. (no. 111332).

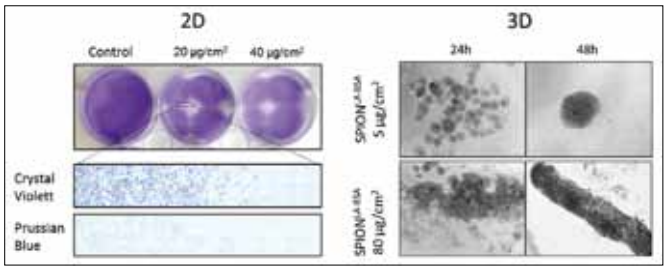

Figure 1: 2D: Vocal fold fibroblasts treated with no (control), 20 and 40 µg/cm^2 SPIONs seeded on a 6 well plate with magnets below (4 magnets per well). Rectangle: enlarged section with crystal violet (upper) or Prussian blue (lower) staining. 3D: Formation of three-dimensional cell structures with 5 or 80 µg/cm^2 after 24 and 48 h.

POLYMERIC MAGNETITE NANOCOMPOSITE TARGETED AGAINST HUMAN GLIOMA INTRACEREBRAL MURINE XENOGRAFT MODEL

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Therapy for chemo/radio resistant malignant gliomas are limited by factors such as blood brain barrier, transport within the brain interstitium, and complexities in delivery of therapeutic agents specifically to the tumor cells and highly invasive quality of gliomas leading to poor prognosis. Nano trigger based imaging and therapeutic agents are aimed to deliver versatile payloads with favorable pharmacokinetic and pharmacodynamics. This approach capitalizes enhanced specificity, efficacy and improved safety levels. In view of this, our study formulated an optimized polymeric magnetite nanocomposite of Temozolamide, primarily focusing on specific tumor localization using an intracerebral glioma model.

A multifunctional polymeric magnetite nanocomposites were engineered with entrapped Temozolomide along with a ligand to bypass the blood brain barrier using transferrin/polysorbate-80 along with a targeting moiety anti-nestin antibody for active targeting of nestin on to the glioma (U-251 MG) cell surface. This nanocomposite was radio-labeled with 99mTc for bio-distribution study and analyzed by SPECT-CT. This non-invasive imaging procedure indicated the targeted delivery of 99mTc labeled nanocomposite with preferential localization in tumor intracerebrally (Figure 1). The pharmacodinamic experiments indicated even a low dose i.v. administration of Temozolamide with reduced dosage intervals demonstrated increased tumor regression over the pure Temozolamide at a higher dose as indicated by microCT evaluation. This increased tumor regression was further validated by using proliferation markers (PCNA/Ki-67) in an immunohistochemistic analysis.

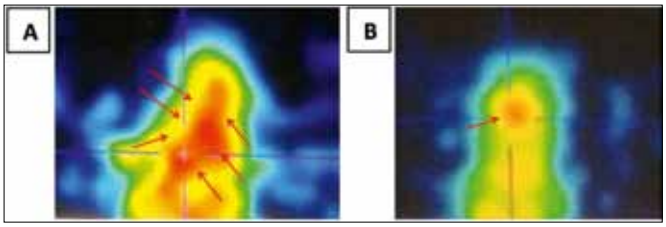

Fig 1: Representative SPECT image showing distribution of pure TMZ (A) v/s anti-nestin + transferrin TMZ nanocomposite (B). Arrows indicate the distribution of radio-labeled drug.

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We thank Manipal University for Providing Dr. T.M.A. Pai Fellowship for Structured Ph.D Program to the first author. We thank Prof. K. Satyamoorthy, School of Life Sciences, Manipal University for the laboratory facilities and ACTREC for facilitating small animal imaging facility

A NON-CELLUAR BARRIER FOR THE PULMONARY DELIVERY OF NANOPARTICLES: THE PULMONARY SURFACTANT LAYER

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INTRODUCTION

Nanoparticles (NPs) reaching the deep lung encounter a biological barrier only few are aware of: the thin layer of pulmonary surfactant (PS). This mixture of mainly (phospho-)lipids and unique proteins constitutes the first line of host defense. Besides its high lipid content (~90 %wt), PS not only possesses a variety of ubiquitous proteins, but also specific proteins, mainly the surfactant associated proteins A-D (SP-A/B/C/D). Two of those (SP-B/C) are highly hydrophobic small proteins and responsible for the surface lowering effect of the PS, without which breathing is impeded. The two hydrophilic large collectins (SP-A/D) are also connected to the lipid layers promoting their organization, but most importantly they are also part of the host defense in terms of surface pattern recognition and are being recognized themselves by professional phagocytes via ligand receptor binding. Inhaled NPs interact with the membranous structure of PS by adsorbing proteins and lipids to their surfaces, resulting in a so-called NP corona which is in this case unlike the commonly investigated plasma protein corona - and which therefore has different effects on NPs and their fate in the lungs. In general, the corona has been found to influence properties such as cell interactions and agglomeration behavior in biological fluids[1,2], as well as an influence on targeting, and therefore, methods to access and modify the corona of NPs are advancing constantly[2]. The plasma corona is being analyzed standardly for engineered NPs intended for medical use in the meanwhile. Interactions of NPs with the proteins and especially lipids of the PS layer are more complex, as it does not display a solution of proteins, but a constantly self-organizing layer system, in which proteins are attached or dispersed. Consequently, it is being recognized that information about the corona formed in PS is urgently needed, not only for the appraisal of drug delivery systems for pulmonary application, but also to assess nanotoxicology *in vitro*[3]. Here, we present a detailed analysis of the corona which forms around NPs of varying surface hydrophobicity after contact with a native PS preparation from porcine source.

METHODS

The poor accessibility of PS is the limiting obstacle for studying nano-PS interactions in the deep lung. While most other studies use the commercially available protein-depleted clinical preparations, such as Curosurf®, we used a preparation of PS which was gained by concentrating lavage from fresh lungs of slaughtered pigs (pPS) and therefore contained all proteins and lipids, present in PS. Different magnetite-loaded NPs with either PLGA (=PLGA-NPs), PEG (=PEG-NPs), or phosphatidylcholine (=Lipid-NPs) surface, were incubated with pPS and had subsequently to be separated from the non-binding supernatant magnetically due to the high density of PS vesicles. Lipid content of the corona was analyzed by normal phase LC-MS after exhausting liquid-liquid extraction; protein composition was quantified using label-free shotgun proteomics after filter-aided sample preparation. Lipid adsorption to NPs in absence of surfactant proteins was determined by thin layer chromatography. A detailed description of the methods can be found in[4]. Additionally, the uptake of polystyrene NPs into a murine alveolar macrophage cell line (MH-S) was determined by flow cytometry in presence of pPS and serum proteins.

RESULTS

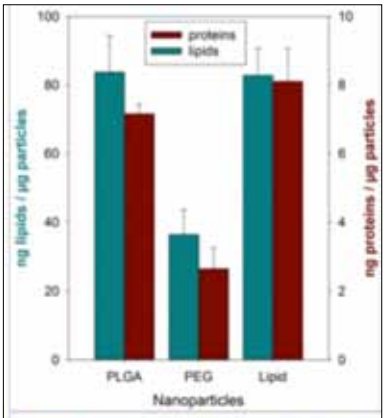

Figure 1: Adsorption profiles of lipids and proteins onto PLGA-, PEG-, and Lipid-NPs

Although the amount of lipids on the surface of the NPs differed, the composition of the lipid corona was conserved on all three used NPs, with phosphatidylcholine (PC) being enriched in comparison to crude pPS. Besides PC, phosphatidylglycerol, -ethanolamine, -serine, -inositol, as well as cholesterol were detected in the samples. The ratio of lipids to proteins was found to be roughly 10:1 (Figure 1). However, up to 417 proteins could be determined in the corona with marked differences between the NPs (Table 1). Among the proteins showing significant differences between the NP coronas, there was a striking prevalence of molecules with a notoriously high lipid and surface binding, such as SP-A, SP-D, and DMBT1. Our data indicate that the selective adsorption of proteins mediates the relatively similar lipid pattern in the coronas of different NPs, making even highly hydrophilic NPs accessible for the lipids of the PS layer. By testing the interaction with a protein-depleted clinical surfactant onto NPs, which resulted in adsorption of lipids only onto Lipid-NPs, this hypothesis was corroborated. Based on our lipidomic and proteomic analysis, we were able to gain a detailed set of quantitative data on the composition of the surfactant corona formed upon NP inhalation. Data confirmed that this structure is unique and markedly different to the plasma corona[4] and can subsequently be used to attract or repulse certain proteins present in PS and the corona. Furthermore, we were able to show in first *in vitro* experiments that the presence of pPS modifies the uptake of fluorescent polystyrene NPs into alveolar macrophages, different from the effects of serum proteins. While at a given concentration of 40 µg proteins and 20 µg NPs per ml, NPs uptake was enhanced in the presence in comparison to plasma proteins, this effect however was heavily depending on concentration of pPS / plasma proteins.

PLGA-NP	PEG-NP	Lipid-NP
Tubulin alpha-4A chain *	Tubulin alpha-4A chain *	Tubulin alpha-4A chain *
Actin, cytoplasmic 1	Actin, cytoplasmic 1	Actin, cytoplasmic 1
Carbonyl reductase [NADPH] 2	Carbonyl reductase [NADPH] 2	Carbonyl reductase [NADPH] 2
Tubulin beta-4B chain *	Tubulin beta-4B chain *	Myosin-9 *
Tubulin beta chain	Myosin-9 *	Tubulin beta-4B chain *
DMBT 1 protein	Tubulin beta chain	SP-A
Tubulin alpha-1A chain	Fibronectin *	DMBT 1 protein
SP-A	GAPDH *	Tubulin beta chain
Myosin-9 *	LPLN epithelium protein 1	Tubulin alpha-1A chain
protein 1	Tubulin alpha-1A chain	Fibronectin *

Table 1: Top 10 most abundant proteins found in the corona of PLGA-, PEG-, and Lipid-NPs

CONCLUSION AND OUTLOOK

Our results clearly show that the corona, which obviously forms around nanoparticles not only in plasma, but also at the portals of entry to the body, e.g. the air-blood barrier of the lungs, cannot be expected to be everywhere the same. The NP corona which evolves after contact with PS is unlike the plasma corona with a high lipid content and surfactant specific proteins which might be able to hydrophobize even hydrophilic NPs such as PEGylated ones. By showing the differences of the PS corona in comparison to the plasma corona, we hope to encourage others to have a closer look at the bio-nano interactions of engineered NPs, intended for pulmonary use with the PS layer. Tuning the PS adsorption could lead to a targeted uptake by alveolar cells, as well as to an enhanced residence time without being recognized by phagocytes.

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NOVEL METHOD FOR THE PREPARATION OF COMPLEMENT RECEPTOR TYPE 1 CONSENSUS TRIPLET SCR(1–3), A POTENTIAL INHIBITOR OF NANOPARTICLE-INDUCED COMPLEMENT ACTIVATION

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The utility of certain drug delivery system, such as nanoparticles, is largely compromised by they ability to trigger complement activation. Such physiological effects often overcome the benefits of nanoparticles and/or make them use unreliable in medicine. Thus, regulation of complement actication, particularly its inhibition, has clear pharmacological implications. Our goal is to approximate knowledge has been gained on the ground of classical biochemistry to the pharmaceutical needs to surmount this obstacle.

Complement inactivation by sCR1, or its truncated form SCR1-3 (short consensus repeat 1-3) has the potential to improve *in vivo* performance of nanoparticle delivery systems, however, they use is yet prevented by limitations to produce and purify them in sufficient quantities. We aim to approach this limitation.

Purification of SCR1-3 from bacteria has been described. Bacteria express SCR1-3 as insoluble aggregate and the steps of purification involve resolubilization of aggregate, refolding, buffer exchange by ultrafiltration and chromatography. Some of these steps is not compatible with the necessary industrial scale up. We combine and modify available protocols to substitute these steps to a suitable application. In particular, direct application of the material after refolding to chromatography seems to have the advantage to eliminate the ultrafiltration step that is hard to scale up and usually results in precipitation of significant quantities of the refolded protein. We expect that our approach can be scaled up to yield high quality and high amount SCR1-3 for subsequent downstream applications.

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ABSTRACT: MONTE CARLO SIMULATIONS GUIDED BY IMAGING TO PREDICT THE IN VITRO RANKING OF RADIOSENSITIZING NANOPARTICLES

PAUL RETIF

1. PROBLEM AND MOTIVATION

Radiosensitizing nano-objects are becoming a major innovation in the field of radiation therapy with the promise of a breakthrough in anti-cancer therapies. Since the early '90s, the number of articles published on the subject has kept increasing. Unfortunately, as it has been mentioned in 2013 by Etheridge et al., "Many of the revolutionary nanomedicine technologies anticipated in the literature may be 20 or more years from clinical use". [2] Indeed, only few nanoparticles (NP)-radiation therapy combinations are undergoing clinical trials (only 3 records concerning NBTXR3 devices on clinicaltrials.gov). [3]

2. BACKGROUND AND RELATED WORK

In order to speed up the preclinical development of radiation therapy enhancing NPs, numerous studies have tried to predict their *in vitro* or *in vivo* radiosensitizing effect. The most common method, inherited from the practices in physics, is to use Monte Carlo simulators of particles transport in matter such as BEAM, MCNP, PENELOPE or Geant4. First attempts did not show satisfactory results. Indeed, according to Butterworth et al. biological effects cannot be accurately predicted on the basis of GNP concentration and beam energy and suggest oxidative stress as a central mechanism in mediating response. [1] Yet, it should be nuanced as results are likely to depend on the simulation parameters. That is why in a recent comment McMahon has highlighted the fact that theoretical prediction scenarios and values should be meaningful within a therapeutic context. [4]

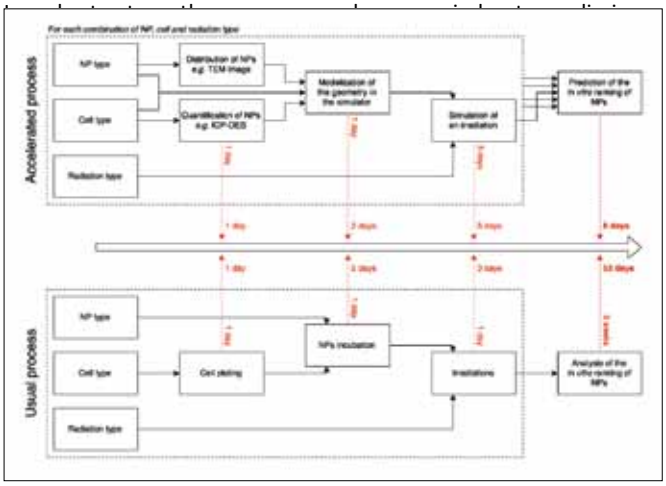
As a step forward towards an efficient prediction of their radiosensitizing effects, we have developed a simple, yet effective method to assess an *in vitro* ranking of radiation enhancing NPs. Our method could make it possible to select the nano-objects performing the most promising results thus speeding up the preclinical development of radiosensitizing NPs.

3. APPROACH AND UNIQUENESS

3.1 Overview of the ranking process

The objective of our work is to elaborate a process that enables us to quickly rank a high number of radiosensitizing NPs with as few experiments as possible. Indeed, biological assays are not

only expensive but also time-consuming. Figure 1 compares two alternative approaches to rank the radiosensitizing effects of NPs: an *in vitro* and classical method versus our in silico and innovative technique. As it can be seen on the Figure 1, in a simple study only requiring 1 clonogenic assay, the accelerated in silico process could save up to 46 days of experiment. Moreover, it should be noticed that for more complex combinations comparing more NPs, cells, sources, the accelerated process would not last much longer whereas the usual process duration would be drastically extended.



3.3 Modeling in the Monte Carlo simulator

For the current study, we used GATE 7.0. The geometry entered in the simulator was highly simple: a water cube (edge size of 1 mm) containing a spherical structure standing for a U87 cell (radius of 6 µm). Then depending on the NP being evaluated, a random distribution taking both the size and the number of clusters into account was generated inside the spherical cell structure. Each cluster contains a fixed number of NPs (spherical structures). Number of clusters, clusters sizes and number of NPs per cluster were determined experimentally using TEM images and ICP-OES quantification. At the end of the simulations the following results were recorded: 3D dose images, secondary species production in the cell structure and a spectrum of the energy deposited in the cell structure. Mean doses in the cell were assessed using 2 methods: a measurement on the 3D dose images and the integration of the energy deposited in the cell spectra.

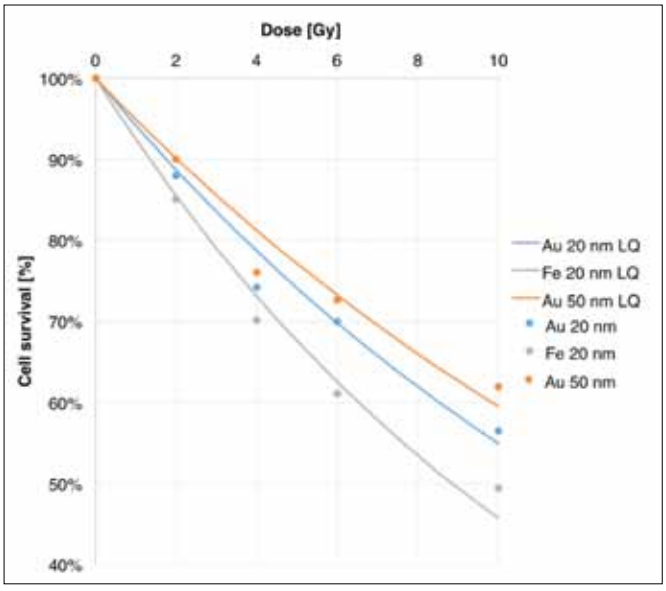


Figure 2: Survival curves with or without NPs

4. RESULTS AND CONTRIBUTIONS

Following the clonogenic assays we were able to plot survival curves and to rank the NPs according to their radiosensitizing effect on U87 cells (Figure 2). It appeared that 20 nm iron NPs showed the best results, whereas 20 nm gold NPs showed slightly similar enhancement and 50 nm gold NPs showed a very poor effect with results equivalent (or worse) to cells without NPs.

Concerning the Monte Carlo simulations, we built a ranking according to the mean dose deposition in the spherical structure standing for a U87 cell (Table 1). Interestingly the order of the ranking was exactly the same that for *in vitro* experiments with the same tendency for the 50 nm gold NPs to show a very poor (or negative) radiation enhancement. Mean dose deposition enhancements in presence of NPs seem surprisingly low. We figured out that NP clusters were causing spots of dose deposition (Figure 3) which, when they are averaged in the whole volume of the NP result in a relatively low mean dose.

NP	*In vitro* ranking	*In silico* ranking	Mean dose enhancement
Fe 20 nm PVP	#1	#1	+ 0,9%
Au 20 nm PVP	#2	#2	+ 0,2%
Au 50 nm PEG	#3	#3	+ 0,1%

Table 1: Ranking results and dose enhancement values

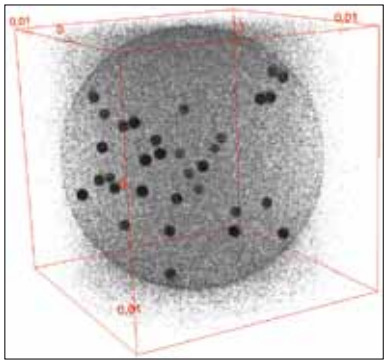


Figure 3: 3D view of a dose image with a visible enhancement inside and around the iron NP clusters (black spheres) which are located inside the U87 cell

This article addresses the in silico-*in vitro* prediction issue of nanoparticle-based radiosensitization enhancement. Conversely to previous studies, the goal is not to predict the X-ray dose effect but to carry out computational experiments to quickly identify non-efficient nanostructures and then to preferentially select the most promising ones for the subsequent *in vitro* and *in vivo* studies. To this aim, this article presents a computational ranking method and tests it by comparison with *in vitro* responses. This comparative analysis has shown equivalent ranking results between the in silico and *in vitro* responses. That corroborates the relevance of such a prior ranking method able to speed up the preclinical development of nanoparticles in radiotherapy and radio-imaging.

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BISPECIFIC PEG ENGAGERS FOR CONDITIONAL DELIVERY OF PEGYLATED NANOCARGOS INTO CANCER CELLS

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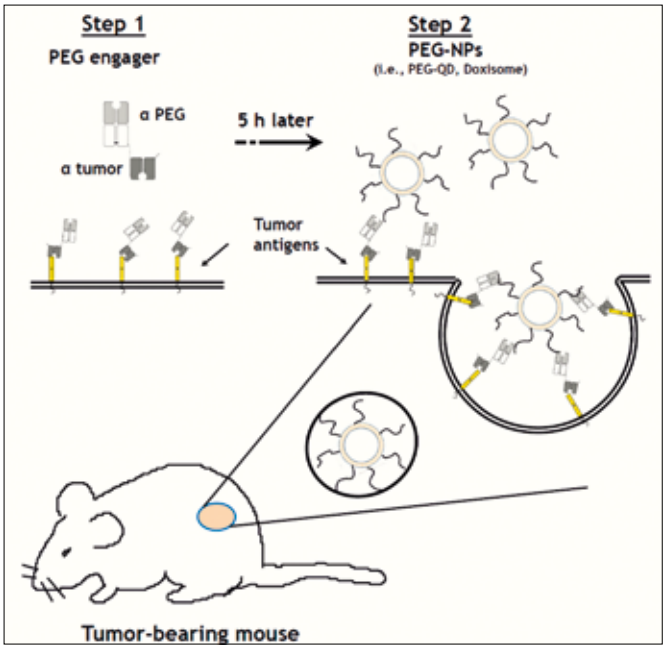

Figure 1. Overview of PEG engager pre-targeting strategy. A two-step pre-targeting approach that enables capture of PEGylated nanocargos to the tumor-pretargeted PEG engagers (PEG-binding bispecific antibodies) and accelerates the internalization of PE-Gylated NPs for multimodality treatment of cancer.

Selective delivery of nanomedicines to target cells may improve treatment specificity and efficacy. Bispecific antibodies are promising tools to facilitate selective delivery of stealth nanomedicines to cancer cells[1,2]. Here, we describe a general targeting approach to deliver PEGylated nanomedicines to cancer cells based on bispecific molecules that bind to both polyethylene glycol on nanomedicines and surface receptors that are overexpressed on cancer cells. We developed polyethylene glycol (PEG) engagers by genetically fusing a humanized anti-PEG 6.3 Fab antibody fragment with anti-tumor antigen single-chain disulfide stabilized Fv fragments (dsFvs) against epidermal growth factor receptor (EGFR, HER1) or CD19 to form bispecific PEG engager^{EGFR} and PEG engager^{CD19}. We tested the antitumor activity of the PEG engager^{EGFR} against triple-negative breast cancer (TNBC), which are clinically negative for expression of estrogen receptors (ER), progesterone receptors (PR) and human epidermal growth factor receptor 2 (HER2). The lack of appropriate markers for targeted therapies has hindered effective therapy of TNBC. Recently, epidermal growth factor receptor (EGFR, HER1) targeted therapies are of interest because up to 50% of TNBC patients overexpress EGFR. The bispecific PEG engager^{EGFR} bound to TNBC tumors via the anti-EGFR scFv arm and stimulated uptake and endocytosis of PEGylated nanocargos by the anti-PEG Fab arm. Pre-targeting of monovalent PEG engager to EGFR positive TNBC cells did not induce internalization until cross-linking with PEGylated nonacargos to trigger conditional endocytosis. PEG engagerEGFR preferentially delivered drug-loaded nanocargos (liposomal doxorubicin) to TNBC cancer cells that expressed EGFR with approximately 90-fold improved cancer cell killing compared to liposomal doxorubicin alone. PEG engager^{EGFR} mediated endocytosis of PE-Gylated nanocargos correlated with the expression levels of EGFR, which may diminish off-target toxicity to normal cells with relative lower EGFR levels. Furthermore, the PEG engager enhanced accumulation of fluorescent PEG probes and significantly increased the antitumor efficacy of PEGylated liposomal doxorubicin in a TNBC xenograft mouse model. PEG engagers may be useful for tumor-specific targeting of PEGylated nanocargos to improve cancer imaging and anti-tumor therapies.

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TARGETED DELIVERY OF ANTI-MIRNA-199A USING SELF-ASSEMBLING PEPTIDE NANO-COMPLEXES INHIBITS PSC ACTIVATION

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INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) presents itself with a strongly abundant tumor stroma which supports tumor growth, invasion and metastasis[1]. Pancreatic stellate cells (PSCs), as precursors of cancer-associated fibroblasts, are the main component of tumor stroma in PDAC[1]. In PDAC, activated human PSCs (hPSCs) promote tumor growth, invasion and metastasis[2]. MicroRNAs (miRNA) play a key role in the regulation of the pro-tumoral activity of activated hPSCs[3]. This makes, anti-miRNA oligonucleotides (AMOs), which block the function of miRNAs, potential therapeutics to diminish the pro-tumorigenic effects of the pancreatic tumor stroma[1]. In the present study, we have designed a dimeric form of a cell penetrating peptide (CPP) to form self-assembling nanocomplexes with AMO via electrostatic interactions, enabling us to efficiently deliver AMOs into hPSCs.

RESULTS AND DISCUSSION

Nanocomplexes were prepared with monomeric or dimeric CPPs (Figure 1A). Dependent on the charge ratio between CPP and AMO, nanocomplexes of approximately 20 – 40 nm size and zeta potentials of approximately 5 – 20 mV were formed, as determined with DLS. Nanocomplexes formed with monomeric (NC-1) and dimeric CPP (NC-2) were structurally different due to the attainment of a different surface charge.

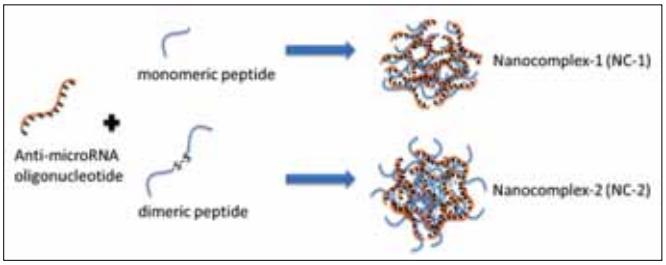

Figure 1: Negatively charged AMO and positively charged CPPs self-assemble to NC-1 and NC-2 nanocomplexes.

NC-2 at charge ratio 10:1 showed about 130-fold higher uptake by hPSCs compared to NC-1 nanocomplexes with the same charge ratio (Fig.2). Remarkably, NC-2 showed significantly higher uptake in hPSCs compared to pancreatic tumor cells (Panc-1, AsPC and MiaPaCa) and human fibroblasts (Fig. 2). To explore the mechanism of uptake we performed heparin competition studies which revealed 97% reduction in the uptake of NC-2 by PSCs upon adding heparin. We demonstrated that the induced uptake of NC-2 by hPSCs is likely due to their interaction with heparin sulfate proteoglycan (HSPG) as receptors for intracellular uptake.

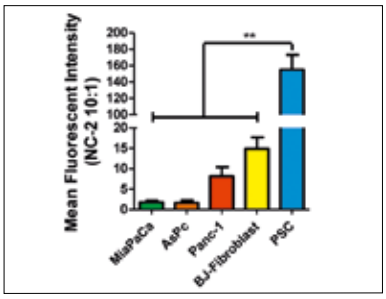

Figure 2: Flow cytometric analysis of NC-2 (10:1) transfection efficiency in PSCs, BJ-Fibroblasts, Panc-1, AsPc and MiaPaCa cells.

Interestingly, both inhibitors for macropinocytosis (EIPA) and receptor mediated endocytosis (phenylarsine oxide) significantly inhibited NC-2 uptake in PSCs (~ 70%). Taken together these findings indicate that the preferential uptake of NC-2 into PSCs is at least partly based on interactions of NC-2 and HSPGs present on transmembrane receptors of hPSCs.

Furthermore, to show the effectiveness of the delivery system, we prepared nanocomplexes with anti-miRNA-199a and delivered them into hPSCs using NC-2 nanocomplexes. Thereby we could significantly inhibit the mRNA and protein expression of hPSC activation markers α-SMA, Col1a1 and PDGFRβ.

CONCLUSION

This study demonstrates that anti-miRNA oligonucleotides can be effectively delivered to human pancreatic stellate cells using our novel peptide based nanocomplexes via a combination of macropinocytosis and receptor mediated endocytosis. Furthermore, we demonstrate that delivery of anti-miRNA-199a using NC-2 nanocomplexes inhibits pancreatic stellate cell activation, a process which is crucial for their tumorigenic activity. Altogether, our self-assembling nanocomplexes can be a valuable tool for intracellular delivery of miRNA into primary hPSCs and potentially be applicable for developing novel miRNA-based therapeutics.

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MASS-BALANCE IN DOXIL-LIKE PRODUCTS BASED ON STATE OF THE ART ANALYTICAL TOOLS:

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STOICHIOMETRY AND MECHANISTIC APPROACH

Doxil-like liposomal doxorubicin products are prepared based on the "active loading" process which involves trans-membrane counter-transport of a number of ionizable species, followed by the drug crystal formation inside the liposome (Fig.1).

Efficiency of the loading, and ultimately, the stability of the structure thus formed, are highly dependent on the careful control of correspondent stoichiometric relationships.

In this work, we attempt to demonstrate full mass-balance accountability of the drug, lipids and auxiliary molecules, based on a combination of analytical techniques, including quantitative Cryoscopic Transmission Electron Microscopy (CryoTEM), Small Angle XRay Scattering (SAXS), nano-particle tracking analysis, regular and ion-chromatography and others.

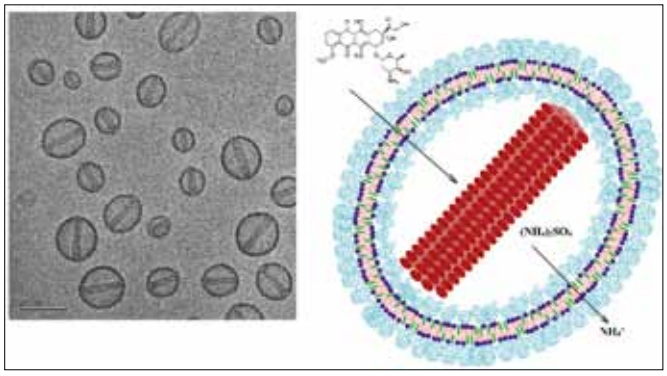

Fig.1. CryoTEM image (left) and a structure of Pegylated Liposome with a crystal of Doxorubicin sulfate (PLD, right) inside based on XRD dataii

On one hand, intraliposomal volume and the total trapped volume are determined via either size and liposome count measurements or via assaying the intraliposomal sulfate content. These parameters may be also confirmed by determination of the lipids membrane volume.

On the other hand, combination of known trapped volume with measurements of intraliposomal concentrations of doxorubicin and ammonium ions, proves close to stoichiometric counter-exchange of the two latter during the loading process.

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CONJUGATES OF PAMAM DENDRIMER, TAXANES AND MONOCLONAL ANTIBODY - A WAY TO INCREASE EFFECTIVENESS IN BREAST CANCER THERAPY

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Breast cancer is the most frequently occurring cancer in women. It has been confirmed that approximately 30% of patients have overexpression of human epidermal growth factor 2 (HER2) on the surface of tumor cells 1. ERBB2 gene encoding the HER2 protein is a proto-oncogene which multiplication (amplification) is a cause of HER2 protein overexpression in tumor cells. Figure 1 shows differences between normal and HER2-overexpressing cells.

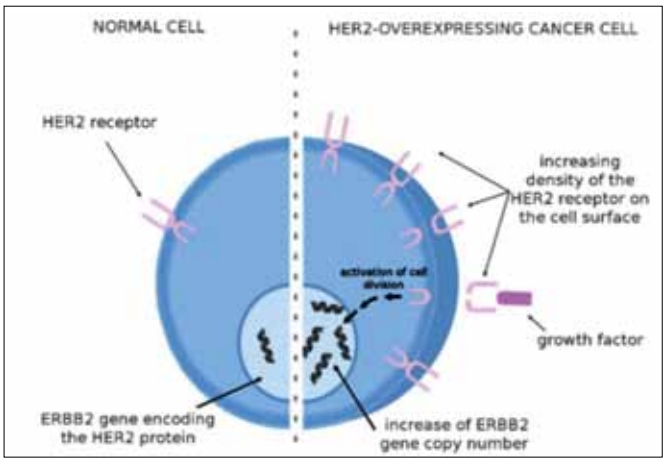

Figure 1. The mechanism of action and overexpression of HER2 in a cell.

Trastuzumab – a recombinant, humanized monoclonal antibody (IgG1 selectively connected with EGFR2) – is directed against HER2 receptor. Trastuzumab blocks the receptor by binding to domain IV of an out-of-cell part of the protein HER2 and inhibits the excessive proliferation of tumor cells. The inhibition of cell proliferation is probably a result of a cell cycle arrest in G1 phase 2,3. Trastuzumab used together with traditional chemotherapy (i.e. with anthracyclines or taxanes) causes an increase of therapy efficiency. However, the systemic toxicity of the anticancer drugs is still a serious problem. Therefore, new solutions are sought, especially in the field of selective drug transporting to tumor cells. Dendrimers are very good candidates to play this role.

Dendrimers are one of the best-known group of monodispersive nanoparticles with a regular and highly branched three-dimensional architecture and a strictly predictable molecular weight. Many dendrimers can easily penetrate the cell membrane and increase cellular uptake of drugs complexed or conjugated with them. Therefore, we decided to use dendrimers not only as carriers of anticancer drugs but also as a connecting link between the monoclonal antibody trastuzumab and the different anti-cancer drugs. We believe that the synthesis of immunoconjugates trastuzumab-dendrimer-drug has some fundamental, but decidedly important advantages. First, the drug is protected against premature release in the circulatory system and too rapid transformation in the liver, therefore, it provides a possibility of dose reduction while maintaining a therapeutic effect 4. Secondly, the direct release of active substance in the tumor environment allows to avoid the toxicity to normal cells, especially the cardiotoxicity and nephrotoxicity occurring after the administration of taxanes used in conventional therapy. Application of the dendrimer conjugate also allows to avoid the use of toxic solvents and antihistamines administered during treatment with this group of drugs 5-7. Moreover, the presence of trastuzumab on the surface of the dendrimer-drug conjugate might allow for efficient transport of the active substance direct to the cells overexpressing HER2.

SYNTHESIS AND CHARACTERIZATION

The synthesis of PAMAM-NH2 dendrimers with 1,4-diaminobutane core with drugs and/or with the monoclonal antibody was carried out according to the scheme presented in Figure 2 and stoichiometry[(1)] PAMAM-trastuzumab (1:1);[(2)] PAMAM-docetaxel-trastuzumab (1:2:1);[(3)] PAMAM-paclitaxel-trastuzumab (1:2:1). Then the compounds were characterized with use of mass spectroscopy (ESI-MALDI-TOF), NMR, FTIR, HPLC.

In order to obtain the conjugate of the dendrimer with taxanes (paclitaxel, docetaxel) a multi-stage reaction was performed (Figure 2). Modification of a hydroxyl group on the taxane molecules surface by succinic acid was the first step. Secondly, the surface of the dendrimer was linked to the drug by amide bond. In further reaction steps, the monoclonal antibody was covalently bound to the surface of the dendrimer. In order to obtain the trastuzumab-PAMAM conjugate, specially selected from Sigma-Aldrich base, linker molecule was used. The linker has on its ends functional groups which react both with amines and with thiols. In order to have the reaction process going fluently, it is very important to introduce earlier -SH groups on the surface of the dendrimer. Traut's reagent was used in the reaction. Addition of monoclonal antibody, previously coupled to the linker was the final step in PAMAM dendrimer modification.

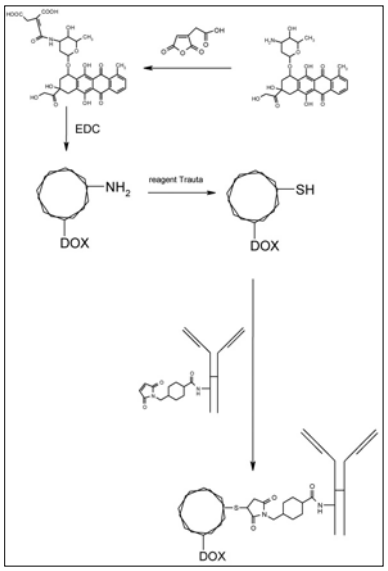

Figure 2. The synthesis of PAMAM-drug dendrimer conjugates with monoclonal antibody.

CYTOTOXICITY STUDIES

A panel of human tumor derived cell lines representing the most common types of breast cancers was obtained from a cell bank source: MCF-7 (breast cancer cells) and SKBR-3A (breast cancer cells overexpressing HER2). Cellular toxicity was assayed using MTT assay (mitochondrial enzyme assay) to determine and compare IC50 concentrations in both cell lines.

A preliminary results of PAMAM-trastuzumab conjugates cytotoxicity towards chosen breast cancer cell lines indicated that the conjugates of dendrimers with the monoclonal antibody enhanced its toxic effect in comparison to free antibody (Table 1). The effectiveness of dendrimer with conjugated monoclonal antibody was confirmed by Miyano et al. They used PAMAM dendrimers generation six modified on the surface with lysine and glutamic acid (KG6E) and with attached trastuzumab monoclonal antibody. Studies with this conjugate were carried out also on the two human breast cancer cell lines: SKBR-3A (HER2 positive) and MCF-7 (HER2 negative). The results proved the effectiveness of selectively penetration of the conjugate to cells overexpressing the HER2 8. In breast cancer treatment, trastuzumab is administered with taxanes: paclitaxel or docetaxel 9. Unfortunately, the IC50 values have not confirmed synergistic effects of drugs and antibodies administration, but only an additive effect on the tested cell lines MCF-7 and SK-BR3 4. Therefore, our results obtained for conjugates PAMAM-drug-trastuzumab are so unique because they show an increase in the toxic efficiency towards to cancer cells, several or several dozen times, compared to the free drug or the conjugate PAMAM-trastuzumab. Moreover, the use of a combination of trastuzumab, dendrimer and drug allows to obtain the effect of the targeted therapy and to provide direct transport of the drug to the tumor cells without previously used toxic solvents. The conjugates are interesting proposition which might lead to improvements in the effectiveness of the therapy of the most common cancer in women, However, their application in the future in anticancer therapy requires still further testing.

Table 1. Comparison of IC50 values for MCF-7 and SKBR-3A cell lines.

IC_{50} µM/L	MCF-7 24h	MCF-7 48h	SKBR-3A 24h	SKBR-3A 48h
Trastuzumab	>100	>100	>100	>100
PAMAM·Trast	32,46 ± 4,47	11,92 ± 4,08	4,29 ± 0,06	0,41 ± 0,06
Docetaxel (DOC)	23,76 ± 4,81	9,19 ± 3,36	10,75 ± 1,50	2,00 ± 0,44
PAMAM·DOC·Trast	>100	48,85 ± 4,82	2,03 ± 0,07	0,004 ± 0,002
Paclitaxel (PTX)	7,82 ± 0,18	2,24 ± 0,33	7,31 ± 1,54	0,49 ± 0,13
PAMAM·PTX·Trast	0,585 ± 0,18	0,09 ± 0,01	0,72 ± 0,21	0,002 ± 0,001

ACKNOWLEDGEMENTS

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body - a new opening in target tumor therapy" UMO-2015/19/N/NZ3/02942).

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POLYMERIC MICELLE-LIKE NANOCARRIERS BASED ON THE CONJUGATION OF AMPHIPHILIC DIBLOCKS TO THE SURFACE OF A MULTIFUNCTIONAL NANODIAMOND ANCHOR

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The poor aqueous solubility and the physicochemical stability of many drugs is one of the most challenging issues in pharmaceutical development. Polymeric micelles (PMs) are largely used for increasing the solubility of drugs and more recently to improve their oral bioavailability(1). However, a main challenge is preventing the disassembly of PMs under extreme dilution in the body fluids, which leads to uncontrolled release of the encapsulated cargo. We are investigating a new amphiphile architecture, namely core-anchored PMs, to improve the physical stability and performance of these nanocarriers. The rationale relies on the conjugation of amphiphilic copolymers to the surface of multifunctional organic or inorganic particulate and molecular anchors to form corona-core nanomaterials that due to covalent bonding do not disassemble upon dilution. In this conceptual work, carboxylated nanodiamonds (cNDs) were used as molecular anchor for the conjugation of amphipathic diblock oligomeric chains made of oligo(epsilon-caprolactone) (oligoCL) and poly(ethylene glycol) monomethyl ether (mPEG) as hydrophobic core and hydrophilic corona, respectively (mPEG-oligoCL). Complementary analysis by ^{1}H-NMR, FTIR, DSC and TGA confirmed the conjugation of amphiphiles to the surface of the cNDs. TEM revealed the presence of a thick polymeric layer that conferred the cNDs a "curly" outlook (Figure 1).

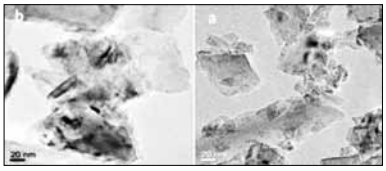

Figure 1. TEM micrograph of mPEG-oligoCL-modified cND (a) and cNDs (b). Scale bar = 20 nm. Dynamic light scattering (DLS) and nanoparticle tracking analysis were used to test the size, size distribution, zeta-potential and the stability of the cNDs before and after the conjugation stage (Figure 2). Conjugation increased the hydrodynamic diameter of the particles. Then, the cytotoxicity was evaluated in Caco2 cell monolayers, an in vitro model of the intestinal epithelium.

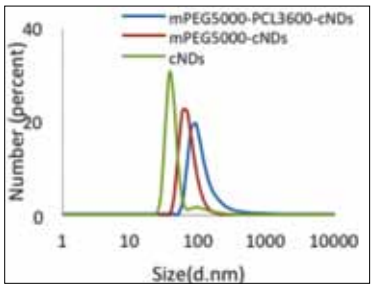

Figure 2. Size and size distribution of pristine cNDs and their counterparts modified with mPEG and a mPEG-oligoCL diblock. The conjugation of the copolymers increased the compatibility with respect to the pristine cNDs. Finally, the encapsulation of the antihelmintic drug nitazoxanide approved for the treatment of parasitosis of the gastrointestinal tract was studied. Overall, preliminary results highlight the potential of this novel approach to extend the applicability of PMs in drug delivery.

ACKNOWLEDGEMENTS

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MECHANO-SENSITIVE LIPOSOME CONTAINING HYDROGELS: TOWARDS AN INTRA ARTICULAR DRUG DELIVERY SYSTEM

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INTRODUCTION

Osteoarthritis is a degenerative disease that affects the synovial joint, causing chronic joint pain. Current treatment range from physical therapy to complete joint replacement with parenteral and oral administration of pain killer being the main tools to manage the pain.[1] Here, we propose an in situ crosslinking hydrogel containing liposomes able to release pain killer inside the joint thus reducing the side effect. Periodate oxidized dextran makes a Schiff base formation reaction with both an amine bearing liposome (i.e. containing the aminolipid DPPE) and polyethyleneimine (PEI) to create a polymer network that contains covalently bound liposome. The force applied by the joint on the hydrogel deforms the polymer network disrupting the bound liposome thus making them release their cargo (fig. 1).

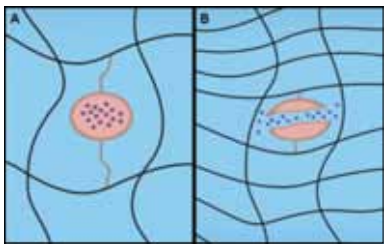

Figure 1 Schematic representation of the behavior of covalently bound liposomes inside the hydrogel network, (A) in the relaxed state the liposomes are intact, (B) upon compression the liposomes break and deliver their payload.

RESULTS

Preliminary results for DextCHO_5 (5% of oxidized glucose units) show a significant difference in release between liposomes formed from non-reactive DPPC and liposomes containing 20 mol% of the reactive lipid DPPE. Non-reactive liposome are not influenced by the stress put on the hydrogel in contrast to the amine containing liposomes which are disrupted and release the encapsulated sulfo-rhodamine B.

DISCUSSION AND CONCLUSION

The preliminary results show a working concept. Further research

will focus on the factors that influence the release of the encapsulated cargo under conditions found in synovial joints.

ACKNOWLEDGEMENTS

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PREPARATION AND CHARACTERIZATION OF WOUND DRESSING USING SURFACE MODIFIED CHITOSAN NANOFIBERS BY ARGININE

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INTRODUCTION

Wound healing is a complex process including different stages. A suitable dressing could protect the injury and contributes to the recovery of damaged tissues. In comparison with conventional wound dressings, nanofiber-based wound dressings have different advantages such as haemostasis induction, good absorption of wound exudates and facilitation of cell growth due to their nanofibrous structure. In addition of using suitable wound dressing, the use of wound healing agents such as growth factors could improve the wound healing process rate[1]. Nitric oxide (NO) and its precursors such as arginine are promising wound-healing agents that could regulate collagen formation and wound contraction. Delivery of wound healing agents in controlled release manner could promote wound healing process efficiently. Among different polymer, chitosan and its derivatives are widely used in the preparation of hydrocolloid wound dressings[2]. In current study we prepared surface modified chitosan nanofibers by arginine as an effective product with easy application for the treatment of wounds.

METHODS

Chitosan nanofiber gel (1.5%) was prepared by chemical and mechanical method. 200 mg arginine was added to 1 mL of alginate sodium solution (10%) and was stirred for 4 h until arginine was conjugated to alginate by electrostatic interaction. The prepared arginine-alginate complex was added to 1 g of chitosan nanofiber gel and was stirred for 24 h until the arginine-alginate complex was conjugated to chitosan nanofiber by electrostatic interaction. The percent of attached arginine to chitosan nanofibers was determined. The effect of pH on the amount of attached arginine was evaluated in three different pH; 5, 6 and 7. The viscosity of surface modified chitosan nanofibers by arginine were evaluated. To determine the spreadability of formulation, 0.5 g of surface modified nanofibers gels was placed within a circle of 1 cm diameter pre-marked on a glass plate of 20 × 20 cm, over which a second glass plate was placed. A weight of 500 g was allowed to rest on the upper glass plate for 5 min. The increase in the diameter due to gel spreading was noted. The release of arginine from surface modified chitosan nanofibers was evaluated for 24 h at 32 ° C at phosphate buffer pH 7. The wound healing effect of prepared surface modified chitosan nanofibers was evaluated *in vivo* by wound excision method on Wistar rats (275 ± 25 g) in comparison with mixture of non-modified chitosan nanofibers and alginate, arginine solution and normal saline solution for 9 days. Skin tissue (1.5 cm × 2.5 cm) was surgically removed using sterile surgical tools to create full thickness wound on the back of the animal. Materials was locally applied to the full thickness skin wounds, from day 0, once a day for 9 days. The images of wounds were captured using a digital camera. The images of wounds were analyzed for the wound area using Photoshop (versus CS5) software (day 0, 3 and 9).

RESULTS

The results showed that arginine could sufficiently conjugated to chitosan nanofibers by electrostatic interaction. 90.16±2.1%, 97±1.2% and 95.94±1.7% of arginine could attached to chitosan nanofibers in pH 5, 6 and 7. The viscosity of gel was reported to be significantly decreased by decreasing of the pH of the gels. The spreadability of the surface modified chitosan nanofibers was 8.3±0.5, 7.9±0.4 and 7.1±0.3 for surface modified chitosan nanofibers with pH 5, 6 and 7, which was proved the ease of applicability of gels on skin. Moreover about 83.95±2.5%, 81.62±2.1 and 76.2±3.4 of arginine was released from surface modified chitosan nanofibers with pH 5, 6 and 7 after 24 h in a controlled release manner (Figure 1).

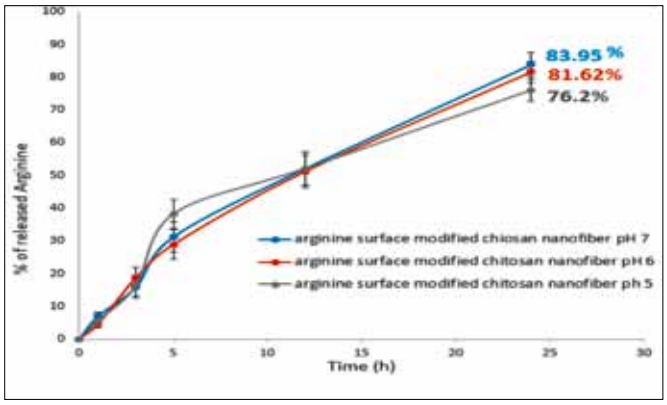

Figure 1. Effect of pH of chitosan nanofiber on the % of released arginine from chitosan nanofibers

In vivo wound healing effect of surface modified chitosan nanofiber was performed using arginine–alginate pH 7. The results clearly substantiate the beneficial effects of the topical application of surface modified chitosan nanofiber with pH 7 containing arginine in the acceleration of healthy wound healing process with less scarring in comparison with non-modified chitosan nanofibers and alginate, arginine solution and normal saline solution (Figure 2).

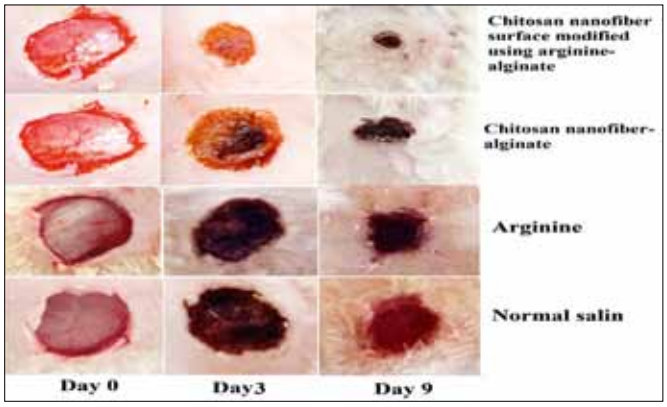

Figure 2. In vivo wound healing effect of surface modified chitosan nanofiber, non-modified chitosan nanofibers and alginate, arginine solution and normal saline solution

CONCLUSION

Nanofibers from natural polymers such as chitosan are promising materials for preparation of wound dressing. These results suggest that this arginine-releasing nanofibers have the potential to use as a novel topical wound-healing therapy in acute wounds.

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A NANOTOXICOLOGY EVALUATION OF NANO-HYDROXYAPATITE-CHITOSAN CONTAINING SI AND AG, AS AN ANTIBACTERIAL BIOMATERIAL SUBSTITUTE FOR BONE REGENERATION

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The restoration of defective bone tissue and complications related to surgery and fracture site infection are major concerns in orthopedic surgeries. However, it is crucial to develop osteoconductive and bacteriostatic composites. The aim of this work was to elucidate the effect of size, surface roughness and chemical structure of mentioned nanocomposites on cytotoxicity and bacteriostatic activity via human osteoblast cells and Escherichia Coli, respectively. Particle size, surface roughness, ROS production and bioactivity of nanocomposites were investigated by XRD, AFM, DPPH assay and SEM/UV-Visible spectrophotometer, respectively. Bacterial colony counting test, MTT assay and LDH release were performed as bacteriostatic and biocompatibility tests. The results showed that CT/n-HAp/Ag with smaller particle size (10±0.09 nm) than CT/n-HAp/Si (18±0.14 nm) exhibits higher cell viability and bacteriostatic activity, and lesser LDH release from cell plasma membrane. Integration of Ag into the nanocomposite hindered the release of Ag+ ions and restricts cytotoxic potential on cells. Higher cytotoxic effect of CT/n-HAp/Si might be related to proton concentration derived from nanocomposite and its chemical structure. In conclusion, the strong bone regeneration potential of CT/n-HAp and good biocompatibility and bacteriostatic activity of CT/n-HAp/Ag makes it as potential bacteriostatic bone filler in site of infected bone fracture.

INTRODUCTION

The restoration of defective bone tissue and complications related to surgery and fracture site infection are major concerns in orthopedic surgeries. It is crucial to develop osteoconductive and bacteriostatic composites. The aim of this study was to investigate the effect of size, surface roughness and chemical structure of nano hydroxyapatite/Chitosan (nHA/CT) composites containing of Ag and Si on cytotoxicity and bacteriostatic activity via human osteoblast cells and Escherichia Coli, respectively.

METHODS

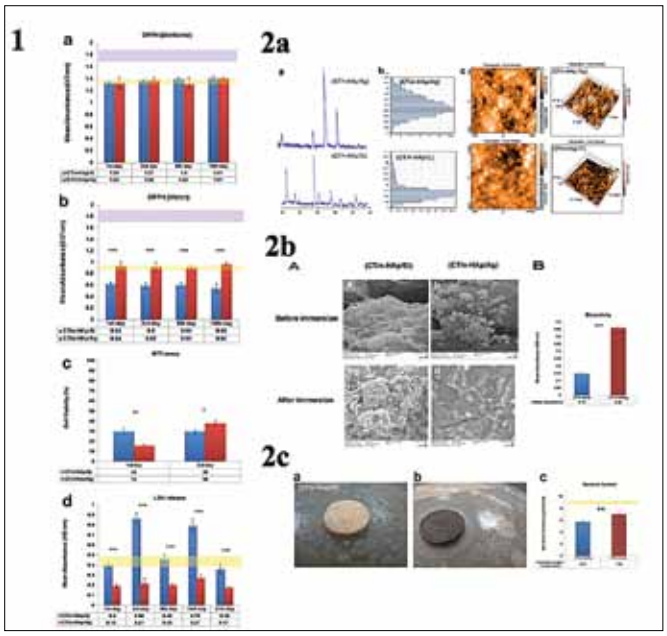
The nHA/CT/Si and nHA/CT/Ag tri-component nanocomposites were prepared by an in-situ hybridization method. Particle size, surface roughness, ROS production and bioactivity of nanocomposites were investigated by XRD, AFM, DPPH assay and SEM/UV-Visible spectrophotometer, respectively. Bacterial colony counting test using E. coli, MTT assay and LDH release using human osteoblast cells were performed as bacteriostatic and biocompatibility tests.

RESULTS

The mean crystallite size was calculated using Scherer's equation in which the approximate crystallite sizes of synthesized samples were about 8.6 and 7.2 nm for nHA/CT/Si and nHA/CT/Ag, respectively (Fig. 2a). The results revealed that the nanoparticles consisting of Ag had higher surface roughness and exhibited less bioactivity than surfaces containing Si. nHA/CT/Ag and nHA/CT/Si exhibited no ROS production (Fig. 1a) and nHA/CT/Si produced significantly higher acidic values compared to nHA/CT/Ag (Fig. 1b). nHA/CT/Ag with smaller particle size (10±0.09 nm) than nHA/CT/Si (18±0.14 nm) (Fig. 2b and c) showed higher cell viability (Fig. 1c) and bacteriostatic activity (Fig. 3), and lower LDH release from cell plasma membrane (Fig. 1d).

Fig.1 a) Measurement of ROS production b) Measurement of H concentration c) Viability of cells treated by two nanocomposites. d) LDH measurement of supernatant of cells treated by two nanaocomposites.

Fig.2 a) Crystal mean size and surface roughness of two nanocomposite. b) Bioactivity analysis of two nanocomposites. c) Antibacterial efficacy of two nanocomposites.



CONCLUSION

Integration of Ag into the nanocomposite hindered the release of Ag+ ions and restricted the cellular cytotoxic effects. Higher cytotoxic effect of nHA/CT/Si might be related to proton concentration derived from nanocomposite and its chemical structure. In conclusion, the strong bone regeneration potential of nHA/CT and good biocompatibility and the integrated bacteriostatic activity of CT/n-HAp/Ag make it a potential bone filler in infected sites of bone fracture.

ACKNOWLEDGMENT

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SIZE AND ZETA POTENTIAL OF SILVER NANOPARTICLES INFLUENCE CHROMATIN STRUCTURES OF HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS

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Recently, it has been disclosed that silver nanoparticles (AgNPs) has potential to inhibit infection and cancerous cells and eventually penetrate from injected site into the capillary due to their small size. This study focuses on the effect of size and zeta potential of bare and citrate-coated AgNPs on human umbilical vein endothelial

cells (HUVECs) as main capillary cells. AgNPs with high and low concentrations and no citrate coating were synthesized by using simple wet chemical method and named as AgNP/HC, AgNP/LC and AgNP, respectively. Citrate coated particles showed larger zeta potential of -22mV and AgNp/HC showed the smallest size of 13.2nm. UV-Visible spectroscopy and DLS were performed to evaluate particle size and hydrodynamic diameter of NPs in water and cell culture media. Results indicated that higher concentrations of citrate decreased hydrodynamic diameter and NP agglomeration. ROS production of all AgNPs was similar at 28ppm although it was significantly higher than control group. Their effects on cell membrane and chromosomal structure were studied using LDH measurement and DAPI staining, as well. Results demonstrated that AgNP/LC was less toxic to cells owing to higher value of IC50, MIC and less release of LDH. Cancerous (Human Caucasian neuroblastoma) and immortal cells (Mouse embryonic fibroblast cell line) were about twice more sensitive than HUVECs to toxic effects of AgNPs. DAPI staining results showed that AgNP and AgNP/HC induced highest and lowest breaking of chromosome. Overall results suggest that viability of HUVECs will be higher than 90% when viability of cancerous cells is 50% in AgNPs chemotherapy.

Keywords: Silver nanoparticle; Particle size; Zeta potential

INTRODUCTION

Cancer and infection are two major problems in the healthcare. Recently, silver nanoparticles (AgNP) have been widely used due to their antibacterial properties. Although, use of AgNPs is expected to continue, antibacterial resistance to AgNP via plasmid gene of pMG101 is suggested. Increase of cytosolic calcium, impairment of respiratory chain in mitochondria and afterward disturbance in synthesis of ATP, breaking of DNA, protein carbonilation and lipidic membrane peroxidation are likely to be the effective mechanisms of bacteriocidal. Size provides important control over many of the physical and chemical properties of nanoscale materials and their effect on biological behavior. NPs with smaller particle size and higher surface/volume ratio can enter easier into the cells and mitochondria. Higher surface/volumes ratio results more active sites on their surface to interact with cell's organelles or biomolecules [1]. Zeta potential of the nanoparticles is another critical factor in determining their effect on biological behavior and can lead to different cell responses as compared to particle size. Zeta potential is electrical charge on the surface of material surrounded in the medium. Higher Zeta potential results in higher stability and assimilation to the body because substances with high zeta potential do not tend to adhere or clump to each other. In the present work, we aimed to investigate the effect of particle size, Zeta potential and ROS production of 3 different AgNPs on cell viability, bacteriocidal efficacy, chromosomal structure and glutathione's conformation.

MATERIALS AND METHOD

AgNPs were synthesized using a simple wet chemical method by chemical reduction of Ag+ ions in water, in presence and absence of tri sodium citrate as stabilizer. Particle size, Zeta potential and ROS production were studied using UV-Visible spectroscopy, DLS and DPPH assay. Cell viability of HUVEC and bacteriostatic activity of NPs against resistant E.Coli and S. Aeurogenosa were measured by MTT assay and micro dilution methods, respectively. For investigation of effect of NPs on chromatin nucleus and on secondary structure of Glutathione, DAPI staining and Circular dichroism were done.

RESULTS AND DISCUSSION

Particle size and Zeta potential data showed that NPs with different concentrations of citrate had approximately equal Zeta potential (-23 and -21 mV) however, AgNP/HC had smaller particle size than AgNP/LC and AgNP/LC was in the particle size range of bare AgNP (17.2 and 17.8 nm). Zeta potential of AgNP was significantly lower than coated NPs (-9 mV). It might be said that citrate coating can slow down transformation and result in decrease of agglomeration and formation of more stable particles as compared to poor citrate particles.

Results of DPPH assay showed that three AgNPs induced non-significant free radical values as compared to each other at 2 ppm. However AgNP induced higher free radical as compared to control group. At 28 ppm, there was a significant increase of free radical of coated and bare particles as compared to control group but this difference was not significant between AgNP, AgNP/HC and AgNP/LC at 28 ppm. At 2 ppm concentrations, AgNP induced more ROS. This might be related to the poor coating on this NP. It is demonstrated that charge of NPs might be influence ROS production. Positively and neutrally charged NPs can induce more intracellular ROS production than negatively charged ones [3].

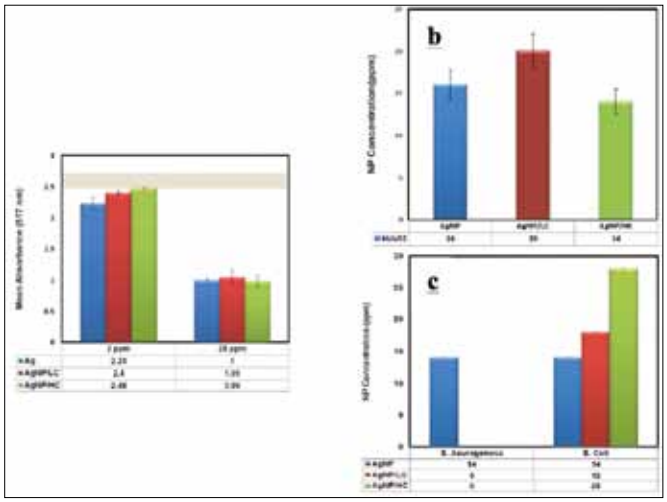
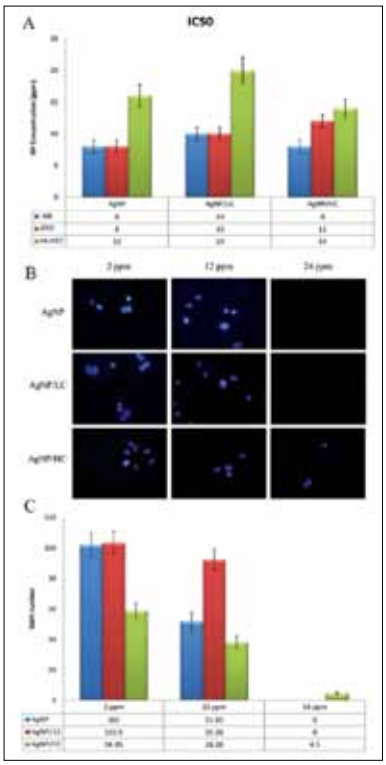


Fig. 1 a) ROS production of NPs measured by DPPH assay. Results showed that at high concentration there is not significant fiffrences between ROS production (P ≥ 0.5): b) IC50 results of HUVEC treated by coated and bare NPs. AgNP/LC induced significantly higher cell viability than AgNP/HC (P ≤ 0.5). c) MIC results of S. Aeurogenosa and E.Coli treated by coated and bare NPs.Results showed that bare AgNP had higher bacteriostatic activity than coated AgNPs.

Although, cell viability of HUVEC treated by AgNP/LC was higher than others, bacteriostatic activity of AgNP was stronger than coated NPs. These effects might be related to potential of ROS production of NPs and eventulally NPs with lower negative charge damaged morphology of mitochondria while higher negatively charged ones did not. Net charge of mitochondrial outer membrane is negative and NPs with lower negative charge such as AgNP can get adsorbed easier onto membrane than higher negative charged particles such as AgNP/LC and thus disruptive effect on mitochondria membrane decreases. Earlier studies showed that AgNPs interact with thiol groups in inner mitochondria membrane and mitochondria dysfunction is an important mechanism towards apoptosis [2]. DAPI staining was done to evaluate apoptotic cells via lighter blue chromatin in nucleus at 2, 12 and 24 ppm concentrations of AgNPs. Results from DAPI staining showed that AgNP and AgNP/LC induced apoptotic cells with lighter blue chromatin staining and AgNP/HC had rarely influenced chromatin even at high concentration. Nuclear membrane pore (NMP) consists of nuclear pore complexes (NPCs). The charge near the pore's wall and the pore's center are negative and positive, respectively. Owing to higher negative charge on the pore's wall it might be speculated that negative particles (AgNP/LC and AgNP/HC) are repelled from the pore's wall as compared to weaker negative charged particle (AgNP). Even if negatively charged AgNP/HC can enter into nucleus, negatively charged DNA tends to interact with positively charged materials and its influence on DNA will be not significant.

This indicated that however, particles at this concentration did not alter cell viability but they can affect biomolecules and use of these materials chronically will show adverse side effects even at concentrations that cell viability is 100 %.

Fig. 2 a) Maximum half inhibitory concentration (IC50) of three different NPs with Human Caucasian neuroblastoma (BE (2)M17) (NB) and Mouse embryonic fibroblast cell line (STO) as immortal cells and HUVEC. There was significant difference between IC50 values of AgNP/



HC and AgNP/LC (P< 0.05). b) DAPI staining of HUVEC at 2, 12 and 24 ppm concentrations of AgNPs. AgNP/HC exhibited less light blue chromatin and damage to nucleus as compared to others. C) Nucleus numbers of of HUVECs at 2, 12 and 24 ppm concentrations of AgNPs. Cells treated by AgNP/LC exhibited higher nucleus number as compared to others. At 2 ppm, the P value was 0.0001, considered extremely significant. There was not significant difference between Bare and AgNP/LC. At 12 ppm, P value was 0.0017, considered very significant. There was no significant difference between Bare and AgNP/HC.

CONCLUSIONS

Overall, it could be suggested that variation of particle size and Zeta potential affect cell organelles and biomolecules. Also, biomolecules are undergone structural and chemical changes at the concentration that cell membrane and mitochondrial function is normal. It is notable that vein endothelia cells are more resistant to the lethal concentrations of AgNPs on above mentioned cancer and immortal cells. Although, it needs more investigation to prove our assumption but the present study disclosed that eventually vein endothelial cells as a cell candidate of capillary will tolerate AgNPs with higher IC50 concentrations of immortal cells in cancer chemotherapy.

ACKNOWLEDGMENT

This work was supported by grant from Student's Scientific Research Center, Tehran University of Medical Sciences, Iran.

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GENERATION AND EVALUATION OF MULTIDRUG RESISTANT, METASTATIC AND IMAGEABLE IRFP-EXPRESSING 4T1 BREAST CARCINOMA XENOGRAFTS

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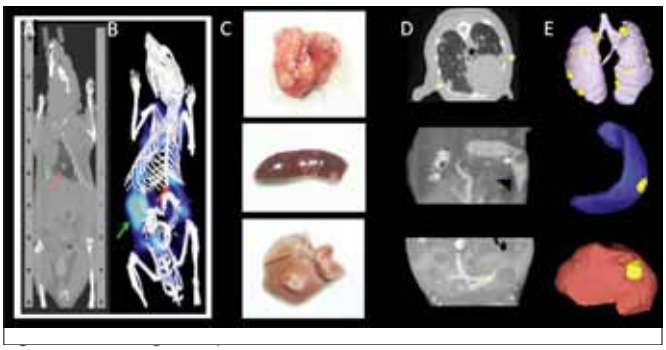
INTRODUCTION:

Multidrug resistance (MDR) is a major limiting event against chemotherapy in cancer treatment. Murine 4T1 breast carcinoma cells are capable of inducing metastatic lesions in several organs, such as lungs, liver, bone and brain. First, we developed an optically imageable metastatic tumor model (4T1/iRFP) to be able to track it in both the primary tumor and metastatic lesions non-invasively in mice. Then, by exposing this parental cell line to low doses of doxorubicin for prolonged periods of time, we developed multidrug-resistant 4T1/iRFP cells, to extend the evaluation of metastases also in multidrug resistant tumor types, for futher studies.

Methods: iRFP-expressing 4T1 cells were orthotopically implanted into mice. Primary tumors and metastatic lesions were monitored using *in vivo* and ex vivo optical imaging (Rizzo et al, in prep). Multidrug resistant 4T1/iRFP cells were developed by stepwise selection using doxorubicin dose increments. In order to indicate differences in cytotoxic response between MDR cells and their more sensitive parental cells, XTT cytotoxicity experiments as well as immunohistochemistry stainings (for prototypic P-gp efflux proteins) were performed.



fluorescent protein (iRFP). Panel (A) shows a CT image, confirming a metastasis in the lung (red arrow). Panel (B) shows the corresponding CT-FMT image, in which both the metastasis (red arrow) and the primary tumor (green arrow) can be identified. Panel (C) shows ex vivo images of the metastases in this mouse two weeks later, clearly showing metastatic lesions in the lung (upper), spleen (middle) and liver (bottom). Panel (D) shows CT images for metastatic lesions in the lung (upper), spleen (middle), liver (bottom) and yellow arrows indicate the specific localization of metastatic lesions. Panel (E) shows metastatic lesions, as yellow areas, in CT organ segmentation.

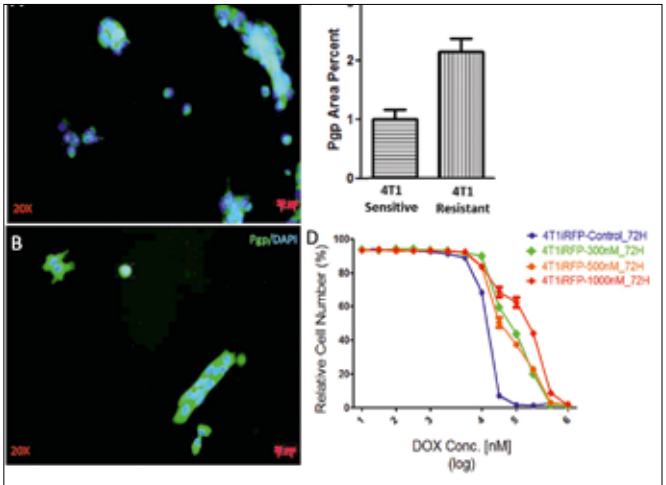


Figure 2: Immunocytochemistry staining for P-gp and cell toxicity in iRFP-expressing 4T1 breast cancer cells. Fluorescence microscopy analyses of P-gp and DAPI staining for doxorubicin sensitive iRFP-expressing 4T1 cells (A) and doxorubicin resistant iRFP-expressing 4T1 cells (B). Graph (C) shows the differences in P-gp area percent in drug sensitive and resistant iRFP-expressing 4T1 cells. Graph (D) shows the XTT cytotoxicity test results after the administration of 14 different doxorubicin doses for 72 hours.

RESULTS:

iRFP-transfected 4T1 cells could be detected *in vivo*, by using computed tomography (CT) and computed tomography-fluorescence molecular tomography (CT-FMT; Figure 1-A, B and D). Ex vivo analyses also demonstrated the metastatic lesions in several organs (Figure 1-C and E). In cell culture, P-gp stainings showed that multidrug resistant cells expressed higher level of P-gp than sensitive parental cells (Figure 2-A-C). According to the results from cytotoxicity test, it was shown that pre-exposed doxorubicin-resistant cells have higher IC50 values (~180 nM) than parental 4T1 cells (~25 nM). These results indicated that doxorubicin resistant 4T1/iRFP cells are approximately 8-fold more resistant against doxorubicin than the sensitive parental cell line (Figure 2-D). Conclusion: Our findings show that 4T1/iRFP cells can be optically visualized in primary tumors and in metastatic lesions in mice. We also provide the evidence that MDR

4T1/iRFP cell are ~8-fold resistant against doxorubicin than their sensitive parental cell line. This increase in drug efflux might be correlated with the induced P-gp level in multidrug-resistant cells. Based on these initial results, using the established 4T1/iRFP model system, we are currently evaluating the impact of MDR on tumor growth, metastasis and treatment responses.

ACKNOWLEDGEMENTS:

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DOXORUBICIN LOADED DUAL PH- AND THERMO-RESPONSIVE MAGNETIC NANOCARRIER FOR COMBINED MAGNETIC HYPERTHERMIA AND TARGETED CONTROLLED DRUG DELIVERY APPLICATIONS

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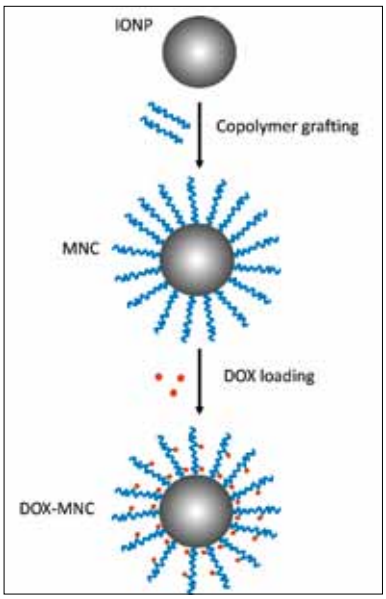

Magnetic nanocarriers have attracted increasing attention for multimodal cancer therapy due to the possibility to deliver heat and drugs locally. The present study reports the development of magnetic nanocomposites (MNCs) made of an iron oxide core and a pH- and thermo-responsive polymer shell, that can be used as both hyperthermic agent and drug carrier.

Fig. 1 Synthesis of the DOX-MNCs.

The conjugation of anti-cancer drug doxorubicin (DOX) to the pH- and thermo-responsive MNCs via acid-cleavable imine linker provides advanced features for the targeted delivery of DOX molecules via the combination of magnetic targeting, and dual pH- and thermo-responsive behaviour which offers spatial and temporal control over the release of DOX. The iron oxide cores exhibit a superparamagnetic behaviour with a saturation magnetization around 70 emu/g. The MNCs contained 8.1 wt% of polymer and exhibit good heating properties in an alternating magnetic field. The drug release experiments confirmed that only a small amount of DOX was released at room temperature and physiological pH, while the highest drug release of 85.2 % was obtained after 48 h at acidic tumour pH under hyperthermia conditions (50 °C). The drug release kinetic followed Korsmeyer-Peppas model and displayed Fickian diffusion mechanism. From the results obtained it can be concluded that this smart magnetic nanocarrier is promising for applications in multi-modal cancer therapy, to target and efficiently deliver heat and drug specifically to the tumour.

COMPARISON OF ENGINEERED NANOSTRUCTURES FOR MULTIMODAL IN VIVO IMAGING

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Multimodality imaging with two or more imaging modalities allows integration of the strengths of individual modalities, while overcoming their limitations. Anatomical imaging technologies, such as MRI, provide unparalleled structural detail; whereas functional modalities such as positron emission tomography (PET) provide insight into morphological and functional behaviors. By incorporating anatomical and functional imaging in a common hybrid imaging platform, a synergism in the imaging capabilities can be achieved, thus making it possible to precisely visualize and delineate structural and functional information. Integrated PET/MRI allows for both spatial and temporal correlation of the signals, differently from using sequentially acquired data. Moreover, the simultaneous acquisition is able to improve the performance and information content of one instrument using the information obtained from the other instrument: the accuracy of the PET estimates might be improved by including the MRI information as the structural framework underlying the distribution of the PET signal. Reciprocally, the strength of PET to provide absolute quantitative information might help validate several MRI techniques *in vivo*. It is important to consider the acquisition times in relation to the bioaccumulation of the contrast agent for MRI and to the half-life of the radiotracers. Even the introduction of nanotechnology has led to the development of many medical applications including the formulation of new nanosystems that can be used for multimodal applications. The resulting nanovectors, capable of diagnosis, drug delivery and monitoring of therapeutic response, are expected to play a significant role in the dawning era of personalized medicine. The use of a theranostic nanovectors gives the possibility to combine different contrast agents or radiopharmaceuticals for diagnostic technologies developed over time with the release of specific drugs or supplements for different types of diseases, tumor lesions, tissue degenerations, neurodegenerative pathologies as Alzheimer`s disease and inflammations. The unusual properties of nanoparticles can be exploited to modify the kinetics of a drug carrier for the transport of hydrophilic and hydrophobic substances, to cross biological barriers as blood brain barrier (BBB) and to detect some diseases as tumor microenvironment or neurodegenerative diseases.

Few results have been presented to obtain biopolymeric nanostructures that can be useful for integrated PET/MRI and even less efforts have been devoted to integration of this two modalities with the optical imaging. Our work aims to synthesize biopolymer nanostructures able to increase long circulating time allowing long time scans, to provide tissue specificity and reduced toxic effects, without any structural modification of the utilized commercial active molecules. In particular in this work will be presented the development of a stable hydrogel nanostructures as multimodal imaging (Optical and integrated PET/MRI) modality for theranostic applications. In particular, the impact of hydrophilic properties of the hydrogel matrix will be exploited when confining Gadolinium based contrast agents and the enhancement of MRI signal and activity of FDG radiotracer will be discussed. Furthermore the release of therapeutic substances in order to have the right therapeutic dose is also evaluated.

Here, we propose some adjustable methodologies to obtain intravascularly-injectable and biocompatible crosslinked polymer nanoparticles. In fact the use of engineered materials and the synthesis through different production approaches allow to obtain

nanostructures characterized by different surface charge, size, shape and texture for different applications. The nanoparticles are differentiated by type of material, for their surface charge, size, shape and method of preparation. The main production methods are Nanoprecipitation, Emulsion-Diffusion, Double Emulsification, Polymer-Coating and Emulsion-Coacervation.
The nanostructures obtained from these processes are different from each other in relation to the type of biomaterial, to the process method used for their production and architecture, such as nanospheres and core shell. In relation to their final application, to the type of substances to encapsulate (hydrophilic and lipophilic) is possible to choose the correct materials and the type of process. The same architecture obtained working the material in different ways provides completely different properties and peculiarities to the nanostructures. Here, we investigate how several nanoparticles characteristics such as hydrophilicity and molecular weight of polymer or crosslinking density and porosity of nanostructures can strongly influence the relaxometric properties, the degradation behavior and the metabolic pathways. The modulation of polymer properties is able to impact on the water dynamics and consequently on the rigidification of contrast agent influencing the relaxometric properties. Through the variation of the crosslinking degree and, therefore, of the water mobility it has been possible to increase the relaxivity of the Contrast Agents, is acting both on the rotational correlation time, TR, and residence life time, TM, the inner-sphere contribution to the relaxivity. The variation of crosslinking degree has the not only the advantage to increase the nanostructures stability in water and to avoid their swelling behavior but, above all, it is essential to control degradation phenomena, the release properties and to allow confinement of the contrast agent. *In vitro* and *in vivo* test have been done on Nps at different concentrations of Contrast Agent after a decoration of the NPs surface with PEG. *In vitro* MRI analysis have been made at 1,5 Tesla to measured T1 and T2 intensity. The results show a reduction of the relaxivity times and so an enhancement of MRI signal of several times respect to the signal obtained with the use of commercial Gd-DTPA. The NPs are tested *in vivo* on C57/BALB mice at increasing concentrations to investigate the variation of the toxicity at short and long term, the biodistribution, the intensity of MRI signal and the presence of an enhancement of the signal itself. It not has been found any toxic effects at short and long period. The biodistribution analysis has been made at different time points for 24h. The animals were anesthetized before the administration of 200 µl of the Contrast Agent-Nps in intraperitoneal and intravenous modes. After administration the blood and tissue samples were collected at predetermined sampling points. Quantitative analysis has been made with the use of ICP-MS. The results show enhancement of the *in vivo* MRI signal of about 10 times.

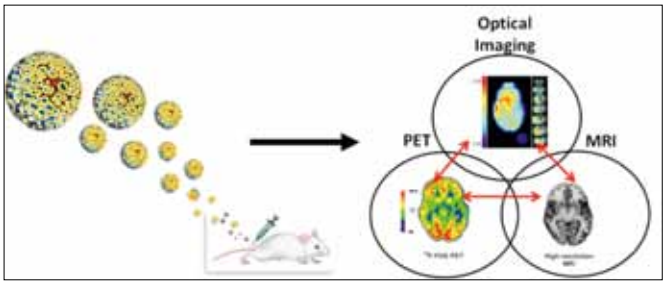

Figure 1. Polymer nanostructures for multimodal in vivo imaging

IMMUNOTOXICITY, IMMUNOGENICITY OF NANO-DRUGS – NANO-CARRIERS; TESTING THE PHENOMENON IN A SENSITIVE PIG MODEL AND DEVELOPMENT OF PREVENTION

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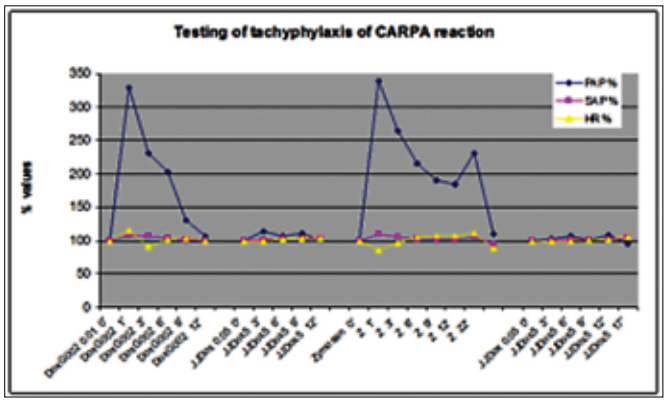
Nanodrugs are promising new therapeutic and diagnostic tools in the medicine, fulfilling the requirements of the modern medicine but besides their advantageous characteristics they may cause immunotoxic and immunogenicity-related side effects.
The immunotoxicity can be manifested in sudden hypersensitivity reaction (HSR), that – albeit rarely – can be even lethal. The symptoms vary from mild skin and respiratory changes to severe cardiopulmonary distress. The second side effect, the immunogenicity may initiate humoral immune response, leading to production of specific IgG, IgM antibodies and decreasing the efficiency of treatment after repeated application.
The immunotoxicity evokes a pseudoallergic reaction that is at least partly caused by complement (C) activation and called C activation-related pseudoallergy (CARPA, Szebeni et al., 1999). The poster presents the acute immunotoxic reactions of different nanodrugs, nanocarriers and presents the sensitive *in vivo* animal pig model to detect this potentially life-threatening side-effect. The pseudoallergic reaction (also called anaphylactoid, idiosyncratic or infusion reaction) resembles the symptoms of Ig-E mediated anaphylactic reactions, but without the presence of immunoglobulin Ig-E. These reactions arise at first application of nanomaterials and may be less severe or completely absent upon repeated exposures. The sensitive animal for this reaction is the pig. Both, domestic pigs and minipigs are equally sensitive for i.v. application of nanomaterials, but the usual laboratory rodent animals are insensitive: e.g. in case of mice or rat has to be applied 2-3 magnitude higher doses to evoke reaction. The pigs are so sensitive like the most sensitive humans. Following the application of the first marketed successful nanomaterial the liposomal Doxorubicine (Caelyx) about 2-4% of patients are reactogenic. If we inject this drug to pigs, 99% is reacting.



Besides the CARPA effect of already mentioned PEGilated Doxorubicin through other liposomal, micellar drugs, polymers, dendrimers and gold nanoparticles will be presented. Some nanomaterials show tachyphylaxis that means that after the first injection the animals show tolerance to subsequent treatments. This provides a possible preventive method to develop a toleration protocol, to avoid the life-threatening reaction. The background of this phenomenon will be addressed.

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CONNECTION BETWEEN COMPLEMENT ACTIVATION AND HEMOSTASIS IN PORCINE CARPA REACTION

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One of the major risk of administering nanomaterials in the blood stream consists of innate immune reaction (complement activation) which may lead to the so called C activation-related pseudoallergy (CARPA) symptom complex. The nanomaterial-induced immune reactions can be associated with cardiovascular and laboratory abnormalities along with secondary activation of the coagulation system resulting in increased risk of thrombosis. Links between complement activation and hemostasis are widely discussed, but detailed role of the molecular components involved in the cross-talk between the two system is less known in porcine model.

The porcine model is highly sensitive to nanomaterial-induced pseudoallergic reactions and predicts well the reactions of hypersensitive humans. In both species, very low nanomaterial doses can trigger immunotoxic reactions, but these occur much more frequently in pigs than in humans (99% versus 2-7 %, respectively), and the risk of false negative results is low. Another advantage of this model lies in the similarity of the CARPA symptoms in both pigs and humans and the reproducibility of the measured endpoints.

The aim of this study was to shed light on the connection between coagulation and complement activation associated with the immunotoxic hypersensitivity in healthy pigs. For this purpose, we tried to evaluate the effect of complement activator Zymosan A positive reference substance used in experiments testing CARPA-genic potential of nanomaterials. We would like to know whether enhanced coagulation observed during nanomaterial-induced CARPA reactions is associated with the complement activation as a secondary effect of the innate immun reaction, or elevation in blood clotting is triggered by other conditions (e.g. by the presence of the nanomaterial as foreign surface in the blood or nanomaterial aggregation).

We measured cardiovascular parameters (pulmonary arterial pressure [PAP], heart rate [HR], and systemic arterial pressure [SAP]), hematological indicators (red blood cell and white blood cell counts, hemoglobin amount, and platelet count), biochemical changes (positive regulator of platelet activation and aggregation, Thromboxane (TxB2) level; terminal complement effector, sC5b-9 level as an indicator of complement activation, and positive regulator of coagulation activation (inhibitor of fibrinolysis), Serpine-E1 (PAI-1) level), as well as activated clotting time (ACT) during Zymosan-A-induced CARPA reactions. Among all hemodynamic symptoms, the rise of PAP is the most prominent and reproducible effect of porcine CARPA. A transient, massive pulmonary hypertension (> 2-fold increase from the baseline) occurs 0.5-2 min after i.v. injection of reactogenic nanomaterials, likely due to the activation of the complement system and the pulmonary intravascular macrophages (PIMs) with the consequent release of vasoactive mediators (TxB2). Hematological changes usually run parallel with the CV reactions. They typically include initial, short lasting leukopenia followed by protracted leukocytosis and thrombocytopenia. The rise of TxB2 (2-20-fold increase) is often reported in porcine CARPA showing a strong correlation with the increase in the PAP within 0.5-2 min post i.v injection of the reactogenic nanomaterials.

We observed typical CARPA reactions in response to bolus injection of Zymosan-A along with initial increase in the TxB2 level and signs of complement activation. However, enhanced coagulation was not observed during the 30-minute-long follow-up period. Increase in the ACT was measured 1-3 minutes postdose indicating temporal dysfunction of the system or consumption of the intrinsic coagulation factors by fast, early clotting activation. These changes occurred parallel with the PAP peak, along with decrease in the PAI-1 level. PAI-1 concentration remained low during the observation period with a small, transient increase 10 minutes postdose (see Figure 1). These findings suggests that initial steps of complement activation may inhibit coagulation for a short period of time (~3 minutes). Presence of other, complement-independent triggering factors in the nanomaterial-induced blood clotting can not be excluded.

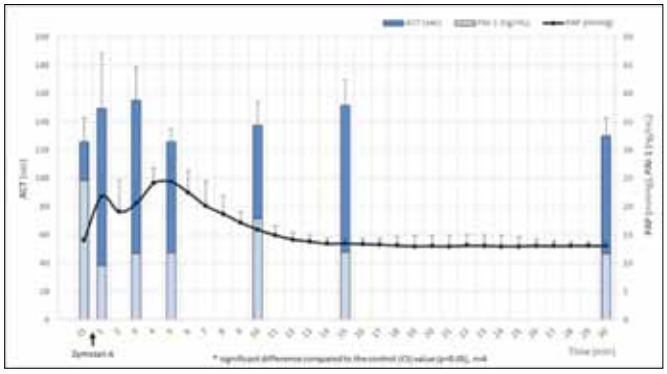

Figure 1. Changes in the Pulmonary Arterial Pressure (PAP -, mmHg), Activated Clotting Time (ACT -, sec) and PAI-1 plasma concentration (ng/mL; % of 0' control) in response to complement activator, Zymosan -A treatment (0.1 mg/kg dose) in 12-14 weeks old pigs. (n=4).

ACKNOWLEDGEMENTS

We would like to thank Maria Velkei for her excellent laboratory assistance. This research was realized in the frames of TÉT 13 IL-2-2014-0001 „Pharmacological reduction of the risk of serious adverse immune effects of liposomal medicines". The project is supported by the Research and Technology Innovation Found Hungarian Goverment. Participation of Hungarian authors on CLINAM Summit 2016 was supported by the SCOPES programme of the Swiss National Science Foundation.

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MULTI-CROSSED MICRO-IMMUNOELECTROPHORESIS: APPLICATION TO THE DETERMINATION OF COMPLEMENT ACTIVATION AT 50% BY NANOPARTICLES

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Abbreviations: $C3A_{50}$: Protein C3 activation at 50%, IE: Immunoelectrophoresis, NM: Nanomedicine, NP: Nanoparticle, XC-µIE: Multi-crossed micro-immunoelectrophoresis

Keywords: Complement system, crossed immunoelectrophoresis, protein C3, robustness
In a context of clinical translation of nanomedicines (NMs), high throughput methods allowing their characterization are needed. Indeed, characterization is mandatory to warrant quality hence safety of the treatment for a patient. The synthetic identity of the nanomedicine is generally defined by its size, surface charge and chemical composition. Since several years, it became obvious that interactions between NMs injected intravenously and biological components encountered in the blood are determinant for their *in vivo* fate, being at the roots of mechanisms that control their biodistribution. From these interactions, the NMs acquire a biological identity that is believed interesting to investigate as a characteristic influencing the biodistribution of the NM[1]. Another option consists in investigating biochemical reactions that can be triggered from the contact of blood components with NMs. One of these is related to the immune system including the complement system. Indeed, it is now well established that nanomaterials circulating in the blood are subjected to recognition by the immune system while the activation of the complement cascade may be the first event that is involved in this recognition[2,3]. The consequence of the activation of the complement cascade by NMs is their clearance from the blood associated with macrophage uptake. Investigating the potential of NMs to activate the complement cascade is then a relevant mean to anticipate their removal from the blood by the immune system. This can be performed by evaluating the activation of the protein C3 of the cascade that is the most abundant protein of the complement system and a protein having a central role in the cascade during the activation process. C3 protein is activated regardless of the pathway followed for the cascade activation. Activation of the protein C3 results in the cleavage of this protein into a series of smaller fragments (C3a, iC3b, C3c, C3dg) with molecular weights that are much smaller compared with that of the native protein (MW 185kDa). The cleavage of the protein C3 can be highlighted by means of a 2D-immunoelectrophoresis in which the electrophoresis of the first dimension achieves a separation of the proteins according to their molecular weights[4] (Figure 1). This method allowed a direct determination of the ratio between the cleaved (iC3b, C3c, C3dg) and native C3 protein from samples of serum that were incubated with NMs. 2D immunoelectrophoresis methods are generally quite complex in handling and time consuming. A low number of samples can be processed in the same time. This was identified as a limitation of the method that could only be proposed so far to compare NM samples studied in the same conditions. Thus, the aim of our work was to revisit experimental modalities of the methods that hampered its spread to be used at a large scale with only 5 samples being able to be analysed over a period of 19 hours. The proposed multi-crossed micro-electrophoresis (XC-µIE) allows the analysis of 35 samples on a single plate, giving stamp-size electrophoregrams (2.8 x 3 cm) maintaining the performance of the original method performed on 5 x 7 cm gel slabs. The robustness was proved for different factors (analyst, day, position of the sample on the gel plate, electrophoresis system). Modalities defined by the new experimental conditions brought several improvements compared with the original method. Time requires to perform one run with the same equipement was divided by 4 (4h45min compared to 19h15min in the original conditions), the number of analysis was increased by a factor 7 (35 analysis instead of 5), reagent consumption per analysis was reduced hence the cost of reagent to perform one analysis was decreased by a factor 4 (0.14€ against 0.57€), the handling was also improved as it will be shown during the presentation.
Due to its potential to be used to analyze a large number of samples at a time, it was suggested to apply this method to the determination of a new parameter. It could serve characterizing nanoparticles about their status regarding their capacity to trigger activation of the complement system. The parameter that was suggested to evaluate is the surface of NM that induced the cleavage of 50% of the protein C3 (C3A50: Activation of protein C3 at 50%). The nanoparticles are tested at different concentrations expressed in surface area and the C3A50 is determined at a concentration the produced 50% of activation, as evaluated in the conditions of the experiments. The method was applied with different nanoparticles with an example given in figures 1 and 2. This parameter could become an indicator of the interactions of a given NMs with the complement system acknowledging their ability to prevent or induce an immune response.

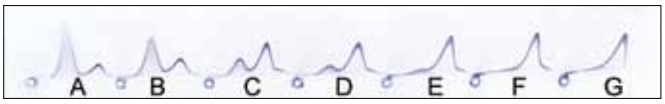

Figure 1: Serie of electrophoregrams obtained for increasing surface of NPs made with Isobutylcyanoacrylate grafted with Dextran 67 kDa, 1.3%[5].
Serum were incubated with NPs concentrations of 950 (A), 1500 (B), 1800 (C), 1900 (D), 2100 (E), 2500 (F) and 2800 cm^2/mL (G).

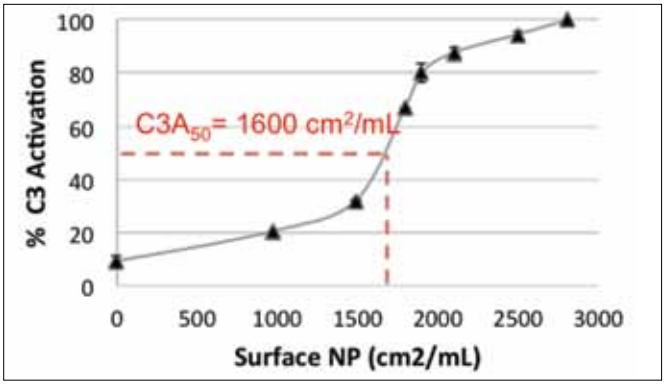

Figure 2: Pattern of activation for different concentration of the NP tested in Fig. 1. Determination of its $C3A_{50}$

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"SMART LIPOSOMES" FOR REMOTE CONTROL DRUG RELEASE

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In this study, we developed new approach to control drug release from liposomes under super low frequency alternating magnetic field (MF). Synthesised Fe_3O_4 magnetic nanoparticles (MNPs) with the core diameter 10 nm were incorporated into inner part of liposomes. Transmission electron microscopy (TEM) and infrared (IR) spectroscopy data showed that under MF liposomal membrane became more flexible and disintegrated. Our study suggests the possibility of bilayer permeability increasing through MNPs under external MF exposures. The efficiency of the model system was shown by fluorescent label release from liposomes.

INTRODUCTION

Liposomal drug delivery systems are widely applicable containers for protection and delivery of active molecules. Liposomes consists of biocompatible lipids, are stable during blood circulation and can concentrate at a target site. Despite their advantages, the major shortcoming of liposomes is incomplete drug release. such as low loading and partial release of drug, especially hydrophobic. To solve the problem of drug release liposomes, containing sensitive to pH, temperature changes polymers are created. These approaches have limitations too. In recent years, progress in magnetic nanoparticles technologies led to creation of magnetic nano-formulations including liposomes. Such magnetic liposomes with special thermosensitive polymers are activated by high frequency MF, i.e. using the effect of hyperthermia (which is caused through magnetic particles). In this case, high- frequency magnetic field with high intensity are used during a long time, that makes the method inconvenient. In this work we try to develop a new method to improve drug release through MNPs and super low frequency MF. We suppose that after low frequency the relaxation of nanopraticles magnetic moments after MF exposures proceeds via particles' mechanical rotation (Brown relaxation) or via the energy of crystallographic anisotropy (Neel relaxation). In the case of MNPs with 10 nm diameter, Brown relaxation proceeds faster than Neel. According to Y.I.Golovin et.al review[1] spherical MNPs can cause deformations of the membrane through their mechanical rotation and thereby increase drug release. The aim of this work is to create magnetic liposomes loaded with fluorescein as a model and investigate the effect of super low frequency MF on this drug container.

METHODS

Liposomes were prepared as follow: MNPs, fluorescein, phosphatidylcholine and cholesterol were dispersed in chloroform, dried to get film and then dispersed in phosphate buffer, sonicated and free MNPs were separated by passing the emulsion through extruder with pore size filter 400 nm. Excess of fluorescein was removed by centrifugation through NAP-25 desalting column.

RESULTS

After loading magnetic liposomes had average diameter of 160±14 nm and polydispersity index (PDI) was 0.25±0.10. Liposomes are stable in DW and PBS at pH=7.4 at 37^0C during a week. Free magnetic liposomes poses low toxicity and T2- relaxation. TEM analysis showed that MNPs are in inner part of liposomes. According to microphotos samples after 3 and 25 min MF exposures MNPs for the first time aggregate into clusters, then these clusters rotate in liposome and destroy membrane. IR- spectroscopy showed that MF application caused "melting" of liposomes membrane and the "melting" range depends on MF exposures time and MF intensity. For example, under 50 Hz MF membrane started "melting" after 5 min exposure, the maximum of peak shifting was after 15 min exposure, then the effect decreased, that could be caused by liposomes' destroying. These data are correlated with release experiments. Release experiments were conducted on the model of liposomes loaded with fluorescein. Under external MF conditions release was about 80% for 40 min in comparison to 45% without MF application. Moreover, the most part of fluorescein was released for the first 10 min, in the case of MF exposures. It could be caused by destroying and/or reorganization of lipid bilayer, that leads to changes in release of drug in comparison with control without MF exposure.

CONCLUSION

In the case of magnetic liposomes, IR-spectroscopy and TEM data showed that MF application caused "melting" of liposomes membrane and the "melting" range depends on MF exposures time and MF intensity. These data are correlated with release experiments. It could be caused by destroying and/or reorganization of lipid bilayer, that leads to changes in release of drug in comparison with control without MF exposure. TEM showed that MNPs are in inner hydrophilic part of liposomes. After short time exposures particles arrange in clusters (lines) and rotate destroying membrane. After long time exposures magnetic liposomes are destroyed and free MNPs are out of lipid membrane. We describe a new application and mode of remote control of MNP and its application in drug delivery.

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QM BASED REGULATORY SCIENCE

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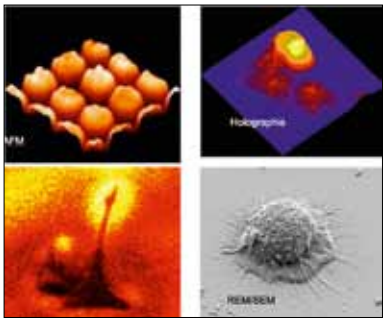
You manufacture or distribute innovative products containing nanomaterials and need customized analytical solutions assess quality or safety of your product? You prefer dealing with just one contract partner and have to rely on quality management?



The companies forming the NCL-Muenster work at the frontiers of nanoscience and develop new technologies, equipment and services for surface and cell analysis as well as microbial and molecular diagnostics. The special competences in high resolution analysis with ToF-SIMS mass spectrometry, Near Field Scanning Probe analysis, XPS and electron microscopy are matched with specific know-how on medical diagnostics and cell analysis. This combination enables us to analyse the complex reactions of cells and tissues to nanometarials necessary to develop and evaluate new *in vitro* and *in vivo* toxicology assay tailored to your material. Each individual company is an absolute specialist in its core technology and the combination of their certified know-how in a common portfolio is the unique selling point of the consortium. Acting as one single „Principal Investigator" we will tailor a specialized consortium to meet your demands, solve your analytical problems, and interpret complex data according to highest quality and secrecy standards.

STUDYING NANOPARTICLE TRANSLOCATION AND EFFECTS AT THE HUMAN PLACENTAL BARRIER USING EX VIVO AND ADVANCED IN VITRO MODEL SYSTEMS

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The use of medicines in pregnancy is widespread despite the fact that comprehensive drug safety and efficacy data for pregnant women are often lacking. Consequently, novel drug delivery therapies to specifically treat the mother, the fetus or placental disorders with reduced or absent off-target effects and fetotoxicity are of major interest[1]. Nanoparticles (NPs) are promising candidates for targeted drug delivery but the knowledge on how particle characteristics and functionalization affect placental NP translocation and effects is very limited. Therefore, we aim to acquire a mechanistic understanding on the interaction of NPs with the placental barrier.
We investigated placental NP translocation using the ex vivo human placental perfusion model to circumvent uncertainties in the extrapolation of animal data. This is particularly relevant due to substantial differences in the architecture, function and development of the placenta between different species. For mechanistic studies and to pre-screen a larger variety of different NPs, we are developing novel advanced *in vitro* models of the placental barrier that mimic the highly dynamic microenvironment and the multicellular structure of the human placenta. These include a perfused Transwell co-culture system for NP translocation studies as well as an organotypic 3D co-culture microtissue (MT) for NP effect and uptake/penetration studies.
In ex vivo perfusion studies, we identified a size- and surface modification-dependent transfer of polystyrene (PS) NPs across the human placenta (Figure1)[2,3]. Moreover, bidirectional transport studies demonstrated an increased transfer of PS beads in reverse (fetal to maternal direction) perfusions and an accumulation of PS beads in the syncytiotrophoblast layer of the placental tissue[3]. These studies indicate that the syncytiotrophoblast is the key player in regulating transplacental PS NP transfer, and that the underlying mechanism is not based on passive diffusion but involves active, energy-dependent translocation pathways. Establishment of novel *in vitro* placenta models is ongoing: 1) we successfully developed a placental co-culture Transwell model with confluent monolayers of BeWo cells (trophoblast derived human choriocarcinoma cell line) and HPEC (human placental venous endothelial cell line) on either side of the insert to represent the two key cellular barrier layers of the human placenta. First translocation studies with the paracellular marker sodium fluorescein indicate that the trophoblast layer constitutes the main barrier and addition of endothelial cells did not further reduce permeability. In contrast, transfer of the transcellular marker antipyrine was equally high across the trophoblast and endothelial monolayer or the co-culture barrier. For the larger paracellular marker FITC-Dextran (40kDa) and for 49 nm or 70 nm PS NPs, BeWo trophoblasts as well as endothelial cells formed a major barrier for the passage of these particles. Simple shaking of the inserts to mimic maternal and fetal blood circulation did not lead to any differences in the transport kinetics of all markers or NPs as compared to static conditions. Consequently, a truly perfused Transwell system with two individually pumped circulations will be established and compared to the ex vivo placenta perfusion model and to static culture conditions in order to understand if a perfused condition is improving the predictive value of the perfused Transwell model. 2) We developed 3D MTs consisting of a fibroblastic core (human villous mesenchymal fibroblasts, HVMFs) surrounded by a trophoblastic cell layer (BeWo choriocarcinaoma cells), resembling the *in vivo* placental barrier structure. BeWo cells released significantly more ß-hCG when cultured in 3D co-culture MTs than in 2D monocultures in the presence and absence of forskolin, a known inducer of trophoblast differentiation. Interestingly, TiO2, CuO and COOH-CdTe NPs were more toxic to 2D BeWo cultures than 3D MTs. Thus toxicity studies in 3D co-culture MTs provide more tissue-like dose-response curves as they represent a morphology and exposure scenario closer to the *in vivo* situation than 2D monocultures.

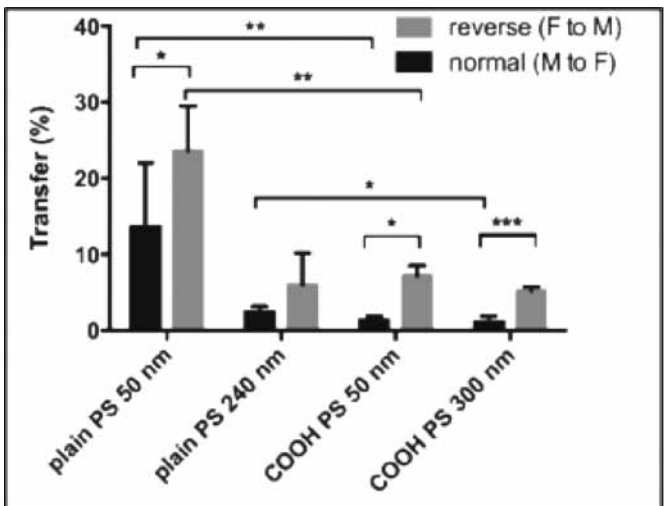

Figure 1: Transfer rates of plain- or COOH-PS beads in *ex vivo* human placental perfusion studies after 6 h of perfusion either from the maternal to fetal (M to F) or reverse from the fetal to maternal (F to M) circulation (C and D); adapted from [3].

Conclusively, our studies indicate that physicochemical properties and functionalization of NPs have a major impact on their interaction with the placental barrier. However, more detailed studies using a larger variety of well-characterized NPs that only vary in one characteristic are required to effectively support the safe design of targeted particle-based therapies in pregnancy. Moreover, identification of the relevant parameters (e.g. perfusion, co-culture, 3D structure) that have a key impact on NP translocation and effects will be essential to design highly predictive and cost-effective *in vitro* models.

ACKNOWLEDGMENTS:

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MRNA INCORPORATED IN LIPID MATRICES AS CONTROLLED RELEASE SYSTEMS

ANTJE ZILLER, Sara S. Nogueira, Eva Hühn, Heinrich Haas, Sergio Funari, Gerald Brezesinksi, Hermann Hartmann, Sahin Ugur, Peter Langguth

Controlled-release drug-delivery systems play an important role in the treatment of various diseases. Understanding the impact of the molecular organization is fundamental to ensure model systems for development of new therapeutic agents.
This study reports on incorporation of mRNA in zwitterionic lipid matrices (DOPC, EPC) using a cationic lipid (DOTAP) as an anchor for mRNA insertion. Structure and activity of the formulations was investigated for different DOTAP and mRNA fractions.
The structure of the systems was investigated by small-angle x-ray scattering (SAXS) measurements, phase transition temperature was measured with Differential Scanning Calorimetry (DSC), zeta potential measured with Dynamic Light Scattering (DLS). Exemplary, D-spacing of different DOPC/DOTAP- gelplexes is shown in Figure 1.

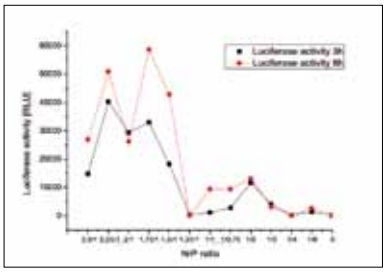

Figure 1: D-spacing of different DOPC/DOTAP-gelplexes dependent on N/P-ratio

Free and bound mRNA was determined and the functionality of the mRNA was proven in cell culture experiments. Transfection efficiency in C2C12-cell line after 3 and 6 hours is illustrated in Figure 2.

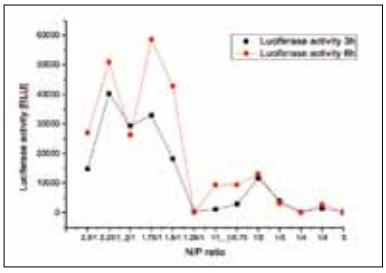

Figure 2: Transfection efficiency in C2C12-cell line of DOPC/DOTAP-gelplexes dependent on the N/P-ratio

The data indicate, that the mRNA could be efficiently loaded to the lipid matrices. Both the structure and the biological activity depended sensitively on the ratio between DOTAP and RNA. A structural model was developed, which can be seen in Figure 3.

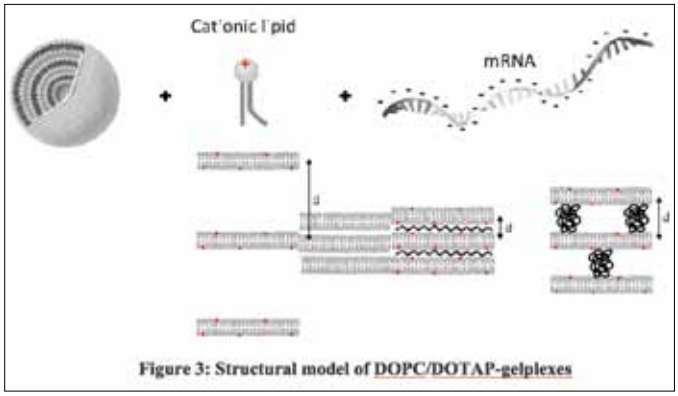

Figure 3: Structural model of DOPC/DOTAP-gelplexes

For all formulations, the integrity and the biological activity of the mRNA was maintained. The formulations may be of interest for local administration of the RNA as they may enable accurate control of the form and the time course of release.

EXPLORING SHEAR-SENSITIVE LIPOSOMES

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[2]Biomaterials Science Center, University of Basel, 4123 Allschwil, Switzerland.

Abstract. Mechanosensitive liposomes are representative of a new class of drug delivery nanocontainers[1]. The vesicles retain their cargo when they are left untouched but do release their contents when vigorously shaken. Mechanosensitivity as a drug release trigger can therefore be used as an attractive alternative to the classical triggers such as changes in temperature or pH, light, redox reactions, enzyme activation or application of ultrasound[2]. Understanding mechanosensitivity of liposomes calls for a systematic study probing the forces at play in vesicle self-assembly. In this contribution we explore several new classes of artificial phospholipids. We present their synthesis as well as biophysical studies utilizing three model systems ranging from monolayers and stacked bilayers to curved bilayers. A matrix of phospholipid interactions emerges, leading to valuable insights that will be used for the design of future generations of artificial phospholipids[3].

Keywords. Phospholipid liposome, drug carrier, organic synthesis, small-angle X-ray scattering, Langmuir-Pockels monolayers, grazing incidence X-ray diffraction.

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[1] M.N. Holme et al., (2012), Nat. Nanotech, 7(8):536-43.
[2] D. Mellal et al., (2014), J. Mater. Chem. B. 2(3), 247-252.
[3] R. Tanasescu et al. (2016), Langmuir, 32(19),

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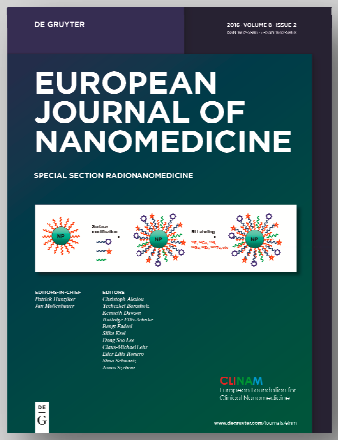

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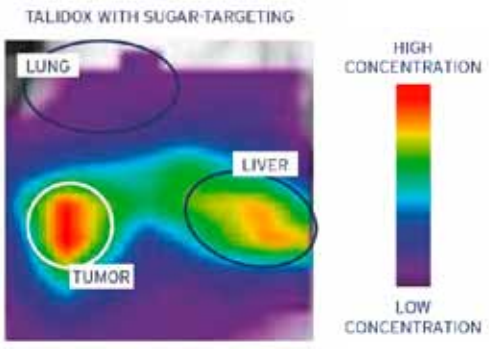
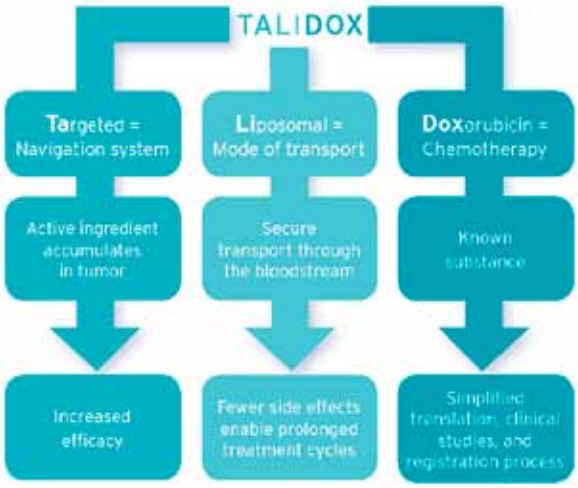
TALIDOX – TARGETED CANCER THERAPY

At some point in their lives, the vast majority of people are unfortunately confronted with the issue of cancer in their family or among friends. InnoMedica's goal is to significantly improve cancer therapy. InnoMedica's Talidox (Targeted Liposomal Doxorubicin) uses liposomal sugar targeting technology to deliver chemotherapy directly at the tumor site. This leads to a more efficient cancer treatment while simultaneously reducing side effects for patients.

A comparison of substance distribution with and without sugar targeting shows that Talidox significantly increases accumulation of medicine in tumor tissues, whereas the untargeted chemotherapy medicine mainly accumulates in the liver. The targeting technology of InnoMedica thus actively influences the distribution of drugs in the body. The dual impacts on chemotherapy effectiveness (more chemotherapy in the tumor) and side effects (less chemotherapy in the liver) occur simultaneously.





OBJECTIVES OF INNOMEDICA:

- Development, production and marketing of Talidox, a novel cancer therapy which
  - targets tumor cells directly
  - reduces side effects to a minimum
  - has broad therapeutic application possibilities at reasonable costs
- Building a drug pipeline for other medical applications using InnoMedica's targeting technology.
- Continuing independence of InnoMedica through a broad public shareholder base supported by a stable shareholder core.
- Public listing on the stock market following successful human clinical trials of Talidox.

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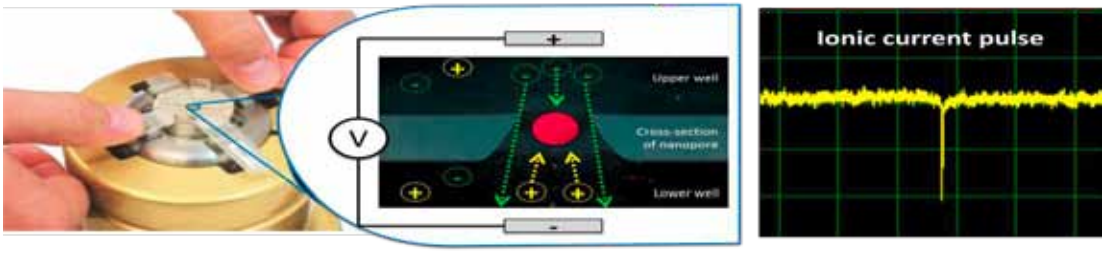
Izon Science provides instrumentation for accurate particle-by-particle measurement of concentration, size and charge of 40nm to 20 micron sized particles. The measurement principle used is Tunable Resistive Pulse Sensing (TRPS). Unlike light scattering methods, TRPS produces the necessary resolution, accurate and reproducible results for medical grade analysis. Measurement is in absolute (SI) units not arbitrary numbers. Reliable data of the highest quality from TRPS allows researchers to meet strict measurement and QA requirements of the medical world.

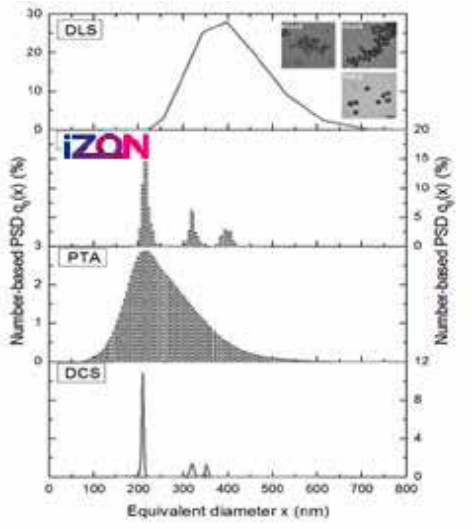


Particle by Particle measurement with TRPS: *When a particle enters the pore it blocks a proportion of the ions flowing through the pore – this causes a pulse in the ionic current the magnitude and duration of the pulse is proportional to the particle size and velocity, respectively. The pulse of every particle passing through is directly compared with the pulse of a traceable calibrated standard.*

TRPS is recognized as the most accurate technology for the simultaneous characterisation of size, concentration and charge properties of nanoparticles by a wide range of organisations including academic, research institutions and scientific companies. Particle types measured with TRPS include: lipsomes, polymeric drug delivery PLGA lipid, micelles, red blood cells and blood platelets.

TRPS enables accurate measurements and provides several benefits to users:

- Calibrated method ensures validation of data and repeatability
- Long term stability of particle solution can be better understood with precise measurement of aggregation
- Accurate measurements of size and charge facilitate in distinguishing different particle solutions



The chart on the left displays measurement of a known trimodal mixture of standard particles by DLS, TRPS, PTA and DCS. Of the 4 techniques, only TRPS was able to resolve the 3 particulate systems in the mixture. In addition, only TRPS can directly measure particle concentration for each distribution. *(Image reference: Journal of Colloid and Interface Science, Will Anderson, Darby Kozak, Victoria A. Coleman, Åsa K. Jämting, Matt Trau).*

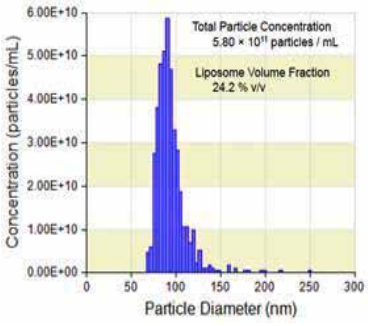


Figure 1: Size specific concentration with TRPS

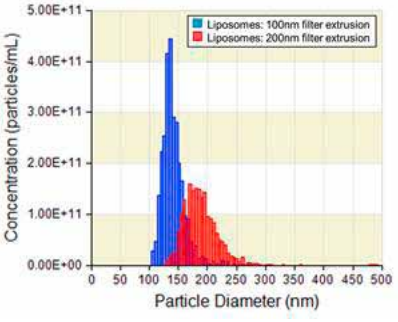


Figure 2: TRPS size analysis for liposome synthesis

Find out more about the qNano Gold instrument upgrade based on TRPS technology for accurate size, concentration and surface charge measurement and analysis: www.izon.com

Company Profile

Leica Microsystems develops and manufactures microscopes and scientific instruments for the analysis of microstructures and nanostructures. Widely recognized for optical precision and innovative technology, the company is one of the market leaders in compound and stereo microscopy, digital microscopy, confocal laser scanning and super-resolution microscopy with related imaging systems, electron microscopy sample preparation, and surgical microscopy.

Founded as a family business in the nineteenth century, the company is now a global enterprise. Its leitmotif is “with the user, for the user”, put into words by Ernst Leitz I, the entrepreneur who led the business to world renown. A historically close collaboration with the scientific, medical, and industrial communities is the key to Leica Microsystems’ tradition of innovation. Drawing on users’ ideas and developing solutions tailored to meet their requirements, the company continually sets new standards in markets it serves. This also applies to the services Leica Microsystems provides customers with: The company offers expert technical services, but also application support for all application-related questions.

Users of Leica Microsystems’ instruments and services can be found in both clinical and life science research, several surgical specializations, disciplines associated with material sciences, the manufacturing industry, and forensics services as well as in classrooms around the world. Instruments and workflow solutions for electron microscopy sample preparation for life sciences and industrial applications alike complete the company’s product portfolio.

Leica Microsystems has seven major plants and product development sites located in Wetzlar and Mannheim (Germany), Vienna (Austria), Heerbrugg (Switzerland), Cambridge (UK), Shanghai (China), and Singapore. The company is represented in over 100 countries, has sales and service organizations in 20 countries, and an international network of distribution partners. Its headquarters are located in Wetzlar, Germany. Leica Microsystems is part of Danaher.

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Lipoid

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Company Profile

Lipoid is a German-based company offering the whole range of pharmaceutical grade Lecithins and Phospholipids under the trade names:

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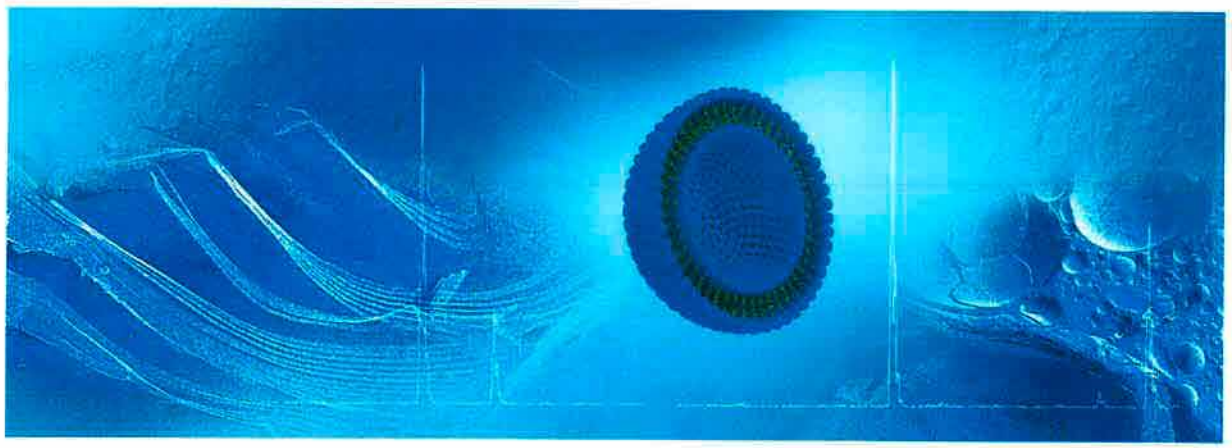
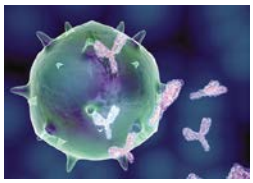
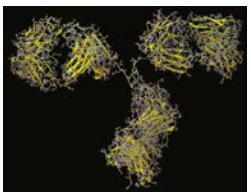
The products are manufactured under certified GMP conditions in three independently operating plants in Germany, analyzed comprehensively and are fully documented (including DMFs). They are used worldwide in large quantities in all kinds of parenteral, oral and topical pharmaceuticals.



Malvern Instruments Ltd – (rep. by Instrumat AG in Switzerland)


The analytical systems from Malvern deliver information that is essential for the understanding and **control of the behavior of nano-scale materials**.

They are used to: determine nanoparticle size and molecular weight, count particles in samples, detect aggregate formation, measure zeta potential to assess stability, and differentiate components on the basis of buoyancy.

Dynamic and electrophoretic light scattering, fundamental to Malvern's Zetasizer systems, are standard techniques for measuring the size and zeta potential of dispersed nanoparticles. Nanoparticle Tracking Analysis in Malvern's NanoSight systems, Resonant Mass Measurement in Archimedes and Taylor Dispersion Analysis in Viscosizer TD provide complementary and orthogonal characterization.

- The **Zetasizer Nano ZSP**: used to: determine nanoparticle size and molecular weight, count particles in samples, detect aggregate formation, measure zeta potential to assess stability.
- The **NanoSight** ***(displayed on booth #4)***: using Nanoparticle Tracking Analysis uniquely **visualize, size and count exosomes, microvesicles and all kind of nanoparticles** in pharmaceutical industry. The fluorescence measurement mode enables detailed studies of fluorescently labelled vesicles in complex media.The role of exosomes and microvesicles as mediators of intercellular communication is a research focus. Pathophysiological roles are being recognized in areas such as cancer, infectious diseases and neurodegenerative disorders, providing potential targets for therapeutic intervention. Knowing exosome and microvesicle size and concentration is valuable in elucidating their roles in disease and their potential exploitation in diagnostic or therapeutic applications
- The **Archimedes**: an automated biophysical characterization tool that uses Resonant Mass Measurement technology to differentiate components on the basis of buoyancy (i.e. **mass and density**).
- The **Viscosizer TD**: an automated biophysical characterization tool that uses microcapillary technology and Taylor Dispersion Analysis to deliver solution-based **molecular sizing from 0.2 nm to 50 nm, and relative viscosity measurements**. These capabilities, in combination with ultra-low sample requirements, accelerate drug stability screening and formulation studies in complex media.
- The **HELIX**: a hyphenated DLS-Raman system provides insight into **stability at the microscopic structural level** and develops mechanistic understanding of protein aggregation.

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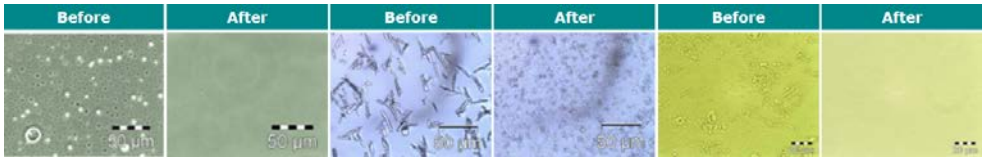

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Nanobiose is a startup dedicated to the *in vitro* immunotoxicity assesment of nanomaterials and nanoparticles used in the formulation of drug candidates, implantable medical devices, contrast agents and cosmetic products.

Due to their size comparable to biological objects like proteins or nucleic acids, nanoparticles display amazing physico-chemical properties, defining a new scope of interactions with the body. In the case of the immune system cells, the accumulation of nanoparticles may lead to dysfunctions that can trigger unwanted side-effects and therefore immunotoxicity.

Nanobiose combines the unique expertise of its seasoned team of experts in immunology and immunotoxicology with a customer-oriented project management to offer a complete portfolio of services to customers :

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Nanobiose services are delivered in accordance with a quality management system to offer the best results quality to customers. For more reliable and more predictive results Nanobiose promotes the use of primary cells in functionnal assays (NK cells activity, lymphocytes proliferation, macrophages / neutrophiles functions, cytokines dosage (ex vivo / in vitro), hematotoxicity, platelets functions, etc.) and also develops its proprietary assays which can be customized.

Nanobiose *in vitro* immunotoxicology services help customers in the life science area :

- To strengthen the toxicology data of their nano-enabled products
- Make the most appropriate strategic decisions
- Mitigate risks and reduce costs
- Accelerate time-to-market
- Answer regulatory authorities' requests
- Secure approval to launch clinical phases and market their nano-products.

Nanobiose headquarters are located in Chambery, France, at the alpine crossroads of Europe.

For more information, please contact us : contact@nanobiose.com

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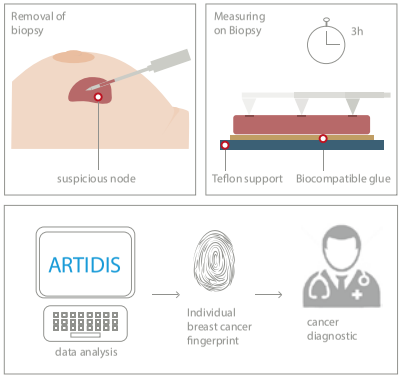
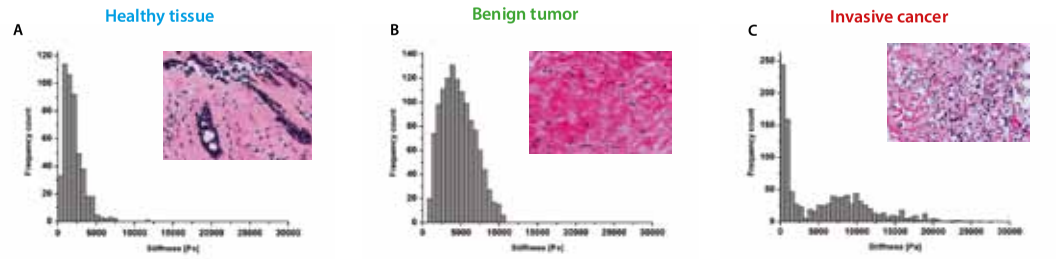
Nano - fabricated 20nm fine tip of ARTIDIS® (Automated Reliable Tissue Diagnostics) probing a neoplastic lesion.

Detecting the nanomechanical fingerprint of breast cancer

Nuomedis is dedicated to uncover the mechanical profile of (breast) cancer at a nanometer scale. ARTIDIS®-technology uses a 20 nm – fine tip to indent at several thousand locations across a biopsy and harvest its specific nanomechanical profile. This gives an information how stiff the tissue structures are, which is used as marker to differentiate healthy, benign and malignant tissue. As seen in the figure below, specific stiffness distributions are related to different disease conditions. In parallel, distinctive nanomechanical stiffness profile across the tumor provide a prognostic indicator of tumor progression and aggressiveness.

By adding this new dimension to breast cancer diagnostics and prognostics, ARTIDIS® technology helps clinicians with faster diagnosis, better determination of the aggressiveness of the disease and improved treatment plan. Patients benefit from more efficient therapy and less overtreatment, which directly promotes better outcome of the disease and helps to sustain quality of life.





Healthy
Unimodal distribution with peak stiffness from 1.13 to 1.83kPa

Benign
Unimodal distribution with stiffness values from 1.91 to 3.68kPa

Cancer
Multimodal distribution with an initial peak at 0.31-0.75kPa, second peak at 1.54 – 1.99 kPa, third peak up to 20kPa

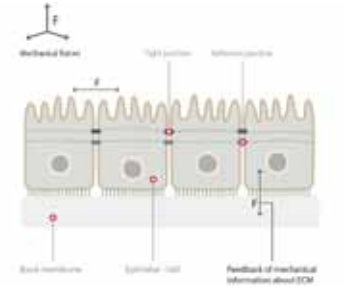
Stiffness histograms of breast biopsies. On breast biopsies, different signatures are found depending on the health state of the tissue. A: Healthy tissue shows a uniform soft peak. B: Benign tumor shows higher stiffness. C: Malignant tumor exhibits very soft and very hard tissue. [doi: 10.1038/nnano.2012.167.g001].

nuomedis®

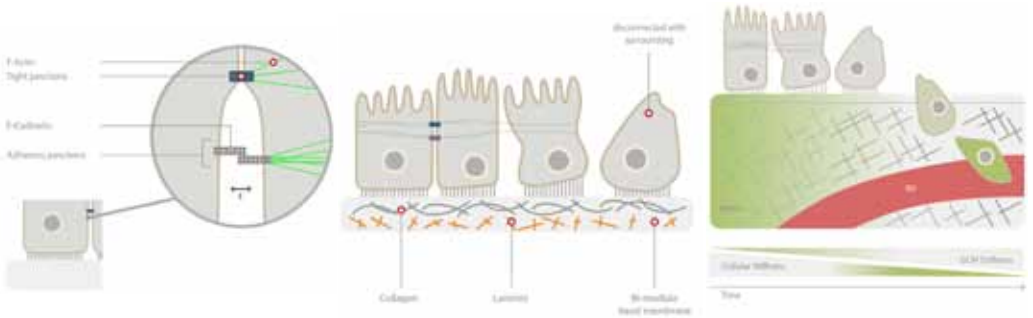
Why nanomechanics matters?

The biophysical approach to cell biology yields better understanding and new insight of cancer.

The complex cancer biology reflects the array of somatic, genetic and epigenetic alterations. Nanomechanical assessment of a tissue directly addresses the outcome of the complex interplay of numerous biological pathways leading to a phenotype. In comparison, gene-expression profiling or single protein analysis are methods typically used to asses only specific segments of biological pathways, which are leading to a given cancer phenotype.



One prominent example of how biochemical signals manifest themselves in biomechanical phenomena is epithelial-mesenchymal transition (EMT). One of the biochemical essentials for EMT is the loss of E-cadherin, which alter physical and mechanical properties of cells as well as the intercellular adhesion and cell morphology. An abolishment of physical contacts between cells unambiguously leads to aberrant mechanical properties and acquisition of migratory and invasive properties. Such mechanical alterations are being detected by the ARTIDIS® technology.



Nuomedis Team

Nuomedis AG was founded in 2014 with the goal to develop nanomechanics based *in-vitro* medical device for applications in cancer diagnostics. The executive management brings together over 30 years of business experience in the field of nanotechnology and scientific experience from renowned institutions to perform at its best.

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Gräubernstrasse 14
4410 Liestal
Switzerland
www.nuomedis.com
info@nuomedis.com

A remarkable approach:

Particle Metrix Nanoparticle Analyzers

With its titration efficiency, Stabino® opens a new field of particle charge fingerprinting. Associated aggregation can be simultaneously followed with the in-situ pobe of NANO-flex®. NTA Nanoparticle Tracking Analysis with ZetaView® extends the portfolio of information to low particle concentrations, fluorescence and sub-population analysis.

ZetaView® NTA

Nanoparticle Tracking Analyzer for Concentration, Particle Size and Zeta Potential, in scattering and fluorescent mode

This laser scattering video microscope provides classical micro-electrophoresis zeta potential and Brownian diffusion size in a single system. The particle concentration is monitored continuously. „Measure what you see“ is the charme oft he software when it comes to in sub-population evaluation. Auto-alignment and sub-volume scanning make robust results of 1000s of individual particles. Built-in-rinsing allows the measurement of 30 to 150 samples a day. Among key applications, the study of extracellular vesicles (EV's) is made an extremely easy process.

NANO-flex®

In-situ DLS Size and Concentration Analysis System

Simultaneous observation of smallest ang big size particles is offered in the 180° DLS NANO-flex®. Its measurement probe is idealy suitedl to follow reactions in-situ. The sytem is offered in combination with stability formulation work in the Stabino® and as a size and concentration extension for ZetaView® NTA. Key applications are protein aggregation and stability formulation of colloids.

Stabino®

Efficient Stability Analysis System for Colloids and Dispersions

In contrast to optical zeta potential analyzers, particle charge mapping is made in minutes. During titration, the dipped-in NANO-flex® controls the critical coagulation point. It is applicable to macromolecules and particles. The combined system provides a new dimension of stability mapping.

Particle Metrix wishes CLINAM an extraordinary exchange of ideas.

June 2016

Liposomal Formulation of Drugs

Liposomes protect, transport and release your drug at the right place and time. By this, a reduced dose achieves better efficacy and avoids side effects with a non-invasive application. A liposomal formulation can clearly improve the therapeutic index of your drug.

Polymun offers the development of liposomal formulations for all kinds of pharmaceutically active ingredients such as oligo-nucleotides, small molecules and proteins as well as vaccine antigens. A broad spectrum of analytical methods has been established for this purpose. Polymun produces GMP material including all necessary documentation for IMPD/IND.

We assist in planning and implementation of clinical trials. Finally, license agreements are offered for the respective substance on an exclusive basis. Contracts can be arranged step by step – proof of concept, in-depth analysis, GMP production, product license – or all in one.

MAIN CHARACTERISTICS OF OUR TECHNOLOGY

FULL SCALABILITY

The injection module is the heart of the liposome production. The process parameters determine the size of the liposomes regardless of the scale. Production of 250 liters of liposome preparation takes only 1.5 hour. Large scale also can be achieved by using several injection modules in parallel.

ASEPTIC PROCESS

A closed system is used for production. All components can be added via sterile filtration. Subsequent concentration by crossflow filtration is possible as well.

HOMOGENEOUS, UNIFORM VESICLES

All process parameters are controlled precisely. This results in a very narrow size distribution, necessary for reliable targeting and transport characteristics.

SINGLE STEP PROCESS

Liposome size is adjusted by modulating the process parameters during vesicle formation. No additional downsizing is required.

EXCELLENT BATCH TO BATCH CONSISTENCY

High quality of raw materials and precisely controlled process parameters guarantee excellent reproducibility – essential for pharmaceutical products.

MILD PROCEDURE – STABILITY

The crossflow injection technique is a very mild procedure that allows the processing of sensitive drugs. Together with the high quality of raw materials and narrow size distribution, we achieve long term stability of liposomes even at room temperature.

SERVICES

- Formulation Development
- Analytical Method Development
- Process Development
- GMP Production
- Filling
- Clinical & Regulatory Support

CLINICAL & REGULATORY SUPPORT

- IMPD / IND, IRB Submission
- Pre-/Clinical Development Concepts
- Organisation of Clinical Studies
- Legal Representative
- Requests for Scientific Advice

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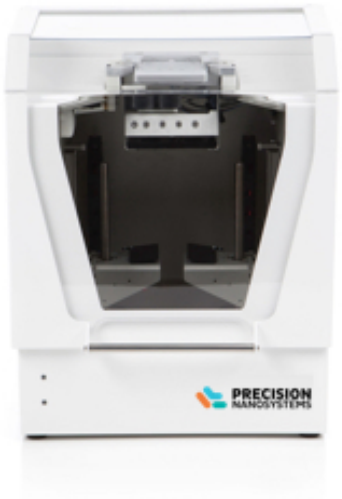
PRECISION NANOSYSTEMS

Precision NanoSystems inc. provides scientists with an integrated platform of products and support, helping advance innovative nanotechnology solutions to understand the molecular basis of disease, and to develop new treatment models.

We are introducing a variety of products throughout the second half of 2016 that will empower researchers to propel from the bench to the clinic



- **NanoAssemblr™ Spark**: Small volume nanomedicine manufacture (25 µL - 250 µL). Kits for delivery of nucleic acids (siRNA, mRNA) to sensitive cells in vitro (e.g primary neurons).
- **NanoAssemblr™ Benchtop**: Controlled and tunable nanoparticle manufacture (1 mL - 15 mL) for formulation development and in vivo applications.
- **NanoAssemblr™ Blaze**: Pre-clinical instrument for nanoparticle batches of 10 mL - 1 L seamlessly scaled using our continuous flow microfluidics.
- **GMP Scale-Up System**: Clinical grade microfluidic manufacturing of nanoparticle therapeutics for phase trial study without the risk of process redevelopment.

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SeroScience Ltd (*SRS*)

is a small/micro biotech enterprise (SME), spin-off company of Semmelweis University Medical School in Budapest, Hungary, founded in 2006 by medical scientists. It provides research services and regulatory testing for pharmaceutical companies in regards to the safety of drugs in R&D. Specifically, *SRS* utilizes state-of-art in vitro technologies and large and small animal models for assessing the potential of drug candidates (most importantly intravenous drugs, biologicals, nanomedicines and contrast media) to cause hypersensitivity (allergic, pseudoallergic or infusion) reactions and other adverse immune effects, including antibody production (immunogenicity).

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36-30-415-0007
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Expertise

SeroScience Ltd. has special expertise in the prediction of complement activation-related pseudoallergy (CARPA) that represents a major barrier to the clinical use of many nanomedicines and biologicals.

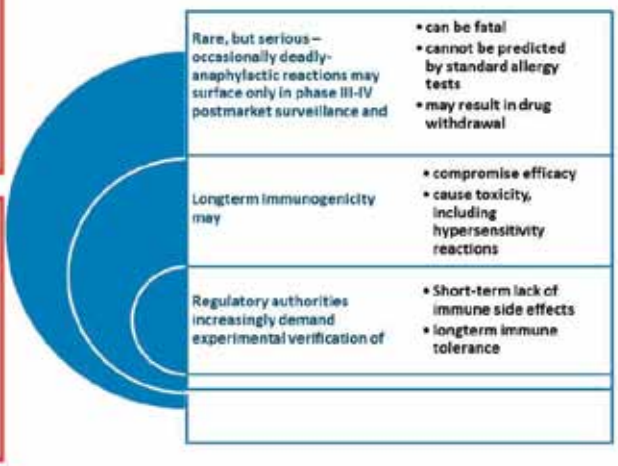

Guidance for Industry

S8 Immunotoxicity Studies for Human Pharmaceuticals


Guidance for Industry

Immunotoxicology Evaluation of Investigational New Drugs

Why CARPA is important?



Services

IN VITRO STUDIES

- **Complement activation assays in vitro in human/animal sera/plasma/whole blood**
- **ELISA for human C3a, C5a, C4d, Bb, SC5b-9**
- **ELISA for all animal C3 (PAN-C3)**
- **SRBC (CH50) hemolytic assay for all animals**
- **ELISAs for serum/plasma TXB2, PAF, histamine, leukotrienes, triptase in man and animals**
- **FACS analysis of basophil activation**
- **ELISAs for anti-drug antibody (ADA) measurements**

IN VIVO STUDIES

- **Analysis of test drug-induced hemodynamic, hematological, laboratory and skin changes in pigs/minipigs/dogs/rats/mice (CARPA assays)**

- **REGULATORY REPORTS**
- **R&D WITH LIPOSOMES AND OTHER NANOCARRIER FORMULATIONS**
- **SAFETY COUNCELING**

See SPECIAL SECTION ON CARPA IN THE CLINAM ISSUE OF THE EUROPEAN J. NANOMEDICINE

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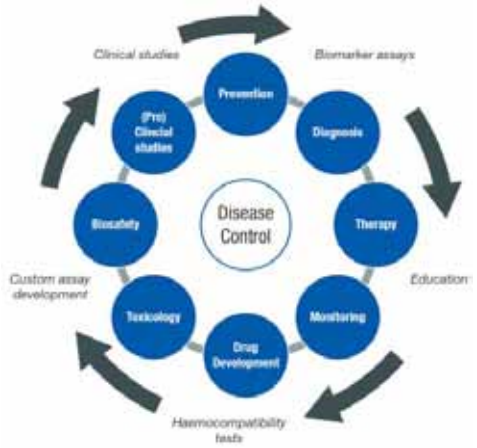

Founded in 1984, the Swiss-based TECOmedical Group and the subsidiaries in Germany, France, Austria and Benelux provide assays and services for (pre)clinical studies, biosafety and toxicology studies, medical research and in vitro diagnostics.
We offer an extensive portfolio of specialty assays, assay systems and services to Pharma and Biotech companies, CROs, medical and research centers.

Specialty assays for (pre-)clinical and medical studies
Drug development, research, diagnostics and therapy control
- bone/calcium/cartilage metabolism
- diabetes/obesity/metabolic syndrome
- liver disease & apoptosis
- drug-induced liver & kidney injury
- complement system
- cardiovascular disease
- oxidative stress

Specialty assays and test systems for biosafety of medical devices, transplants, implants, pharmaceuticals and blood products
- Haemocompatability related to activation of the complement (C) system – Anaphylatoxins
- Complement C activation related to pseudoallergy (CARPA)
- Complement activation in animals (in vitro & in vivo)
- Cytotoxicity

Specialty assays for toxicology
- Detection of drug induced liver injury (DILI)
- Detection of drug induced kidney injury (DIKI)
- Vitellogenin assay for endocrine disruption potential of chemical substances according to OECD for laboratory and environmental use. This is the first Vitellogenin fish assay allowing non-destructive, non-invasive sampling from epidermal mucosa.

Custom Assay Development & Services
Custom ELISA Assay development and Services are offered to organizations like Biotech companies, CROs, Pharma and Research institutions, requiring specialty assays and studies based on customer specifications.
- Host Cell Protein testing for recombinant protein pharmaceuticals
- Immunogenicity assays to test for Anti-Drug Antibodies (ADA)
- High sensitive ELISAs
- Food safety assays
- Veterinary assays
- Environmental assays

Assay Services include measurement of study samples, validation of new and existing assays, test adaption, pilot to medium size manufacturing of ELISA kits and assay components.

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9/2016

Basel, June 26–29

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