CLINAM **European Foundation for Clinical Nanomedicine**

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The European Summit for Clinical Nanomedicine and Targeted Medicine

The Translation to Knowledge Based Medicine

8th European CLINAM-Conference, Foyer-Exhibition and University Village, Basel, June 28 – July 1, 2015

CONFERENCE PROCEEDINGS 8/2015

In Collaboration with:

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CLINAM European Foundation for Clinical Nanomedicine

CLINAM 8/15
European Summit for Clinical Nanomedicine and Targeted Medicine

Basel, Switzerland, Sunday, June 28 – Wednesday, July 1, 2015

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INTRODUCTION ON BEHALF OF THE EUROPEAN FOUNDATION FOR CLINICAL NANOMEDICINE (CLINAM)

Dear Participants of the 8th European Summit for Clinical Nanomedicine

The CLINAM-Foundation and its collaborators for the conference have the pleasure to welcome you to the European Summit for Clinical Nanomedicine and Targeted Medicine in Basel. This Summit takes place the eighth time this year, and it evolved into a worldwide interdisciplinary platform of Nanomedicine and Targeted Medicine. This year again, many pioneers and worldwide experts will participate in the debates, presenting the highlights of their research, discuss the application of Nanomedicine and Targeted Medicine and thus shape the medicine of the future.

CLINAM's major goal, the support of development and application of Nanomedicine and Targeted Medicine from the stage of basic research all the way to the clinic for the benefit of the patient and humankind has developed well. The CLINAM Summit has achieved a unique position in bringing together all stakeholders in the fields of Nanomedicine and Targeted Medicine, including regulatory authorities from all continents, clinicians, researchers and industrial innovators.

The global scope of CLINAM now reaches far beyond its modest roots in the Nanomedicine Research Group of CLINAM and the University Hospital Basel, embedded in the Science and Medical Faculties of the University of Basel, while Nanomedicine at the same time is developing well also at the local level, leading to new scientific results, patents and startups.

The CLINAM Summit has emerged as valuable interaction place to launch collaboration, get new ideas and learn about novel methodologies and technologies as well as novel projects, including numerous EU-wide efforts, in Nanomedicine and Targeted Medicine. The field represents one of the most exciting and promising arenas 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 globally. Nanomedicine and Targeted Medicine are the building blocks of the medicine of the future, being catalyst disciplines for developing diagnostics and treatments that account for the nanoscale, molecular and cellular origin of disease. Nanomedicine is thus a key enabling discipline for the Knowledge-Based Medicine of tomorrow.

In the next decades, medicine will experience a transformation to personalized diagnosis and treatment, taking the individual aspects of the patient and his/her disease into consideration. Key roles play high-resolution molecular profiling techniques, having been enabled and made increasingly cost-effective by Nanomedicine.

The Board of the CLINAM Foundation is this year especially grateful for the collaboration with many organizations that were willing to bring into this meeting their skilled expertise and to transform the Summit into an international melting pot for the medicine of the future on the floor of the neutral, non-profit platform of the European Foundation for Clinical Nanomedicine.

We look forward to three days of inspiration, good debates and many new results for each participant in this Summit and wish you a very fruitful stay in Basel.

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

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

PROGRESS OF NANOMEDICINE IN HORIZON 2020

The CLINAM Conference is an excellent opportunity for global networking and for discussing the unmet medical needs and novel solutions that can be offered by the application of nanomedicine and other novel technologies.

The European Union's Horizon 2020 Framework Programme for Research & Innovation is now well underway. With € 80 billion EU funding for the period 2014 – 2020 it will support the competiveness of the EU industry, address Societal Challenges such as Health, stimulate investments and the creation of growth and jobs for the citizens.

The first Call for Proposals from the Horizon 2020 Workprogramme 2014 resulted in at least ten new innovative nanomedicine projects. They include the building blocks to implement the core of the 'Translation Hub' that was proposed by the European Technology Platform for Nanomedicine. This 'Translation Hub' will have a central role in helping to translate novel nano-medicine laboratory concepts into new medical products, either for diagnosis or therapy, for the benefit of patients.

The Horizon 2020 'EU-Nano-Characterization Laboratory' Infrastructure project is creating a network of European laboratories for chemical, structural, biological, safety and toxicity characterization of nanomedicines. EU-NCL is created in strong international partnership with the US Nano-Characterization Laboratory. EU-NCL and US-NCL will help SME's and other organizations to characterize their novel nanomedicines, supporting regulatory requirements.

The programme 'Leadership in Industrial Technologies' funds the Coordination and Support Action ENATRANS. This project aims to support spin-off companies, SMEs and researchers to identify issues that are important for future translation of new nanomedicine concepts. It will help them to be aware of regulatory issues from the early stages of a new development, thereby avoiding costly failures.

'Leadership in Industrial Technologies' also funds three new pilot line projects for up-scaling of nanopharmaceuticals production, from small laboratory quantities to the larger GMP production quantities needed for late pre-clinical and clinical testing.

The nanomedicine community was also very successful by winning grants for 5 projects from the HEALTH Challenge ' Personalised Health and Care', in direct competition with other proposals. These projects use nanotechnology for diagnostic in-vitro devices and assays and for novel therapeutic approaches.

Nanomedicine products have a long and very costly development path from the laboratory to the market. Extensive pre-clinical and clinical testing is needed for proving efficacy, safety and quality before getting the marketing authorisation. The role of medical regulation is therefore extremely important in the translation process. Strong and robust regulatory science is needed for evaluating the efficacy, safety and quality of new nanomedicine products. Close international cooperation is needed to develop new testing guidelines, methods and standards.

The regulatory agencies are already actively coordinating their activities in order to reduce duplication of efforts for obtaining approvals in different countries in the world and they also provide scientific advice to applicants. The important role of regulation will be discussed in a regulatory session during this conference.

The application of nanotechnology, biotechnology, novel biomaterials, cell technologies, micro- and nano-electronics, photonics, genomics, big data and other advanced technologies offer tremendous opportunities for advancing medicine. The integration of technologies and cooperation between the different technical and scientific communities and industry will be especially needed for the new more personalised medical products and therapies of the future.

I look forward to lively discussions and a very interesting conference.

Dr. Rudolf W. Strohmeier
Deputy Director-General Research Programmes

CURRICULA VITAE OF SPEAKERS

Dong June Ahn

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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.

Khuloud T Al-Jamal

Dr. Khuloud T. Al-Jamal, BSc (Honour), PhD, MRPharmS is a senior lecturer in Nanomedicine since April 2013. 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 under the supervision of Professor Alexander T Florence (2005).
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 held numbers of positions such as Senior Research and Teaching Fellow in Nanomedicine and a Deputy Lab Leader of the Nanomedicine Lab at The School of Pharmacy, University of London (now known as UCL-School of Pharmacy) (2007-2010). She joined KCL as a lecturer in January 2011.
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. 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 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, Association for International Cancer Research, EPSRC, BBSRC, 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.

Christoph Alexiou

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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 Universityhospital 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.

María José Alonso

María José Alonso is full professor of Biopharmaceutics and Pharmaceutical Technology at the University of Santiago de Compostela (USC).
She has made critical contributions to the design and development of novel nanostructures for the targeted delivery of drugs and vaccines and to the understanding of the interaction of nanoparticles with biological barriers.
She has been the coordinator and PI of several consortia and cooperative projects financed by the WHO, the “Bill & Melinda Gates Foundation” and the European Commission. Currently, she is involved in 4 European Projects and she is coordinating the TRANSINT consortium (oral peptide delivery).
She is the author of 222 international scientific contributions with more than 10,000 cites (H factor 60). Because of the quality of her papers she has been among the TOP TEN in Pharmacology, according to the Times Higher Education international ranking. She has also been the inventor of 20 patents.

She is part of the scientific boards of a number of societies and a member of the executive board of the Controlled Release Society. She has also received 15 Awards, among them the "King Jaume I Award" given to the best researcher in the area of new technologies in Spain and the Maurice Marie Janot Award 2014 (APGI).

Mansoor M. Amiji,

PhD, RPh

Dr. Mansoor Amiji is currently the Distinguished Professor and Chairman of the Department of Pharmaceutical Sciences and Co-Director of Northeastern University Nanomedicine Education and Research Consortium (NERC) at Northeastern University in Boston, MA. NERC oversees a doctoral training program in Nanomedicine Science and Technology that was co-funded by the National Institutes of Health (NIH) and the National Science Foundation (NSF). Dr. Amiji received his BS degree in pharmacy from Northeastern University in 1988 and a PhD in pharmaceutical sciences from Purdue University in 1992.

His research is focused on development of biocompatible materials from natural and synthetic polymers, target-specific drug and gene delivery systems for cancer and infectious diseases, and nanotechnology applications for medical diagnosis, imaging, and therapy. His research has received over $18 million in sustained funding from the NIH, NSF, private foundations, and the pharmaceutical/biotech industries.

Dr. Amiji teaches in the professional pharmacy program and in the graduate programs of Pharmaceutical Science, Biotechnology, and Nanomedicine. He has published six books and over 200 book chapters, peer-reviewed articles, and conference proceedings. He has received a number of honors and awards including the Nano Science and Technology Institute's Award for Outstanding Contributions towards the Advancement of Nanotechnology, Microtechnology, and Biotechnology, American Association of Pharmaceutical Scientists (AAPS) Meritorious Manuscript Award, Controlled Release Society's (CRS) Nagai Award, and the AAPS and CRS Fellowships.

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Anthony Amaechi Attama is 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 Technical University 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 articles published in peer-reviewed journals. He is currently the Deputy Director, Education Innovation Unit of the University of Nigeria, Nsukka. 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.

Konstantinos Avgoustakis

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Konstantinos Avgoustakis obtained his Diploma in Pharmacy from the Aristotle University of Thessaloniki, Greece in 1985 and his Ph.D. in Pharmaceutics/Quality Control of Medicines, from the same University in 1991. He received a scholarship for Ph.D. studies abroad (1989–1992) from Greek State Scholarships Foundation and he obtained in 1992 a Ph.D. degree in Pharmaceutical Technology from King's College, University of London, UK. In 1993-1994 he served as research assistant in the Institute of Radioisotopes in National Centre for Scientific Research "Demokritos". Since 1994 he has joined the Department of Pharmacy in University of Patras (Greece), where he teaches subjects related to Pharmaceutics and Drug Delivery. His research interests lie on the controlled, targeted drug delivery using engineered nanoparticles based on biodegradable polymers and copolymers and magnetic hybrid inorganic/organic nanocarriers, on the development of novel prophylactic or therapeutic vaccines based on biodegradable and biocompatible, polymeric nano- and micro-particles and on the development of formulations for the efficient delivery of drugs with limited aqueous solubility. He is the author/co-author of 50 articles in peer-reviewed journals and 1 article in biomaterials encyclopedia. He has been Guest Editor for the special issue of "European Journal of Pharmaceutics and Biopharmaceutics" on "engineered polymers and polymeric systems in controlled drug delivery and targeting" (2009). He is also the author (inventor) of 1 European patent. His published research has received over 1000 citations (h index 17). He has participated in 9 research programs in collaboration with academic and industrial organizations (in 7 as coordinator). He serves as reviewer for major pharmaceutical and nanoscience/nanotechnology journals and he is Assistant Editor of "Current Nanoscience" and member of the Editorial Board for the "Open Drug Delivery Journal" and for the "Journal of Excipients and Food Chemicals".

Lajos (Lou) P. Balogh

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Dr. Lajos (Lou) Balogh is the Editor-in-Chief of the journal Nanomedicine: Nanotechnology, Biology and Medicine (Elsevier, 2012 Impact Factor=6.93, 5-year IF=7.46, www.nanomedjournal.com) and member of numerous USA, European, and International expert committees including the Steering Committee of the American National Standard Institute Nanotechnology Panel and the US Technical Advisory Committee to the International Standard Organization on Nanotechnology (TC-229). Lou is one of the five Founders of the American Society for Nanomedicine. (http://www.amsocnanomed.org)

Lou is Chief Scientific Advisor and Principal of AA Nanomedicine & Nanotechnology Consultants, North Andover, MA (baloghl@prodigy.net), providing expert advice, scientific evaluation, and feasibility assessments for nanomedicine related R&D projects, business plans, as well as technology due diligence for private companies, government agencies, and investors in Nanomedicine, Nanobiotechnology, and Nanotechnology since 2000.

Dr. Balogh is the former Co-Director of the NanoBiotechnology Center and Director of Nanotechnology Research in the Department of Radiation Medicine at the Roswell Park Cancer Institute, Buffalo, NY. He received his Ph.D. with honors from the Kossuth L. University in Hungary in Chemical Technology and was invited to the University of Massachusetts Lowell as a Visiting Professor in 1991. Later he worked at the Michigan Molecular Institute as a senior scientist, and had faculty appointments at the University of Michigan, Ann Arbor, and later at the University at Buffalo, SUNY. Dr. Balogh is an Adjunct Professor of Pharmaceutical Sciences at Northeastern University, Boston, MA, and Professor and Distinguished International Scientist of the Chinese Academy of Sciences. He authored or coauthored over 150 scientific publications and six book chapters, delivered more than 120 presentations was awarded 12 patents in various disciplines, including nanotechnology and nanomedicine.

Yechezkel Barenholz

Professor 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 an dnano-drugs based on drug delivery systems (DDS) best exemplified by the anticancer Doxil®, the first nano liposomal drug and the first FDA-approved (1995) nano-drug used world-wide. Professor Barenholz is an author of more than 385 scientific publications totalling more than 17,000 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 Special Issues, and is on the editorial board of five scientific journals.
Professor Barenholz is a founder of NasVax LTD, PolyPid Ltd, Moebius Medical 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.

Manuel Battegay

Prof. Dr. med. Manuel Battegay is Chief of the Division of Infectious Diseases & Hospital Epidemiology, acting Chief of Medicine with 20 clinics and Professor of Internal Medicine & Infectious Diseases at the University Hospital, University of Basel, Switzerland. He has co-authored over 400 scientific papers cited in PubMed in the field of HIV-Medicine, other viral diseases such as hepatitis C and general Infectious Diseases.

Manuel Battegay has studied Medicine in Basel and as an extern at the Maimonides Medical Center, Brooklyn, New York. He received his MD degree in 1985 from the University of Basel. He then worked in Internal Medicine and Infectious Diseases in the Canton Hospital Liestal and University Hospital Zürich. He is board certified in Switzerland in Internal Medicine and Infectious Diseases.
From 1990 to 1994 he worked in basic research in the field of viral immunology on the LCM Virus and hepatitis C in the laboratories of Rolf M. Zinkernagel at the Institute of Experimental Pathology, University Zürich and of Jay H. Hoofnagle and Stephen M. Feinstone at the Liver Diseases Section, National Institutes of Health, Bethesda, Maryland, USA. His scientific work in these laboratories included the description of CD8 exhaustion upon viral infection in a mouse model lacking CD4 T cells and the first description of HCV epitopes.
As a clinician and clinical researcher Manuel Battegay is active in the field of HIV/AIDS since 1988, particulary with an interest in the clinical course of HIV, namely the response to therapy and immune reconstitution as well as on psychosocial issues. Since 1994 he is a board member of the Swiss HIV Cohort Study (SHCS) Group. He chaired the Scientific Board of the SHCS for four years on a rotational basis from 1998-2002. In the context of the SHCS he contributed to and performed many research projects, be it on epidemiological, clinical or applied basic research questions. Since 2012 he acts as President of of the European AIDS Clinical Society (EACS) He is a faculty member of International Antiviral Society-USA and member of the scientific committee of the biennial Glasgow International Congress on Drug Therapy in HIV Infection. Together with the Swiss Tropical and Public Health Institute he actively participated in the upbuilding of a large rural HIV Clinic at the St. Francis Referral Hospital Ifakara, in Tansania.
His current research focuses on improving the response to antiviral therapy, specifically immune reconstitution and aspects related directly to HIV antiviral therapy. Clinically, he has a special interest in viral diseases.

François Berger

CLINATEC director
Professor of cell Biology and oncology in Grenoble medical university
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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. For the last 4 years he coordinated the Brain Nanomedicine Group in INSERM U 836. 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 CEA-LETI micro-nanotechnology centre. At the interface between technology and medicine, he is the director of Clinatec. Clinatec is a unique clinical-preclinical research facility devoted to the validation of new implanted micro-nanotechnologies at the human brain interface associating biological and imaging facilities to provide the best environment for the first preclinical and human proof of concept.

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

• Since 2011: director of CLINATEC INSTITUE, CEA, 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" evaluated A plus in 2010 by the national evaluation agency.

• 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.

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International patents in the field of nanoproteomic, micro-invasive molecular fingerprints and biomarkers

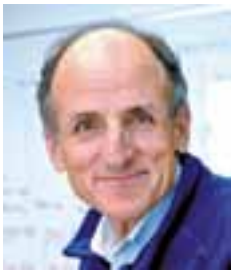
Gerd Binnig

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

Mr. Patrick BOISSEAU is a graduate of the Institut National Agronomique (1983) and of the Ecole Nationale du Génie Rural, des Eaux et des Forêts (1985). He holds an MSc Degree in Human Nutrition. His general academic background is therefore in biology engineering.

FRENCH POSITIONS

He joined the CEA – the French Atomic Energy Commission - in 1987. He had several positions as research fellow in the Life Sciences Division then as an expert on strategy in life sciences and environment at the Foresight & Strategy Division. He became the deputy head of department of biology at the Life Sciences Division, based in Grenoble.

Since 2008, he has been in charge of the business development for NanoMedicine at CEA-Leti, a public Research & Technology organization with expertise in nanoparticles for diagnostics and therapy and more generally on (nano)technology transfer in medical technologies.

In April 2013, he was also put in charge of Strategic Planning in Health Technologies at CEA-Tech, the technological Research Division of CEA.

EUROPEAN ACTIVITIES

From 2004 to 2008, he was the coordinator of the European Network of Excellence in Nanobiotechnology, Nano2Life (www.nano2life.org), with 23 academic partners, 41 companies and 400+ scientists.

In 2006, he became an Executive Board Member of the European Technology Platform on Nanomedicine, and chairman of the working group on "nanotechnology based diagnostics and imaging" (www.etp-nanomedicine.eu). In 2012, he was then elected as Chairman of the Board of the European Technology Platform on Nanomedicine.

Patrick Boisseau coordinates and/or participates in numerous French and European funded R&D projects in nanomedicine.

Patrick Boisseau serves as expert or reviewer for several national/ European bodies: European Commission, European Science Foundation, the European Commission, Oesterreichische Forschungsfoerderungsgesellschaft GmbH (AT), Agence Nationale de la Recherche (FR), EuroNanoMed (EU), CIBER-BBN (ES)…

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.

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.
In the past, Prof. Borchard has served as Scientific Advisor for the Controlled Release Society (CRS), as Scientific Secretary of the European Association of Pharmaceutical Biotechnology (EAPB), and has headed the Academic Section of the International Association for Pharmaceutical Technology (APV). Since 2008, he served as Vice President of the School of Pharmaceutical Sciences Geneva-Lausanne (EPGL) and since 2013 as acting president. In 2012 Prof. h joined the Non Biological Complex Drugs (NBCD) working group hosted at Top Institute Pharma (TIP, Leiden, The Netherlands) and was nominated Chair of the NBCD working party at the European Directorate for the Quality of Medicines & Health Care (EDQM) by Swissmedic.
Prof. Borchard was nominated Fellow of the Swiss Society of Pharmaceutical Sciences (SSPhS) in 2010, and elected President of the Swiss Academy of Pharmaceutical Sciences in 2014. Since 2013, he is also Vice President of the European Federation of Pharmaceutical Sciences (EUFEPS).
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 is fluent in the German, English, Dutch and French languages. Time allowing, he loves to roam the trails and by-roads of the Jura mountains on foot and bike.

Salvador Borrós

Salvador Borrós Obtained his PhD from Institut Químic de Sarria in 1993. He is full professor in Materials Science and Biomaterials at University Ramon Llull in Barcelona. He is the head of the Materials Engineering Group at the same University and Co-founder and CSO of Sagetis Biotech and Sailing Technologies. His main research interest is in the development of smart materials.

Donald Bruce

Dr Donald Bruce is managing director of the independent consultancy Edinethics Ltd., working on ethics of emerging technologies. He holds doctorates in chemistry and theology. From 1976-92 he worked in nuclear energy research, safety and risk regulation, and energy policy. From 1992-2007 he was Director of the Church of Scotland's Society, Religion and Technology Project (SRT), doing pioneering ethical assessment of many emerging technologies including GM crops and animals, cloning and stem cells. He has worked on nano- and converging technologies since 2003, in many contexts, including the ground-breaking EC FP6 Nano2Life project. He is currently doing ethical research on human enhancement in the FP7 ETHENTECH programme, and on stem cells for toxicity testing in ESNATS. He is a member of the advisory board of the Institute of Nanotechnology and gave its Albert Franks lecture at the Royal Society in 2007. He has worked extensively in public engagement with the New Economics Foundation created Democs card games on nanobiotechnology, synthetic biology and human enhancement, and Open-up argument maps. He was a former member of the Scottish Science Advisory Committee, the Societal Issues Panel of Engineering and Physical Sciences Research Council and the Public Affairs advisory group of Bioltechnology Research Council.

Reto Brun

Ph.D., Prof. emer.
Swiss Tropical and Public Health Institute
Basel, Switzerland
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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. As a professor at the University of Basel he supervised over 60 MSc and PhD students and as an author he published over 500 research articles, reviews and book chapters.

Ahuva Cern

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PROFESSIONAL EXPERIENCE

2015– Researcher at the Hebrew University.

2012–2014 Formulation advisor for biotech companies

2007–2010 Director of formulation development at Nextar chempharma solutions, Israel

2000–2007 Research associate and then associate director of formulation department at Pharmos Ltd, Israel

EDUCATION

PhD, Biochemistry, Hebrew University, Jerusalem. Research topic: "Computational models of liposome-based drugs: Discovering candidates for stable remote loading and testing model applicability", 2014 (pending approval)
Supervisors: Prof. Yechezkel Barenholz and Prof. Amiram Goldblum
M.Sc, Biomedical Engineering, Ben-Gurion University, Beer Sheva, 2000
Supervisors: Prof. Smadar Cohen and Prof. Amnon Sintov
B.Pharm, Pharmacy, Hebrew University, Jerusalem, 1996

PUBLICATIONS

1. Cern A, Golbraikh A, Sedykh A, Tropsha A, Barenholz Y, Goldblum A. 2012. Quantitative structure - property relationship modeling of remote liposome loading of drugs. J Control Release 160:147–157.
2. Cern A, Barenholz Y, Tropsha A, Goldblum A. 2014. Computer-aided design of liposomal drugs: In silico prediction and experimental validation of drug candidates for liposomal remote loading. J Control Release 173:125–31.
3. Cern A, Nativ-Roth E, Goldblum A, Barenholz Y. 2014. Effect of Solubilizing Agents on Mupirocin Loading into and Release from PEGylated Nanoliposomes. J Pharm Sci:1–8. Accessed June 11, 2014.

Nam-Joon Cho

Nam-Joon Cho is Nanyang Associate Professor in the School of Materials Science and Engineering at Nanyang Technological University in Singapore and Deputy Director of the Nanyang Institute of Technology in Health and Medicine. In addition, he is a Principal Investigator at the Singapore-MIT Alliance for Research and Technology. His group's research focuses on engineering approaches to solve important biomedical problems and to translate these capabilities into practical applications for global health. Dr. Cho's scientific work has been highlighted by international media organizations such as Reuters, CNBC, and Businessweek, and is leading to major breakthroughs for the treatment of deadly pathogens. He has identified novel classes of drugs to treat hepatitis C virus which affects over 170 million people worldwide. Based on the success of this early work, Dr. Cho's team is now pursuing similar strategies to examine the causes and consequences of infectious diseases, inflammatory disorders and cancer in order to provide improved diagnostic and therapeutic interventions. As part of these activities, Dr. Cho also leads a multi-institution tissue engineering collaboration involving NTU, Singapore General Hospital, and the Stanford University School of Medicine, which focuses on developing an artificial liver platform for regenerative medicine applications. He is a graduate of Stanford University and the University of California, Berkeley.

Sang J. Chung

Sang Jeon Chung received his Ph.D. in Chemistry from POSTECH, Korea, in 1996. Postdoctoral work was performed in the group of Prof. Chi-Huey Wong at the Scripps Research Institute and with Prof. Gregory Verdine at Harvard University. In 2003, he was appointed as a Senior Scientist at the KRIBB and was promoted to Principal Scientist in 2009. In March 2013, he moved to Dongguk University, Seoul, Korea. Currently he is an Associate Professor of the Chemistry Department and director of the Molecular Targeting Research Center of Dongguk University and also head of the Chemical Biology laboratory at Dongguk University.

Daan J.A. Crommelin

PhD
Prof. Daan Crommelin is emeritus-professor 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 also advises venture capital groups. 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).

Lea Ann Dailey

PhD
Lea Ann is a pharmacist by training and gained a PhD at the Philipps University in Marburg, Germany, on the topic of 'Polymeric nanoparticles for aerosol delivery to the lung'. She worked as a post-doctoral fellow for Nektar Therapeutics (now Novartis) in San Carlos, CA, USA, before taking up a position as Lecturer at King's College London in the UK. Currently a Senior Lecturer at King's, Lea Ann's research interests focus on 'safety-by-design' approaches for the development of nanomedicines and nanodiagnostics. She works with a wide range of biomaterials, including polymeric, lipidic and protein-based nanosystems, to characterize the in vitro and in vivo behavior of nanomaterials designed for use as nanomedicines. Knowledge gained through studying first nanomaterial biocompatibility and then biodistribution profiles is used to subsequently select the most promising nanoparticle platforms for drug encapsulation and development towards different therapeutic or diagnostic strategies.

Athanasia Dasargyri

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Athanasia obtained her degree on Pharmaceutical Sciences at the University of Athens, Greece, and subsequently completed a Master's program on 'Pharmaceutical Technology and Biopharmacy' in the Faculty of Pharmacy of Université Paris-Sud in France. She conducted her Master thesis at the Institute Galien Paris-Sud on the 'Formulation and preliminary biological evaluation of Methotrexate-loaded polymeric nanoparticles'. Athanasia is currently a PhD student at the 'Drug Formulation and Delivery' research group of the Institute of Pharmaceutical Sciences of ETH Zurich, under the supervision of Professor Jean-Christophe Leroux. Her current main focus is on the study of the interactions of tumor-targeting ligands with tumor cells.

Colin M Dayan

PERSONAL DETAILS

Title: Professor of Clinical Diabetes and Metabolism, Director, Institute of Molecular and Experimental Medicine
Institution: Cardiff University
Department: IMEM
Address: University Hospital Wales, Heath Park, Cardiff. CF14 4XN, Tel.: 029 2074 2182, E-mail: DayanCM@cardiff.ac.uk
Post-doctoral research experience (for Health Research Awards only)

HIGHER EDUCATION

1978–81 BA Phsyiological Sciences, University College, Oxford. MA conferred 1989
1981–4 MBBS with honours in Pathology and Surgery, Guys' Hospital Medical School, University of London
1987 Membership of the Royal College of Physicians (UK)
1991 PhD in Immunology, Charing Cross and Westminster Medical School, University of London

WORK HISTORY:

2009–2010 Reader in Medicine, University of Bristol (HEFCE funded, tenured).
2009–2010 Clinical Director, Clinical Research and Imaging Unit, University of Bristol
2010– Professor of Clinical Diabetes and Metabolism & Section Head, Cardiff University (30 staff)
2011– Director, Institute of Molecular and Experimental Medicine, Cardiff University School of Medicine (120 staff).

CURRENT GRANTS:

- Diabetes UK. Dayan CM, Waldron-Lynch F, Leslie R.D.G., Todd J. Type 1 diabetes UK – Clinical Engagement and Training Core. £1,068,514 1/1/15 – 30/12/17.
- Juvenile Diabetes Research Foundation (USA). Dayan CM. Monopeptide Trial study extension. $64,000 1/9/14 – 31/7/15
- Juvenile Diabetes Research Foundation (USA) Dayan CM, Birchall J, Coulman S, Wong FS. Microneedle arrays to deliver antigen specific immunotherapy. $320,000. 1/10/14-30/9/16.
- European Union. Dayan CM, Wong FS, Birchall J, Coulman S, Piguet V, Mous J, Verhouni P, Levin Y, Weinbach J, Roep BO, Peakman M, Ludvigssen J. Dayan CM coordinator. FP7 coordinating grant. Enhanced Epidermal Antigen Specific Immunotherapy (EE-ASI). 6m Euros. 1/9/12 – 30/8/16.
- Diabetes UK. Wong FS and Dayan CM. Immune studies of B lymphocyte function in type 1 diabetes. 1/3/12 – 28/2/15. £240,000
- European Union. Mathieu C and colleagues inc Dayan CM. Natural immunomodulators as novel immunotherapies for type 1 diabetes €10,928,000 (€611,700 for CMD) 1/11/09 – 31/10/2014 – extended to 30/4/15

SELECTED PUBLICATIONS:

1. Sayers A, Thayer D, Harvey J, Luzio S, Dayan CM, Wong FS, Gregory J (2014). Evidence for a persistent, major excess in all cause admissions to hospital in children with Type-1 diabetes: results from a large Welsh national matched community cohort study. BMJ Open, in press.
2. Skowera A, Ladell K, McLaren JE, Dolton G, Matthews KK, Gostick E, et al. beta-cell-specific CD8 T cell phenotype in type 1 diabetes reflects chronic autoantigen exposure. Diabetes. 2015 Mar;64(3):916-25
3. Ambery P, Donner TW, Biswas N, Donaldson J, Parkin J, Dayan CM. Efficacy and safety of low-dose otelixizumab anti-CD3 monoclonal antibody in preserving C-peptide secretion in adolescent type 1 diabetes: DEFEND-2, a randomized, placebo-controlled, double-blind, multi-centre study. Diabet Med. 2013 Nov 16. doi: 10.1111/dme.12361. [Epub ahead of print]
4. Williams AJ, Thrower SL, Sequeiros IM, Ward A, Bickerton AS, Triay JM, Callaway MP, Dayan CM. (2012). Pancreatic volume is reduced in adult patients with recently diagnosed type 1 diabetes. J Clin Endocrinol Metab. 2012 Nov;97(11):E2109-13. doi: 10.1210/jc.2012-1815. Epub 2012 Aug 9.
5. Cooper AR, Sebire S, Montgomery AA, Peters TJ, Sharp DJ, Jackson N, Fitzsimons K, Dayan CM, Andrews RC. (2012). Sedentary time, breaks in sedentary time and metabolic variables in people with newly diagnosed type 2 diabetes. Diabetologia. Mar;55(3):589-99. Epub 2011 Dec 14
6. Arif, S. Moore, F. Marks, K. Bouckenooghe, T. Dayan, C. M. Planas, R. Vives-Pi, M. Powrie, J. Tree, T. Marchetti, P. Huang, G. C. Gurzov, E. N. Pujol-Borrell, R. Eizirik, D. L. Peakman, M. (2011). Peripheral and islet interleukin-17 pathway activation characterizes human autoimmune diabetes and promotes cytokine-mediated beta-cell death. Diabetes 60: 2112-9.
7. Andrews RC, Cooper AR, Montgomery AA, Norcross AJ, Peters TJ, Sharp DJ, Jackson N,, Fitzsimons K, Bright J, Coulman K, England CY, Gorton J, McLenaghan A, Paxton E, Polet A, Thompson C, Dayan CM. (2011). The Early ACTID randomised controlled trial of diet versus diet plus physical activity versus usual care in newly-diagnosed type 2 diabetes. Lancet 378(9786):129-39
8. Hermanides J, Nørgaard K, Bruttomesso D, Mathieu C, Frid A, Dayan CM, Diem P, Fermon C, Wentholt IM, Hoekstra JB, Devries JH. (2011). Sensor-augmented pump therapy lowers HbA(1c) in suboptimally controlled Type 1 diabetes; a randomized controlled trial.. Diabet Med. 2011 Feb 5. doi: 10.1111/j.1464-5491.2011.03256.x. [Epub ahead of print]
9. Petrich de Marquesini LG, Fu J, Connon KJ, Bishoph AJ, Mclintock NE, Pope C, Wong FS, Dayan CM. (2010). Interferon gamma and Interleukin-10 islet-antigen specific T cell responses in autoantibody negative first-degree relatives of subjects with type 1 diabetes. Diabetologia 53:1451-60
10. Thrower S, James L, Hall W, Green KM, Arif P, Allen, JS, Van-Krinks C, Lozanoska-Oscher B, Marquesini L, Brown S, Wong DS, Dayan C and Peakman M. (2009). Proinsulin peptide immunotherapy in Type 1 diabetes: report of a first-in-man Phase I safety study. Clinical and Experimental Immunology 55(2):156-65.
11. Anita Skowera, Richard J Ellis, Ruben Varela-Calviño, Sefina Arif, Guo Cai Huang, Cassie Van-Krinks, Anna Zaremba, Chloe Rackham, Jennifer S Allen, Timothy IM Tree, Min Zhao, Colin M Dayan, Wendy Unger, Jan W Drijfhout, Ferry Ossendorp, Bart O Roep, Mark Peakman. (2008). Glucose-regulated cytotoxic T-cell epitope of preproinsulin targets killing of beta-cells in human Type 1 diabetes J Clin Invest 118:3390-3402

Jon de Vlieger

PhD

Jon de Vlieger obtained his doctoral degree in bio-analytical chemistry from the VU University in Amsterdam. He is involved in the strategy department of the Dutch Top Institute Pharma since May 2011. He was responsible for shaping the institutes' process to initiate new Public Private Partnerships and managed the most recent TI Pharma call for SME Partnership projects in 2012. He coordinates several international consortia, scouts for new partners to strengthen these and also engages in teaching activities on Public Private Partnerships and solutions for Neglected Diseases.

Neil P. Desai

PhD

Neil Desai is VP of Strategic Platforms at Celgene Corp. and Founder/CEO of AADi, LLC, a clinical stage start-up developing targeted therapeutics for oncology/cardiovascular application. Prior to its acquisition by Celgene in 2010 for approximately $3B, he was SVP of Global R&D at Abraxis Bioscience (Los Angeles, California, USA), 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 approximately $850M in 2014. 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.

Marc Donath

Name: Donath
First Name: Marc Yves
Date of birth: November 2, 1963
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Marc Y. Donath is Professor and Head of the Clinic for Endocrinology, Diabetes and Metabolism at the University Hospital of Basel, Switzerland.
His main scientific contribution is the description of an inflammatory process underlying the failure of the pancreatic islet to produce sufficient amount of insulin in Type 2 diabetes, with a central role for IL-1ß. On the basis of this he initiated a pioneering clinical trial in patients with Type 2 diabetes that vindicates his hypothesis and opens the way for a causative treatment and prevention of diabetes. These studies have now entered the phase 3 of clinical trials. Furthermore he identified a new endocrine loop by showing that elevated IL-6 mediates a cross talk between insulin sensitive tissues, L cells and pancreatic islets to adapt to changes in insulin. Finally, recently Dr. Donath has uncovered the first monogenic form of type 1 diabetes. Overall this research has contributed to the concept that the innate immune system is part of the regulation of metabolism.

Mike Eaton

After a postgraduate training in nucleic acid chemistry Mike Eaton worked in research in the Pharma industry for more than 35 years. At GD Searle he headed the team that synthesised the gene for Urogastrone and was the first to sequence and express human beta fibroblast interferon in E.coli. He was a founding member of Celltech as Head of Chemistry in 1980; later acquired by UCB. He has worked on a number of marketed drugs - Mylotarg in 2000, the first Antibody drug conjugate and Cimzia in 2009; the first PEGylated antibody. Unusually he has worked with both small molecules and large molecules, including DNA at a technical as well as at a strategic level. He built the first automated DNA synthesiser in Europe, which is now owned by the Science Museum in London. This machine was used for the first cloning of pre-prochymosin, a key ingredient in cheese-making. He has worked on low molecular weight drugs including the first non-emetic PDEIV inhibitor and synthetic vectors for gene therapy. He has maintained his interest in nucleic acid based therapeutics and believes this will be an important class once the delivery issues have been solved.
Mike is an active special professor at Nottingham University and has been an executive board member of the European Technology Platform for Nanomedicine, since its inception in 2005.This is a large network of academics and industries, being the European working group chair for Nano-therapeutics. He left UCB in February 2010 and is now a strategic and technical adviser to a number of large and small companies and organisations, including VCs. His particular interest is commercial translation of nanotechnology into Nanomedicines – real medicines to help patients.
Inter alia he is on the scientific advisory board of Future Medicinal Chemistry, Nanomedicine and Nanomedicine J: Nanotechnology, Biology, and Medicine and CLINAM (www.clinam.org) .
Since leaving UCB, as part of the ETP he has tried to improve the design and translation of open innovation. As part of this initiative he has been involved in publishing a white paper for the EC in 2011 http://www.etp-nanomedicine.eu/public/news-events/news/etpn-white-paper-on-improving-translation-of-public-healthcare-nano-research-in-europe. More technical details including milestones are provided in Nanomedicine -NBM 7 (2011) 371–375 including an online supplement, this is now a key, albeit basic source for SMEs.
As a contributor to the prestigious Else Kröner-Fresenius Symposium in Germany he was invited to contribute in 2011 a chapter to Nanomedicine – Basic and Clinical Applications in Diagnostics and Therapy. With the assistance of John Adair this was published in-Else Kröner-Fresenius Symp. Basel, Karger, 2011, vol 2, pp 185–196.
With a background in both small and large molecules he has contributed to Future Medicinal Chemistry both as a reviewer and as an author Future Med. Chem. (2011) 3(15). His early hands-on pioneering knowledge of developing ADCs has been much sought, after this technology has recently been successfully re-evaluated in the clinic. Lastly he has experience in the courts on litigation relating to IP, having also filed a large number of patents as well as publications over the years.

KEY REFERENCE

Eaton M.A.W., et al., Delivering nanomedicines to patients: A Practical guide. Nanomedicine: NBM 2015;11:1-10, http://dx.doi.org/10.1016/j.nano.2015.02.004

Falk Ehmann

MD, PhD, MSc
Falk Ehmann is currently working at the European Medicines Agency (EMA) on Clinical Pharmacology – Science and Innovation. His main responsibilities include managing the Innovation Task Force promoting Innovation and novel methodologies in drug development with focus in the areas of Pharmacogenomics (Clinical Pharmacology), Nanomedicines, Borderline and Combined Medicinal Products, and other -omics especially in connection with Personalized Medicine.
Further areas of expertise include policy development of Similar Biological Medicinal Products (Biosimilars) with focus on monoclonal antibodies and Vaccines.
He held various positions and responsibilities at the EMA since 2004, including Scientific Advice during product development and working in the Oncology and Anti-Invectives therapeutic areas of the EMA Unit for Human Medicines Development and Evaluation.

Prior to joining the EMA Dr Ehmann studied European and International law at the University Berlin, was a Public Health Researcher at the Robert Koch Institute and Medical Intern at different University Hospitals. Falk Ehmann wrote his PhD thesis on Molecular Intra Cellular Cell Signalling at the Institute of Biochemistry and Molecular Biology at the University Hospital Hamburg-Eppendorf. His Master Thesis discusses coping mechanisms and responses of European Health Care Systems to the 2009 H1N1v Influenza Pandemic. Dr Ehmann published more than 20 articles in peer reviewed journals.

PUBLICATIONS

- Changes and determination of dosing recommendations for medicinal products recently authorised in the European Union; Expert Opinion 2015; Falk Ehmann, Marisa Papaluca, Francesca Di Giuseppe, Luca Pani, Andrea Eskova, Efthymios Manolis & Ralf Herold
- White spots in pharmaceutical pipelines – EMA identifies potential areas of unmet medical needs; Marisa Papaluca, Martina Greco, Enrico Tognana, Falk Ehmann and Agnes Saint-Raymond; Expert Rev. Clin. Pharmacol. Early online, 1–8 (2015)
- Pharmacogenomic information in drug labels: European Medicines Agency perspective; F Ehmann, L Caneva, K Prasad, M Paulmichl, M Maliepaard2,5,6, A Llerena, M Ingelman-Sundberg and M Papaluca-Amati; The Pharmacogenomics Journal (2015), 1–10
- Cancer drug development and the evolving regulatory framework for companion diagnostics in the European union. Pignatti F1, Ehmann F, Hemmings R, Jonsson B, Nuebling M, Papaluca-Amati M, Posch M, Rasi G.; Clin Cancer Res. 2014 Mar 15;20(6):1458-68. doi: 10.1158/1078-0432.CCR-13-1571.
- European Medicines Agency initiatives and perspectives on pharmacogenomics. Falk Ehmann, Laura Caneva & Marisa Papaluca; Br J Clin Pharmacol / 77:4 / 612–617 2014
- Next-generation nanomedicines and nanosimilars: EU regulators' initiatives relating to the development and evaluation of nanomedicines. Ehmann F, Sakai-Kato K, Duncan R, Hernán Pérez de la Ossa D, Pita R, Vidal JM, Kohli A, Tothfalusi L, Sanh A, Tinton S, Robert JL, Silva Lima B, Amati MP. Nanomedicine (Lond). 2013 May;8(5):849-56. doi: 10.2217/nnm.13.68.
- Gatekeepers and enablers: how drug regulators respond to a challenging and changing environment by moving toward a proactive attitude. Ehmann F, Papaluca Amati M, Salmonson T, Posch M, Vamvakas S, Hemmings R, Eichler HG, Schneider CK. Clin Pharmacol Ther. 2013 May;93(5):425-32. doi: 10.1038/clpt.2013.14. Epub 2013 Jan 24.
- Modelling the risk-benefit impact of H1N1 influenza vaccines. Phillips LD, Fasolo B, Zafiropoulous N, Eichler HG, Ehmann F, Jekerle V, Kramarz P, Nicoll A, Lönngren T. Eur J Public Health. 2013 Feb 11. [Epub ahead of print]
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Rutledge Ellis-Behnke

Rutledge Ellis-Behnke is the Director of the Nanomedicine Translational Think Tank at the Medical Faculty Mannheim of the University of Heidelberg in Germany.

In addition, he holds affiliate faculty positions at MIT, as well as Wake Forest and University of South Florida medical schools.

Previously he was Associate Professor in the Faculty of Medicine at the University of Hong Kong, as well as Associate Director of the Technology Transfer Office.
Ellis-Behnke is redefining tissue engineering for nanomedicine. His research is focused on reconnecting the disconnected parts of the brain—with the goal of being able to provide a prescription to restore quality of life after brain or spinal cord trauma, or stroke. In animals he was the first to repair the brain showing reversal of blind-

ness; to stop bleeding in less than 15 seconds without clotting; to preserve stem cells; and to immobilize prostate cancer stem cells.
Ellis-Behnke is an advisor to, and co-founder of, Arch Therapeutics. He has multiple worldwide patent applications and his "Nano Neuro Knitting" and "Immediate Hemostasis" technologies have each been licensed to companies for translation to humans. Technology Review named his "Nanohealing" discoveries one of the "Top 10 Emerging Technologies."
Ellis-Behnke received a PhD from MIT in Neuroscience; a Bachelor of Science from Rutgers University and graduated from Harvard Business School's Advanced Manager's Program (AMP).
Prior to returning to school to pursue his PhD, Ellis-Behnke held various management positions including Senior Vice President of Huntingdon Engineering and Environmental, a public company for testing and consulting services; and in 1995 was co-founder/CEO of one of the first internet companies in the world to do online commerce.
In addition to his work in neuroscience and nanomedicine, Ellis-Behnke introduced the TabletPC to MIT in 2001 and the University of Hong Kong in 2005, as part of the migration to the paperless classroom to deliver all course material and texts to the students digitally. At both MIT and the University of Hong Kong the students learned 25% more material; and the bottom 25% of the class improved by one letter grade.
Ellis-Behnke is Associate Editor for Nanomedicine: Nanotechnology, Biology and Medicine; and also Frontiers in Neurotrauma. He is a founding board member of the International Society of Nanomedicine; and is on the Scientific Advisory Board of the Glaucoma Foundation.

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.

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 Distinguished Adjunct Professor at the King Abdulaziz University in Jeddah, Saudi Arabia. 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 115 papers and holds more than 140 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 three biotechnology companies: BIND Therapeutics (NASDAQ: BIND), Selecta Biosciences, Blend Therapeutics, 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. Dr. Farokhzad was elected to the College of the Fellows of the American Institute of Medical and biological Engineering. 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, and in the same year he was also selected by Thomson Reuters as one of the World's Most Influential Scientific Minds, which recognizes the most highly cited scientists across numerous disciplines such as biology, chemistry, physics, immunology, economics and engineering. In 2013, 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.

Elias Fattal

Elias Fattal is a full professor in Drug Delivery Science at the University of Paris-Sud in Châtenay-Malabry, France and has been President of APGI from 2003 to 2010. He received his Pharmacy Degree (1983) and Ph.D. (1990) from the University of Paris-Sud and followed an internship in Pharmacy at the University of Lille (1984–1986). After visiting the Department of Pharmaceutical Chemistry at the University of California, San Francisco for a post-doctoral position (1990–1991), he became associate Professor (1992) and full Professor at the University of Paris-Sud (2000). He is heading the Institut Galien Paris Sud. Over the past 25 years, he has made outstanding fundamental and applied contributions to the fields of drug delivery using nanotechnologies for targeted or local delivery of drugs and nucleic acids. Several of his inventions have been licensed to the pharmaceutical industry. Recently, he has been involved in the development of an adjuvant to antibiotics able to reduce resistance by adsorbing residual colonic antibiotics (clinical phase I). Prof. Fattal has authored more than 200 refereed articles and 26 book chapters. He has issued 10 international patents and has received the Pharmaceutical Sciences World Congress (PSWC) Research Achievement Award. He serves in the editorial board of several journals among which Journal of Controlled Release and Nanomedicine NMB.

Delphine Felder-Flesch

Institute of Physics and Chemistry of Materials, IPCMS UMR CNRS-UDS 7504, 23 rue du loess BP 43 67034 Strasbourg, France.
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 tumor targeting and cancer diagnostic imaging. She also develops, in collaboration with solid state chemists, dendronized nanoparticles (metal oxides) for MRI and hyperthermia.

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.
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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: 74; **CONFERENCE CONTRIBUTIONS**: 124

Mauro Ferrari

Ph.D.,President and CEO,Ernest Cockrell Jr. Presidential Distinguished Chair, Houston Methodist Research Institute/Director, Institute for Academic Medicine, Executive Vice President, Houston Methodist Hospital System/Senior Associate Dean & Professor of Medicine, Weill Cornell Medical College, New York

Dr. Mauro Ferrari serves as President and CEO of Houston Methodist Research Institute (HMRI) in Houston, Texas USA, where he holds the Ernest Cockrell Jr. Presidential Distinguished Chair. He is also Executive Vice President of Houston Methodist Hospital System and Director of the Houston Methodist Institute for Academic Medicine. In these capacities, he presides over a research program comprising over 1,200 employees, 820 clinical trials, and a yearly budget exceeding $130 M. As Executive Vice President of the Houston Methodist Hospital System, one of the best health care institution in the United States, he oversees the academic activities of seven hospital and approximately 16,000 employees. He concurrently serves at Senior Associate Dean and Professor of Medicine at Weill Cornell Medical College, in New York, and holds Adjunct and Honorary Professorships at many universities around the world.
Dr. Ferrari's degrees are in Mathematics (Padova, 1985, Italy), and Mechanical Engineering (U.C. Berkeley, M.S. 1987, & Ph.D. 1989). He attended medical school at the Ohio State University (2002-04).
Dr. Mauro Ferrari is a founder of biomedical nano/micro-technology, especially in their applications to drug delivery, cell transplantation, implantable bioreactors, and other innovative therapeutic modalities. In these fields, he has published more than 275 peer-reviewed journal articles and six books. He is the inventor of more than 30 issued patents, with about thirty more pending in the US and internationally. He has received many prestigious honors, and research funding from NCI, NIH, DoD, NASA, NSF, DARPA, DoE, the State of Texas, and the State of Ohio, The Ohio State University, and several private enterprises.
Dr. Ferrari has also served in various capacities in the mentorship of prior students and post-doctoral trainees who have secured senior faculty positions at MIT, Oxford (chair), UC-San Francisco (chair), UC Berkeley, Georgetown, Duke, Ohio State, the Universities of Washington, and Florida, among many others.
His primary research is in Cancer Therapeutics and Diagnostics, Nanomedicine, Oncophysics, Nanotechnology, Regenerative Medicine, Drug Delivery, Mechanics of Solids, Mathematical Physics, and Bioethics.

Alke Fink

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Prof. Alke Petri-Fink received her Ph.D. in Chemistry from the University of Ulm, Germany in 1999. After a post-doctoral stay at the University of Gainesville, Florida, she joined the Institute of Materials Science at the École Polytechnique Fédérale de Lausanne (EPFL), first as a post-doctoral researcher, then as a senior scientist. She became an Associate Swiss National Science Foundation Professor in the Department of Chemistry at the University of Fribourg in 2009, and Full Professor in 2011 at the Adolphe Merkle Institute, Switzerland. Her research focuses on inorganic nanoparticles, their synthesis, surfaces, and interactions with biological cells.

Patrick Frederix

Patrick Frederix studied applied physics at Eindhoven University of Technology and has a PhD in Biophysics from Utrecht University. After his PhD he moved to Switzerland and joined the group of Prof. Andreas Engel to work on nanobiology studying membrane proteins by scanning probe microscopy and spectroscopy. In 2010 he started as Application scientist and service engineer at Nanosurf and is now leading this department.

Heico Frima

Heico Frima obtained his Masters Degree in Applied Physics from the Technical University of Delft in 1980 and then worked in various R&D and product management functions in the semiconductor equipment industry. Since 1990 he is Programme Officer in the Directorate-General for Research & Innovation of the European Commission in Brussels in the field of micro-technology and then from 2002 as Programme Officer for nanoscience and nanotechnology. As Programme Officer he contributes to programme policy development, the organisation of research proposal evaluations, contract negotiations and follow-up of research projects that are funded by the European Commission. Presently he is responsible for research policy in the field of nanomedicine, working in the Unit 'Advanced Materials and Nanotechnology'. Heico has the Dutch nationality, is married and has two children.

Katharina M. Fromm

Katharina M. Fromm finished her chemistry studies in Strasbourg, France as valedictorian of her year. After her PhD in 1994 from the University of Karlsruhe, Germany, she became a postdoctoral researcher with Prof. Strähle in Tübingen, Germany and Nobel-Prize winner Jean-Marie Lehn in Strasbourg, France. In 1998, she joined the University of Geneva where she defended her habilitation thesis in 2002. In the same year, she accepted an Emmy Nother Program II from the Deutsche Forschungsgemeinschaft, with which she moved to Karlsruhe University. In 2003, she was awarded with a Swiss National Science Foundation professorship, bringing her to Basel where she build up her research group. Since 2006, she is full professor at the University of Fribourg, Switzerland, and her research spans from antimicrobial coatings for implants to nanoscale battery materials.

Alberto A. Gabizon

Alberto Gabizon received his M.D. from the School of Medicine, University of Granada, Spain, and his Ph.D. in Cell Biology from the Weizmann Institute of Science, Rehovot, Israel. He later completed his training and certification in Radiation and Medical Oncology at Hadassah Medical Center (Jerusalem, Israel). Durign his research fellowship in San Francisco (UCSF Medical Center, Cancer Research Institute), 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 with important pharmacologic and safety advantages over conventional chemotherapy. His most recent invention currently in clinical studies is PROMITIL (pegylated liposomal mitomycin-C prodrug). 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 National Prize of Medicine (Spain, 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 around 150 articles and specialized book chapters.

Dr. Gabizon is Chairman of the Oncology Institute at Shaare Zedek Medical Center, and Professor of Oncology at the Hebrew University-Faculty of Medicine in Jerusalem.

RECENT PUBLICATIONS:

Grenader T, and Gabizon A: "Malignant Epithelioid Hemangioendothelioma of the Liver Successfully Treated with Pegylated Liposomal Doxorubicin". J Clin Oncol, 29(25): e722-724, 2011.

Safra T, Borgato L, Nicoletto MO, Rolnitzky L, Pelles-Avraham S, Geva R, Donach ME, Curtin J, Novetsky A, Grenader T, Lai WV, Gabizon A, Boyd L, and Muggia F: "BRCA mutation status and determinant of outcome in women with recurrent epithelial ovarian cancer treated with pegylated liposomal doxorubicin." Molec Cancer Therap, 10(10):2000-2007, 2011.

• Szebeni J, Muggia F, Gabizon A, and Barenholz Y: "Activation of complement by therapeutic liposomes and other lipid excipient-based therapeutic products: prediction and prevention." Adv Drug Deliv Rev, 63(12):1020-30, 2011.

• Avnir Y, Turjeman K, Tulchinsky D, Sigal A, Kizelsztein P, Tzemach D, Gabizon A, and Barenholz Y: "Fabrication principles and their contribution to the superior in vivo therapeutic efficacy of nano-liposomes remote loaded with glucocorticoids." PLoS One, 6(10):e25721, 2011.

• La-Beck NM, Zamboni BA, Gabizon A, Schmeeda H, Amantea M, Gehrig PA, and Zamboni WA: "Factors Affecting the Pharmacokinetics of Pegylated Liposomal Doxorubicin in Patients." Cancer Chemotherapy Pharmacol, 69(1):43-50, 2012.

• Gabizon A, Shmeeda H, and Grenader T: "Pharmacological basis of pegylated liposomal doxorubicin: Impact on cancer therapy". Eur J Pharm Sci, 45(4):388-398, 2012.

Agnelli G, George DJ, Kakkar AK, Fisher W, Lassen MR, Mismetti P, Mouret P, Chaudhari U, Lawson F, Turpie AG, SAVE-ONCO Investigators: "Semuloparin for thromboprophylaxis in patients receiving chemotherapy for cancer." N Engl J Med, 366(7):601-609, 2012.

Gabizon A, Amitay Y, Shmeeda H, and Zalipsky S: "Therapeutic efficacy of a lipid-based prodrug of mitomycin C in pegylated liposomes: Studies with human gastro-entero-pancreatic ectopic tumor models." J Control Rel, 160(2):245-253, 2012.

• Petersen AL, Hansen AE, Gabizon A, and Andresen TL: "Liposome Imaging Agents in Personalized Medicine." Adv Drug Deliv Rev, 64(13):1417-1435, 2012.

• Grenader T, Rosengarten O, Isacson R, Plotkin Y, and Gabizon A: "Pegylated liposomal doxorubicin/carboplatin combination in ovarian cancer, progressing on single-agent pegylated liposomal doxorubicin." World J Clin Oncol, 3(10): 137-141, 2012.

• Shmeeda H, Amitay Y, Tzemach D, Gorin D, and Gabizon A: "Liposome Encapsulation of Zoledronic Acid Results in Major Changes in Tissue Distribution and Increase in Toxicity." J Control Rel, 167:265–275, 2013.

• Prabhakar U, Blakey DC, Maeda H, Jain RK, Sevick-Muraca EM, Zamboni W, Farokhzad OC, Barry ST, Gabizon A, and Grodzinski P:

”Challenges and key considerations of the enhanced permeability and retention effect (EPR) for nanomedicine drug delivery in oncology.“ Cancer Res, 73(8):2412-2417, 2013.
• Grenader T, Yevgeni Plotkin Y, Maya Gips M, Nathan Cherny N, and Gabizon A: “Diethylstilbestrol for the treatment of patients with castration-resistant prostate cancer: Retrospective analysis of a single institution experience.” Oncol Rep, 31(1):428-434, 2014.
• Parente-Pereira AC, Shmeeda H, Whilding LM, Zambirinis CP, Foster J, van der Stegen SJC, Beatson R, Zabinski T, Brewig N, Sosabowski JK, Mather S, Ghaem-Maghami S, Gabizon A, and Maher J: “Adoptive immunotherapy of epithelial ovarian cancer with Vγ9Vδ2 T-cells, potentiated by liposomal alendronic acid”. J Immunol, 193:5557-5566, 2014.
• Gabizon A, Bradbury M, Prabhakar U, Zamboni W, Libutti S, and Grodzinski P: “Cancer nanomedicines - crossing the translational gap.” Lancet, 384:2175-2176, 2014.
• Cherny, NI, de Vries EGE, Emanuel L, Fallowfield L, Francis PA, Gabizon A, Piccart MJ, Sidransky D, Soussan-Gutman L, and Tziraki C: “Words Matter: Distinguishing Personalized Medicine and Biologically Personalized Therapeutics.” J Natl Cancer Inst, 106 (12), 2014.
• Sabnani MK, Rajan R, Rowland B, Mavinkurve V, Wood LM, Gabizon AA, and La-Beck NM: “Liposome promotion of tumor growth is associated with angiogenesis and inhibition of antitumor immune responses.” Nanomedicine, 11(2):259-262, 2015.
• Nasser NJ, Na’amad M, Weinberg I, and Gabizon AA: “Pharmacokinetics of low molecular weight heparin in patients with malignant tumors.” Anti-Cancer Drugs, 26:106-111, 2015.
• Tahover E, Patil YP, and Gabizon AA: “Emerging delivery systems to reduce doxorubicin cardiotoxicity and improve therapeutic index: Focus on liposomes.” Anti-Cancer Drugs, 26(3):241-258, 2015.
• Saad F, Fizazi K, Jinga V, Efstathiou E, Fong PC, Hart LL, Jones R, McDermott R, Wirth M, Suzuki K, MacLean DB, Wang L, Akaza H, Nelson J, Scher HI, Dreicer R, Webb IJ, de Wit R; ELM-PC 4 Investigators: “Orteronel plus prednisone in patients with chemotherapy-naive metastatic castration-resistant prostate cancer (ELM-PC4): a double-blind, multicentre, phase 3, randomised, placebo-controlled trial.” Lancet Oncol, 16(3):338-348, 2015.
• Scomparin A, Salmaso S, Eldar-Boock A, Ben-Shushan D, Ferber S, Tiram G, Shmeeda H, Landa-Rouben N, Leor J, Caliceti P, Gabizon A, and Satchi-Fainaro R: “A comparative study of folate receptor-targeted doxorubicin delivery systems: Dosing regimens and therapeutic index.” J Control Rel, Epub Apr 11, 2015.

Doris Gabriel

Doris Gabriel is R&D manager at Apidel SA, a pharmaceutical startup company developing innovative (nano)-medicines based on their proprietary technology ApidSOL and ApidCOR. 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.

Eusebio Gainza

Praxis Group R&D Manager
Biopraxis Research AIE General Manager

Eusebio Gainza, PhD in Chemical Engineering in the University of the Basque Country, has a long research career thanks to his experience such as Department manager in the Industry Department of the Basque Country. During 14 years he was managing director of Leia Foundation and Chairman of Laboratorio Sanitatis, S.L. During his time as Dean of the College of the Industrial Engineers of Alava, he directed several doctoral theses. Simultaneously he was President of Iberian Network of Innovation support centers, manager of the Supervisory Board of ALTEC (Asociación Latinoamericana de Tecnología); member of the Basque Council of the European Movement and head of National Platforms of the European Technologic Platform on Industrial Safety.
Since 2008, is the Chairman of BIOPRAXIS and R&D and industry director of Praxis Group. He is involved in quality assurance and regulatory affairs of the pharmaceutical industry for the last 15 years. Moreover, he is the author of several scientific articles and congress contributions and listed as inventor of 5 patents. He has participated in more than 100 research projects funded by regional, national and international organizations, which support his research experience.

Jérôme Galon

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.

Ehud Gazit

PhD FRSC
Department of Molecular Microbiology and Biotechnology
Department of Materials Science and Engineering
Sagol School of Neuroscience
Chair for Biotechnology of Degenerative Diseases
Tel Aviv University

Prof. Ehud Gazit is is Professor and the incumbent of the Chair for Biotechnology of Degenerative Diseases at Tel Aviv University. From 2012-2014 he served as the Chief Scientist of the Israeli Ministry of Science and Technology (MOST). In the years 2008-2012 he served as Tel Aviv University Vice President for Research and Development and the Chairman of the board of directors of Ramot Ltd., the technology transfer company of Tel Aviv University. Prior to his appointment as Vice President, Gazit served in different academic and administrative positions at Tel Aviv University, including the Head of The Chemistry-Biology double major track, a member of the University Committee for Appointments and Promotions, the Head of the Academic Committee of the Ilona Rich Institute for Nano-Biology and Nano-Biotechnology, and a member of the managing board of the Center for Nanoscience and Nanotechnology. Gazit received his B.Sc. (summa cum laude) after completing his studies at the Special Program for Outstanding Students of Tel Aviv University (Currently the Adi Lautman program), and his Ph.D. (with highest distinction) as a Clore Fellow at the Department of Membrane Research and Biophysics, Weizmann Institute of Science in 1997. For his Ph.D. work, he received the John F. Kennedy Award in 1996. He has been a faculty member at Tel Aviv University since 2000, after completing his postdoctoral studies as a European Molecular Biology Organization (EMBO) and Human Frontiers Science Program (HFSP) fellow at Massachusetts Institute of Technology (MIT) where he also had held a visiting appointment (2002–2011).
Gazit's research is directed toward the study of protein folding, misfolding, and self-assembly. His work has resulted in the identification of elements that facilitate the assembly of amyloid fibrils, associated with Alzheimer's disease, and he has identified novel ways to inhibit this process. His laboratory was the first to discover aromatic dipeptides that form nanotubes and nanospheres with unique mechanical and chemical properties. Applications of these nanoassemblies include ultra-sensitive biosensors, energy-storage devices, and metallic nanowires. His work has been published in prestigious journals such as Science, Nature Nanotech., Nature Chem. Biol., and Cell. He is or was on the editorial board of eight journals including Nanomedicine, PLoS ONE, Amyloid and Current Chemical Biology. Gazit had received numerous awards and honors including the Landau Research Award, Dan David Scholarship Award, and TAU Research Council Prize. His technology transfer achievements were acknowledged by inclusion in the 2008 list of "100 Innovations from Academic Research to Real-World Application" by the Association of University Technology Managers (AUTM).

Robert Geertsma

Senior Scientist, Centre for Health Protection, National Institute for Public Health and the Environment (RIVM)

Robert Geertsma has worked at the Dutch National Institute for Public Health and the Environment (RIVM) for almost twentyone 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 is also co-chairing the ISO/TC194/WG17 on Biological Evaluation of Medical Devices – Nanomaterials. 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.

Peter Gehr

Professor Peter Gehr received his PhD in Biology at the University of Bern, held a postdoctoral fellowship at the same University, and has held posts as visiting assistant professor at the Harvard School of Public Health, visiting lecturer at the University of Nairobi in Kenya, head of the Division of Histology at the Institute of Anatomy of the University of Bern, professor and chair of the Institute of Anatomy at the University of Bern. For twenty years he has actively investigated particle-lung interaction, particle-tissue and particle-cell interaction, particle trafficking in and nanotoxicology of cells, cell and molecular biological studies, quantitative (stereological) structural studies with confocal laser scanning microscopy, conventional electron microscopy, energy filtering transmission electron microscopy, electron tomography.

Jozef Glasa

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Prof. Jozef Glasa, M.D., PhD., PhD., physician and clinical researcher, teaches clinical pharmacology, hepatology and medical/health care ethics/bioethics at the Slovak Medical University (SMU) in Bratislava. Deputy head, Institute of Pharmacology, Clinical and Experimental Pharmacology SMU; head, Institute of Health Care Ethics; president, Slovak Society of Clinical Pharmacology; scientific secretary, Slovak Society of Hepatology; director, Institute of Medical Ethics and Bioethics n.f.; editor, Medical Ethics & Bioethics; past chairman, member, Ethics Committee, Ministry of Health, Slovak Republic; 2nd vice-president, Slovak Medical Association (SMA), chairman, SMA Commission on Bioethics; member, CDBI (CoE, Strasbourg); member-past, European Group on Ethics (EGE, EC; Brussels). EF GCP Board member, chair elect, EF GCP Ethics Working Party.

Nicolas Gonzales

Nicolas Gonzalez was born in 1983 in Marseille, France. Graduated with a M. Sc. in Material Science and Nanophysics from Aix-Marseille University in 2007. He landed his first position as an engineer at the Jean Lamour Institute in Nancy, working on deposition process of magnetic thin films and multilayers and their structural and magnetic properties. He then joined the Schaefer group in late 2011 as a sales & application engineer. His work have been focus lately on nanomaterials, their production, characterization and their application in a variety of fields.

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). Since 2004 he is working with the Department Future Technologies and Europe of VDI/VDE-IT (Berlin, Germany). From 2004-2010 he was involved in technology transfer and innovation issues within the Innovation Relay Centre (IRC) and Enterprise Europe Network (EEN). Since 2010 Nicolas is involved in the ETP Nanomedicine, and he took over the management of the platform's secretariat in 2013. Nicolas is currently coordinating ENTRANS, a Coordination and Support Action aiming at enabling Nanomedicine Translation and providing a one-stop-shop service for SMEs to network, interact and share information, experience and advice. From 2012-2014, he coordinated the NANOMED2020 CSA under FP7, which main output was the White Paper "Contribution of Nanomedicine to Horizon 2020".
The ETP Nanomedicine was established in 2005 as a joint venture of the European Commission and CEOs of large industrial companies, SMEs and academic research institutions to investigate and advance joint activities in the area of nanotechnology in medicine. Since 2005 the ETPN published a number of strategic documents outlining the needs and roadmaps for nanomedicine research in Europe. The ETPN contributed to set up numerous European funded projects providing a first impression of the conditions for a suitable social and economic environment and the structural requirements for an efficient translation of R&D results into innovative Nanomedicines. The ETPN supports its members in coordinating their joint research efforts and improving communication amongst the members as well as towards the EC and the European Member States.

Iris Grossman

PhD
Vice President, Global Head of Personalized Medicine and Pharmacogenomics, Teva Pharmaceuticals

Dr. Iris Grossman is Vice president, global head of the Personalized Medicine and Pharmacogenomics (PMP) unit for Teva Global R&D. She has dedicated her research career, in both industry and academia, to the advancement of the field of personalized medicine. Dr. Grossman is currently charged with defining and implementing the global PMP strategy for Teva, a top-10 global pharmaceutical company, covering both discovery and development R&D programs. Israel's leading financial magazine, Globes Magazine, selected Dr. Grossman as one of the country's top 40 professionals under 40 years of age in 2013.
This followed several years of spearheading pipeline pharmacogenetic programs for industry and academia as director of pharmacogenetics at Cabernet Pharmaceuticals Inc. Dr. Grossman moved into consultancy having been responsible for running large-scale pharmacogenetic programs at GlaxoSmithKline, with an emphasis on infectious and neurological diseases.
In academia, Dr. Grossman was a key member of Professor David Goldstein's team at the Center for Population Genomics and Pharmacogenetics, Institute for Genome Sciences and Policy, at Duke University. Dr. Grossman received her PhD from the Technion – Israel Institute of Technology, where her research project, conducted in collaboration with the Weizmann Institute for Science, investigated pharmacogenetic markers of multiple sclerosis treatment response.

James Gubbins

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James obtained his BSc Degree in Biochemistry at Imperial College London in 2011. During his degree, he contributed to the investigation of protein-protein interactions at emulsion interfaces carrying out AFM and rheological analyses at the UK Institute of Food Research. He proceeded to carry out some work at the University of Southern California, assisting a PhD student investigating the role of PTEN in the control of pancreatic beta cell ageing through p16 regulated cellular senescence. He completed his degree with a targeted photodynamic therapy project developing HER2 scFv based photoimmunoconjugates. Following this, he was awarded the Lee Summer Student Fellowship Award by the University of Southern California Research Centre for Alcoholic Liver and Pancreatic Disease to investigate the signalling mechanisms behind liver fibrosis. He returned to Imperial College to complete an MRes in Cancer Biology in 2012 graduating with distinction. He initially investigated cancer cell apoptotic response to therapy for the definition of PET imaging protocol. He then went on to elucidate protein kinases involved in the mechanisms of prostate cancer bone metastasis in collaboration with Cancer Research UK. James is currently working towards a PhD in the Nanomedicine Lab developing theranostic nanoparticle-liposome hybrids for the diagnosis, treatment and monitoring of cancer under the supervision of Prof. Kostas Kostarelos.

Shengrong Guo

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EDUCATION

1992–1995	Ph.D. in Polymer Chemistry and Physics, Zhejiang University, China
1987–1990	M.Sc. in Physical Chemistry, Zhejiang University, China
1983–1987	B.Sc. in Chemistry, Hubei Normal University, China

EMPLOYMENT HISTORY

Jan 2003–	Professor, Shanghai Jiao Tong University (2013–2015, Marie Curie International Incoming Fellow at University of leeds in UK)
2000–2002	Associate Professor, Shanghai Jiao Tong University

1999–2000 Associate Professor, Fudan University
1996–1999 Lecturer, Fudan University (Former Shanghai Medical University)

RESEARCH INTERESTS

- Novel drug delivery systems for cancer treatment
- Drug/Medical device combinations for cancer treatment
- Biomedical polymers for drug delivery

AWARDS AND HONORS:

- Marie Curie International Incoming Fellowship (European Commission, 2013)
- Award for National Scientific and Technological Improvement (2nd prize, The State Council of the people's Republic of China, Dec. 23, 2011)
- Pharmaceutical Science Award (Chinese Pharmaceutical Association, July 22, 2011)
- Natural Science Award (Shanghai Municipal People's Government, Nov. 23, 2011)
- Pharmaceutical Science Award (2008, Pharmaceutical Association of Shanghai)
- Outstanding Life Science Award (2007, Mingzhi Ruye Life Science Awards, Administration Committee)

PUBLICATIONS(2014–)

1. Wei Tian, Jieying Liu, Yuan Guo, Yuanyuan Shen, Dejian Zhou*, Shengrong Guo* Self-assembled micelles of amphiphilic PEGylated rapamycin for loading paclitaxel and resisting multidrug resistant cancer cells. Journal of Materials Chemistry B. 2015, 3:1204
2. Min Li, Li Lv, Aijie Guo, Yuanyuan Shen*, Shengrong Guo* Copolymeric Micelles Loading Curcumin for Enhancing Antitumor Efficacy: Preparation, Characterization and In Vitro Evaluation. Journal of Nanoscience and Nanotechnology. 2015, 15:1-10
3. Yun Wang, Yuan Guo, Yuanyuan Shen, Rongjun Chen, FeihuWang, Dejian Zhou*, Shengrong Guo*. HCl-Retarded Gold Nanorod Growth for Aspect Ratio and Shape Tuning. Journal of Nanoscience and Nanotechnology, 2015, 15.
4. FeihuWang, Yuanyuan Shen, Wenjun Zhang, Min Li, YunWang, Dejian Zhou*, Shengrong Guo*. Efficient, dual-stimuli responsive cytosolic gene delivery using a RGD modified disulfide-linked polyethylenimine functionalized gold nanorod. Journal of Controlled Release, 2014, 196: 37–51.
5. Mingna Li, Xiaofen Xu, Fei Lu, and Shengrong Guo*. Primary in vitro and in vivo evaluation of norcantharidin-chitosanpoly (vinyl alcohol) for cancer treatment. Drug Deliv, 2014, 21(4): 293–301.
6. Aijie Guo, Feihu Wang, Wentao Lin, Xiaofen Xu, Tingting Tang, Yuanyuan Shen*, Shengrong Guo* Evaluation of Antibacterial Activity of N-phosphonium Chitosan as a Novel Polymeric Antibacterial Agent. International Journal of Biological Macromolecules,2014, 67: 163–171.
7. Feihu Wang, Yun Wang, Xiao Zhang, Wenjun Zhang, Shengrong Guo*, Fang Jin*. "Recent progress of cell-penetrating peptides as new carriers for intracellular cargo delivery". Journal of Controlled Release, 2014, 174: 126–136
8. Li Lv, Yuanyuan Shen, Jieying Liu, Feihu Wang, Min Li, Mingna Li, aijie Guo, yun Wang, Dejian Zhou* and Shengrong Guo* Enhancing Curcumin Anticancer Efficacy Through Di-Block Copolymer Micelle Encapsulation. J. Biomed. Nanotechnol. 2014,10:179-193
9. Li Lv, Yuanyuan Shen, Min Li, Xiaofen Xu, Mingna Li, Shengrong Guo*, Shengtang Huang* Preparation and in vitro Evaluation of Novel Poly(anhydride-ester)-based Amphiphilic Copolymer Curcumin-loaded Micelles. J. Biomed. Nanotechnol. 2014, 10:324-335
10. Chen Weiluan , Wu Zhenghua , Yang Han , Guo Shengrong*, Li Dawei , Cheng Liang. In vitro and in vivo evaluation of injectable implants for intratumoral delivery of 5-fluorouracil. Pharmaceutical Development and Technology. 2014, 19(2):223-231
11. Yun Wang, Feihu Wang, Yuan Guo, Rongjun Chen, Yuanyuan Shen, Aijie Guo, Jieying liu, Xiao Zhang, Dejian Zhou, Shengrong Guo*. Controlled synthesis of monodisperse gold nanorods with different aspect ratios in the presence of aromatic additives. Journal of nanoparticle Research. 2014, 16:2806

Heinrich Haas

Vice President Dug Delivery, Head of IMP Manufacturing, 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 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 DARPin technology. During his PhD, he developed genetically engineered viruses for the 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 loading of the liposome and the addition of linkers and 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 drug delivery system suited for a number of key medical applications like the chemotherapeutic treatment of cancer or the anti-inflammatory treatment of arthritis.

Steffi Hansen

PhD, pharmacist
Dr. Steffi Hansen is a project manager at VPM since November 2013.
She studied pharmacy at Ernst-Moritz-Arndt University Greifswald and graduated with a diploma thesis on the "Characterization of a ferrofluid for magnetic drug targeting" dedicated to improving therapy of superficial head and neck tumors. Dr. Hansen is furthermore board certified as a pharmacist. Dr. Hansen received her PhD from Saarland University, the Department of Biopharmacy and Pharmaceutical Technology. The topic of her thesis as well as a research period of several months at Winkle College of Pharmacy, University of Cincinnati, was the development of a diffusion model to predict drug transport across the skin. Afterwards, Dr. Hansen joined the Helmholtz-Institute for Pharmaceutical Research Saarland in the Department Drug Delivery where she became team leader in the field of transdermal vaccination. Her research focused on the development of innovative drug delivery systems based on nano- and microtechnology as methods for needle free immunization or for improving bioavailability of biopharmaceuticals and anti-inflammatory drugs. Furthermore Dr. Hansen gained experience as freelancer for PharmBioTec GmbH in planning and conducting bioequivalence and biodistribution studies especially of topically and transdermally applied drugs.

Jennifer Hare

PhD,
Jen received her B.Sc. (Hon.) in Pharmacology at the University of Alberta (Canada) in 2005. In 2011, she received a Ph.D. in Pharmacology from the University of Alberta for her research exploring the use of liposomes in combination cancer chemotherapy. Using pre-clinical mouse models, she investigated a dual-targeted (tumour vasculature and tumour cell) combination liposomal drug approach to the treatment of HER2-positive breast cancer, and a combination of liposomal irinotecan plus free 5-FU for the treatment of pseudo-metastatic colorectal cancer. In 2012, Jen began her current position as an industrial post-doctoral research scientist at AstraZeneca in the United Kingdom. Her present research focus is improving our understanding of the EPR effect by gaining insight into the influence of tumour phenotype on nanomedicine accumulation, distribution, and retention in tumours.

Jens Hasskarl

Jens Hasskarl is senior Global Clinical Leader (Senior Director) for development of CTL019 (CD19-directed chimeric antigen receptor T-cell therapy) for lymphomas at the Cell and Gene Therapies Unit (CGTU) Novartis in Basel, Switzerland. In this role he is responsible for planning and execution of clinical trials in various lymphoma types. Prior to joining the CGTU he worked as Medical Director for an oncology CRO in Freiburg, Germany. Before that he held several positions at Novartis Oncology Global Clinical Development. Jens Hasskarl is board certified hematologist and oncologists and holds a teaching position at the Freiburg University Medical School in Freiburg, Germany.

Michael Hehenberger

After earning a Dipl.Ing. in Physics from the Technical University of Vienna, Dr. Hehenberger moved to Sweden and obtained Ph.D./Dr.Sc. degrees in Quantum Chemistry from Uppsala University. He also spent two years at the University of Florida, Gainesville, as Visiting Associate Professor.
In 1985 he joined IBM in Stockholm where he initiated academic partnerships in computational chemistry and biology, structural engineering, computer networks and high performance computing. Throughout his IBM career which took him to Paris, California (San Jose/Almaden Research), and New York, he has led collaborations with academic and industrial life sciences organizations. The partnerships were based on the joint desire to extend the frontiers of molecular biology, information based medicine, biopharmaceutical research, genomics and nanomedicine. His efforts have been documented in over 40 publications and book chapters.
At the end of 2013, Dr. Hehenberger retired from IBM Research and started the HM NanoMed Partnership where he has been focused on writing books and advising clients on nanomedicine.
His first book, "Nanomedicine: Science, Business, and Impact", published in 2015, covers the steps needed to take a new scientific breakthrough all the way from concept to patient benefit.
His second book will explore the role that computational science and big data analytics are playing in current and future developments of the field of nanomedicine.

Clemens Helmbrecht

Head of Research and Development
Particle Metrix GmbH, Diessen
Name: Dr. Clemens Helmbrecht
Date of birth November 3rd, 1977
Nationality: German
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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

Enara Herran

PhD (University of the Basque Country)
PostDoc position-Department of Pharmaceutical Technology, Pharmacy Faculty-University of the Basque Country
Enara Herran was born in Vitoria-Gasteiz (Spain) in 1985. She studied Pharmacy (2003–2008) at the University of the Basque Country (UPV-EHU) in her hometown. During the last year of her degree, she started collaborating with the Department of Pharmaceutical Technology in the development of nano and microspheres for protein and drug delivery, where she finally started his PhD in the group of Jose Luis Pedraz and Rosa Maria Hernandez, thanks to a grant from the Basque Government (2009-2012). During this period, she works in the development of different nano and microparticulate systems to encapsulated different growth factors to treat Alzheimer's and Parkinson's diseases. In 2012 she made an internship at the Neuroscience Group of the Research Institute of the Hospital 12 de Octubre (Madrid). There he collaborated with Dr. Eva Carro in the treatment of APP/Ps1 mouse model of Alzheimer's disease with VEGF loaded PLGA nanospheres obtaining promising results published in Journal of Controlled Release. In 2014 she defended her doctoral thesis work "Angiogenic and neurotrophic factors encapsulated in PLGA micro and nanospheres as therapeutic approach to treat neurodegenerative diseases" and was graded with honors. After the defense of her doctoral thesis she continued to work in the Department of Pharmaceutical Technology with a PostDoc position conducting different research projects in collaboration with Praxis Pharmaceutical related to: nano and microtechnology, drug nose-to brain targeting to treat neurodegenerative disease and Alzheimer's and Parkinson's diseases.
Her main research works published in international journals are:

- Argia Acarregui; Enara Herran Martinez et al. Multifunctional Hydrogel-Based Scaffold for Improving the Functionality of Encapsulated Therapeutic Cells and Reducing Inflammatory Response. Acta Biomaterialia (2014).
- Enara Herran et al. Novel Drug Delivery Systems for Releasing Growth Factors to the CNS: Focus on Alzheimer's and Parkinson's Diseases. Mini-Reviews in Medicinal Chemistry (2014) 14 - 7, pp. 557 - 566.
- Enara Herran et al. Increased antiparkinson efficacy of the combined administration of VEGFand GDNF-releasing nanospheres in a partial lesion model of Parkinson's disease. International Journal of Nanomedicine (2014) 9, pp. 2677 - 2687.
- Enara Herran et al. In vivo administration of VEGF- and GDNF-releasing biodegradable polymeric microspheres in a severe lesion model of Parkinson's disease. European Journal of Pharmaceutics and Biopharmaceutics (2013) 85 - 3, pp. 1183 - 1190.
- Ainhoa Murua; Enara Herran et al. Design of a composite drug delivery system to prolong functionality of cell-based scaffolds. International Journal of Pharmaceutics (2010) 407, pp. 142 - 150.
- Enara Herran et al. Enhanced hippocampal neurogenesis in APP/Ps1 mouse model of Alzheimer's disease after implantation of VEGF-loaded PLGA nanospheres (Undereview). Current Alzheimer Research.

In addition she has performed two Master of Science (M. Sc.):

- M.Sc. Pharmacology. Evaluation, Development and Rational Use of Drugs. University of the Basque Country (UPV-EHU). Vitoria-Gasteiz, Spain (2009)

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 group of Professor Wendelin Stark at the ETH Zurich, she developed a nanomagnet-based blood purification technology in collaboration with the University Hospital Zurich. After her PhD in biomedical engineering and postgraduate studies in clinical trials management, she worked at the Centre of Clinical Research at the University Hospital in Zurich, the University of Illinois (visiting scientist) and at the Imperial College London. Since March 2015, she works as a group leader at the Swiss Federal Laboratories for Materials Science and Technology (EMPA). Her research interests include therapeutic applications of magnetic nanoparticles, organ-protective small molecules with applications in sepsis and ischemia reperfusion injuries and the development of point-of-care biosensors.

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 official 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.

Martin Hobe

Dr. Martin Hobe
Was born in Düsseldorf 1971, studied Biology in Mainz and Cologne and did his promotion at the Institute for Developmental Biology in Cologne. 2004-2006 he worked as Postdoc am Salk Institute, Plant Molecular and Cellular Biology Laboratory, Prof. Jeffrey Long. 2006 he went to Industry and became Global Product Manager bei Intavis AG. Since 2009 he

is at Bayer Technology Services as Technology Package Manager Computational Biology. More than 8 years of experience in steering and leading life-science based business. Strong background in plant, animal and human genetics, gene expression analysis and pharmaceutical drug development. Global responsibility for bio-based product lines since 2006, shaping innovation and business: Strategy, industrial marketing, product development, contract drafting and negotiation, business development and management. Initiative, pronounced analytical and networking skills and team player.

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 Hole

Dr Patrick Hole

Patrick is currently Engineering Manager for the NTA range of products at Malvern Instruments having previously spent 8 years in various roles and finally as Head of Development for Nanosight Limited, now part of Malvern Instruments.

He was responsible for managing team of up to 14 people (across development, support and production) and has developed and brought two new product lines into production, along with multiple other developments, including software, fluidics, electronics, temperature control, optics etc.

He is an author on 24 publications across a range of technical developments and applications and has numerous conference papers.

Alexander Huber

Dr. Alexander Huber started his career in the pharmaceutical industry at the research department of F. Hoffmann- La Roche Ltd in Basel, Switzerland in 2002. He held several positions with increasing responsibility and became plant manager for lyophilized products in 2007. 2009 he joined Novartis Pharma technical research and development (TRD) as director of the global centre of excellence for parenteral clinical supply. In 2013 he changed to the newly founded “Cell & Gene-Therapy Unit” as technical project leader. There he is mainly responsible for the management of CMOs to ensure supply of critical materials for Novartis portfolio of cell- and gene therapy projects.

Alexander received his PhD from the Federal Institute of Technology (ETH) Zürich in the field of molecular neuropharmacology, developing a first ex-vivo gene therapy approach against focal epilepsy in an animal model. He also holds a MBA from the same university.

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.

Pavel S. Ignatyev

Pavel S. Ignatyev graduated from the Moscow Institute for Radio engineering, Electronics and Automation in 2006. He is presently the CEO "AMPHORA Laboratories LLC" and a Ph.D. candidate. His major research fields are now laser interference microscopy of bioobjects and investigation of liquid media.

Wenlei Jiang

Ph.D.
Dr. Wenlei Jiang is the Acting Deputy Director of the Office of Research and Standards in the Office of Generic Drugs. She provides oversight on Generic Drug User Fee Act (GDUFA) regulatory science research activities to help develop ANDA review standards and ensure the therapeutic equivalence of generic drug products. She has been mainly responsible for developing bioequivalence standards of generic complex drug products such as liposomes and nano drug products, revising ANDA review policy of narrow therapeutic index drugs, and initiating post-market generic drug research including generic product bioequivalence in patient populations, generic drug surveillance methods, and patient perception about generic drug usage. She used to work in the Division of Chemistry, OGD to review the chemistry and manufacturing control (CMC) sections of ANDAs. Prior to joining FDA, she was at Novartis Pharmaceutical Corporation where her responsibilities included formulation development of conventional liquid and solid dosage forms, as well as parenteral drug delivery systems. She received her PhD in Pharmaceutics and Pharmaceutical Chemistry from The Ohio State University in 2001.

Sangyong Jon

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Education: 1999: Ph.D, Chemistry, KAIST
1995: M.S, Chemistry, KAIST
1993: B.S, Chemistry, KAIST

BIOGRAPHICAL SKETCH

Dr. Sangyong Jon received his B.S. in 1993, M.S. in 1995, and Ph.D. in 1999 from the Department of Chemistry at KAIST, Korea. He had experienced his postdoc career in the Department of Chemical Engineering at M.I.T. in the United States. In 2004, he joined Gwangju Institute of Science and Technology (GIST) as an Assistant Professor of Life Sciences and promoted to a Professor in 2010. He moved to KAIST in 2012 and is currently a Professor in the Department of Biological Sciences at the institute. Dr. Jon has published over 110 papers with a total citation over 7,900 and h-index of 40, numerous chapters, and over 60 patents. His proprietary technologies have been licensed to numerous companies. He sits on the editorial board of 4 peer-reviewed journals and is a regular reviewer for over 30 journals. He founded a startup company, Aptide Inc, in 2010 and is also a CTO of AnyGen Corp since 2005. He won a number of national and international awards. His research interest lies at the interface of medicine, biotechnology, nanotechnology, and biomaterials. A major research focus over the last 5 years has been development of i) novel peptide aptamer-based biologics, ii) multifunctional nanoparticles for combined cancer imaging and therapy, and iii) nanoparticle vaccines for various diseases targets.

Nazila Kamaly

Nazila Kamaly is currently an Instructor in the Laboratory of Nanomedicine and Biomaterials at Harvard Medical School, a Research Scientist at Brigham and Women's Hospital and a Research Affiliate at the Koch Institute at MIT. Prior to this she carried out postdoctoral research at Harvard Medical School and Imperial College London. She obtained her undergraduate/masters degree in Medicinal Chemistry from University College London and her PhD in Bioorganic Chemistry from Imperial College London. Her research is highly multidisciplinary and involves the development of nanomedicines for drug delivery and imaging applications for a variety of diseases including cancer and for the treatment of inflammation.

Dr. Jin Won Kim

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OCCUPATION

Professor of Cardiology at Korea University

EDUCATIONAL BACKGROUND

- Feb. 2005 Ph.D., Department of Medical Science, Korea University
- Aug. 1999 Ms., Department of Medical Science, Korea University,
- Feb. 1995 M.D. & Bachelor, Department of Medical Sciencei, Korea University

WORK EXPERIENCE (JOB HISTORY)

- 03/1995–02/1996 Full rotating Internship Training, Korea University Medical Center
- 03/1996–02/2000 Residency Training of Internal Medicine, Korea University Medical Center,
  a) 3-year for General Internal Medicine
  b) 1-year for General Cardiology
- 05/2000–05/2003 Military Service for 3 years,
  a) 2000.5–2001.5: Korean Navy Lieutenant
  b) 2001.5–2003.5: Director, Department of Cardiology, Capital Military Hospital
- 05/2003–05/2004 Fellowship/Clinical Instructor, Cardiology Division, Department of Internal Medicine, Korea University Medical Center, Anam Hospital, Seoul
- 05/2004–03/2006 Clinical Assistant Professor Cardiology Division, Department of Internal Medicine, Korea University Medical Center, Guro Hospital, Seoul
- 03/2006–02/2009 Assistant Professor, Cardiology Division, Department of Internal Medicine, Korea University Medical Center, Guro Hospital, Seoul
- 09/2009–08/2011 Postdoctoral Research Fellow, Cardiovascular Research Center Harvard Medical School, MGH, Boston, MA, USA
- 03/2009–Present Associate Professor, Cardiology Division, Department of Internal Medicine, Korea University Medical Center, Guro Hospital, Seoul, Korea

MAJOR PROFESSIONAL ACTIVITIES

- Educational Committee Board, Korea Nanomedicine Society
- Scientific Committee Board, Korea Society of Circulation
- Scientific Committee Board, Basic Science Working Group, Korea Society of Circulation

- Intelligent Committee Board, Korea Society of Interventional Cardiology
- Faculty, TCT-Asia Pacific, International Conference
- Faculty, Encore, International Conference
- Faculty, K-Imaging, International Conference
- Faculty, IMAGING & PHYSIOLOGY SUMMIT, Internatinal Conference
- Faculty, Imaging anf Physiology on PCI, International Conference
- Member, America Heart Association, ATVB Council
- Member, Korean Society of Lipidology and Atherosclerosis
- Member, Korean Society of Internal Medicine
- Member, Korean Society of Echocardiography
- Member, Korean Society of Hypertension

AWARDS, FELLOWSHIPS

- Nov. 2014 Astrazeneca Research Award
- Sep, 2012 Yuhan Medical Prize
- Dec, 2011 BRIC, Korea Outstanding Scientist
- Mar, 2010 Fellow American College of Cardiology (F.A.C.C.)
- Aug, 2009 BWH-Japan-Asia Dialogue Symposium, Best Research Award
- May, 2009 BRIC, Korea Outstanding Scientist
- Apr, 2008 APTCT, Outstanding Research Award
- Oct, 2008 Korea Lipid-Atherosclerosis Society, Reseach Award
- Sep, 2006 Korea Lipid-Atherosclerosis Society, Research Award
- Mar, 2005 Japanese Circulation Society. Travel Grant
- Oct, 2002 Korean Circulation Society, Best Research Paper Award
- Dec, 1996, KUMC, Best Resident of The Year

MAIN RESEARCH INTERESTS

- Development of optical nanoimaging in vivo for vascular application
- Deveopment of catheter based, integrated OCT-NIRF structural-molecuar imaging
- Innovative therapeutic approach for vulnerable coronary plaque

Costas Kiparissides

Costas Kiparissides is a full time Professor of Chemical Engineering Department at Aristotle University of Thessaloniki since 1981. During the period 2001-2006, he was Director of Chemical Process Engineering Research Institute (CPERI) at CERTH and in the period 2005–2010 Director of Centre for Research & Technology Hellas (CERTH). In 1971, he received his diploma Degree in Chemical Engineering from NTUA. In 1978, he received his Ph.D. Degree from McMaster University in Canada. From 1978–1983, he taught as an assistant and associate Professor at University of Alberta in Canada. He has also been a visiting Professor of University of Newcastle in U.K. and Queen's University in Canada. He has been member of the Expert Advisory Group (EAG) of the NMP programme, member of the Mirror Group of the European Technology Platform for Sustainable Chemistry, member of the Ad-hoc Advisory Group on Industrial Nanotechnologies, vice-chair of the working group: Nanopharmaceutics of the European Nanomedicine Technology Platform, etc. He has supervised more than fifty Ph.D. graduate students, 160 diploma theses and has presented more than three hundred invited seminars and lectures at international scientific conferences, industrial research centers, institutes and universities in Europe and North America. In addition he has published 200 papers in refereed journals, 350 conference papers and 24 books and reports. His published work has received more than 3100 citations. His current research interests are in the areas of advanced multi-scale modeling of chemical and biological systems, functional materials, novel micro- and nano-encapsulation technologies, molecularly imprinted polymers for selective recognition and separation of biological molecules, nanotechnology applications in targeted delivery systems, and microbial production of functional biopolymers from renewable sources.

Ingrid Klingmann

MD, PhD, FFPM, FBCPMy
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.

Felix Kratz

Ph.D., Vice President of Drug Discovery
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 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. As Vice President he heads the CytRx Drug Discovery Branch located in the Innovation Center Freiburg, Germany.

Silke Krol

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Since 2011 Dr. Silke Krol is with the 2009 funded Center of Nanotechnology@Fondazione I.R.C.C.S. Istituto Neurologico "Carlo Besta" in Milan, Italy. She is now 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 Udine and Trieste since 2008 and as guest lecturer for "Nanotoxicology". 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. She serves as external expert reviewer for National projects in France, Italy, and Greece. She is frequently peer-reviewing for Nanoscale, Nanomedicine, Nanoletters, and others.

John M. Lambert

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John Lambert is an Executive Vice President at ImmunoGen, Inc., and in 2015 was appointed Distinguished Research Fellow at the company. Dr Lambert first joined ImmunoGen in 1987 when the company established laboratories in Cambridge, Massachusetts. Prior to this, he was an Assistant Professor at the Dana-Farber Cancer Institute, Harvard Medical School (1982-1987), working on the ImmunoGen-funded programs to develop antibody-drug conjugates (ADCs) and immunotoxins as anti-cancer therapeutics. Dr Lambert has served on the executive team of ImmunoGen since 2008, and was Chief Scientific Officer from 2008 until 2015. Dr. Lambert holds a BA in Natural Sciences (1972) and a Ph.D. in Biochemistry (1976), both from the University of Cambridge, UK, and completed postdoctoral training at the University of California, Davis (1976-1980), and at Glasgow University, Scotland (1980-1982).

Twan Lammers

Twan Lammers, PhD, DSc
Dept. of Nanomedicine and Theranostics
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Twan 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 and the Helmholtz Institute for Biomedical Engineering at RWTH Aachen. In 2014, he was promoted to full professor of Nanomedicine and Theranostics at RWTH Aachen. Since 2012, he has also worked as a part-time assistant professor at the Department of Targeted Therapeutics at the University of Twente. He has published over 100 research articles and reviews, 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 therapies.

Karin E. Lason

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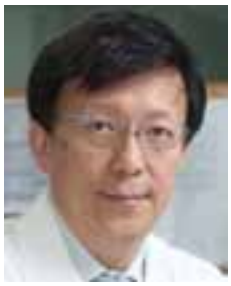
Karin E. Lason initially studied veterinary medicine at the Free University of Berlin and the École Nationale Vétérinaire d´Alfort in Paris. Soon she became interested in the world of science culminating in a transdisciplinary doctorate thesis on the evolutionary ecology of lactation energetics in two recent wild ruminant species at the Leibniz Institute of Zoo Biology and Wildlife Research and the Natural History Museum of Berlin. After some time working and studying in Spain she rediscovered her great interest in science communication. After professional initiation at the central press office of the German Leibniz Association and an advanced training course in online journalism she started working for the scientific Publisher De Gruyter as a Journal Editor. Karin Lason is Managing Editor of the European Journal of Nanomedicine since its transfer to De Gruyter in 2011.



Dong Soo Lee

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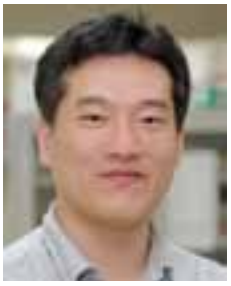
Dong Soo Lee is the Professor in the Department of Nuclear Medicine of Seoul National University (SNU) and SNU Hospital. He is also the Professor and Chairman of the Department of Molecular Medicine and Biopharmaceutical Sciences.

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 published over 300 articles in SCI journal and has been working in the Editorial Board of Journal of Nuclear Medicine, European Journal of Nuclear Medicine and Molecular Imaging, Journal of Nuclear Cardiology and as Special Associate Editor of Nanomedicine: Nanotechnology, Biology, and Medicine and 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.



Yun-Sang Lee

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Current Position: Research associate professor

EDUCATION:

3/2001-8/2006: Seoul National University, College of Pharmacy, Seoul, Korea
Ph.D. in Pharmaceutical Manufacturing Chemistry. Thesis: Complex preparation of group VIIB radiometal (99mTc, 188Re) with diaminedithiols and their biological evaluation.
3/1996-2/1998: Kyunghee University, College of Sciences, Seoul, Korea
M.S. in Department of Chemistry
3/1990-2/1996: Kyunghee University, College of Sciences, Seoul, Korea,
B.S. in Department of Chemistry

RESEARCH EXPERIENCE:

2014–Present: Research Associate Professor, Seoul National University, Dept. of Molecular Medicine and Biopharmaceutical Sciences
2008–2014: Research Assistant Professor, Seoul National University Medical Research Center
2007–2008: BK21 postdoctoral fellow, Seoul National University
1998–2006: Research Scientist, Seoul National University College of Medicine, Department of Nuclear Medicine
1996–1997: Korea Institute of Science and technology, Life Sciences Division, Seoul, Korea, Research assistant in Biochemicals Research Center.

Claus-Michael Lehr

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Claus-Michael Lehr is Professor at Saarland University, and also cofounder and head of the department "Drug Delivery" of the Helmholtz Institute for Pharmaceutical Research Saarland (HIPS) as well as of two companies (Across Barriers and PharmBioTec). HIPS is the first permanently installed institution explicitly dedicated to Pharmaceutical Research in Germany and is part of the Helmholtz Centre for Infection Research (HZI) in Braunschweig.
The main focus of research of Prof. Lehr's team 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. The expansion of this approach by developing new in vitro models of higher sophistication and relevance, based on the epithelial cells and tissues concerned, is in advanced progress. In parallel, the nanotechnology approach is improving and broadened in terms of formulating multifunctional nanocarriers that allow in vivo tracking of the system, targeting to the site of action, release the payload in a controllable manner and last but not least being safely biodegraded and excreted from the body.
Prof. Lehr has published some 300 papers, which have been cited ~ 9,600 times (h-index 52). He was the recipient of the CRS Young Investigator Award (2001), the APV Research Award (2006) and the International Price of the Belgian Society for Pharmaceutical Sciences (2008). 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 of the Controlled Release Society (2013). In 2014, he has been appointed to the Advisory Boards of The Catalent Applied Drug Delivery Institute and of the European Centre for the Validation of Alternative Methods (ECVAM). Prof. Lehr serves on different editorial boards and is co-editor of the European Journal of Pharmaceutics and Biopharmaceutics. At Saarland Universty, he has been the initiator of Galenos EuroPhD program and the biannual Conference and Workshop "Biological Barriers" which typically attracts 200+ attendees, and going to take place in February 2016 for the 11th time

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 (ERIT-M 0204), 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, in European FP7 projects and since 2013 as European coordinator in NMP "NanoAthero" large scale program project (16 partners, 10 countries – http://www.nanoathero.eu).
D Letourneur is the author of 126 international publications, 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 the Medical Research Foundation, and OSEO/BPI emergence 2012 & Creation-Dev 2013 for start-up creation.
He has more than 80 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 is the vice-chairman for Regenerative Medicine at the European Technology Platform for Nanomedicine (http://http://www.etp-nanomedicine.eu). Since 2009, he is President of BIOMAT, French Society for Biomaterials.

Julianna Lisziewicz

Dr. Julianna Lisziewicz is the President and Chief Scientific Officer of eMMUNITY, a US-Hungarian company. eMMUNITY has an enabling technology of personalizing immunotherapy that will fundamentally change the prevention and treatment of cancer and infectious diseases. eMMUNITY's team has developed immunogenetic tests and an associated algorithm for selecting the most effective treatment for cancer patients and designed DNA-based nanomedicines that are 10 times more immunogenic than any vaccine or immunotherapy tested to date.
Dr. Lisziewicz has previously founded two successful companies in the US: Research Institute for Genetic and Human Therapy to discovery and clinical investigation of new treatment approaches for HIV, and Genetic Immunity, Inc for clinical development and commercialization of a HIV therapeutic vaccine product developed at RIGHT. She has been directing all the vaccine-related R&D from discovery to preclinical and through clinical trials. She raised over $40 million funding from grants, contracts, investment and loans. She sold Genetic Immunity to Power of the Dream Ventures, Inc. in 2012. Dr Lisziewicz has >100 peer reviewed publications in leading scientific journals including New England Journal of Medicine, Science, AIDS, JID..

Beat Löffler

Beat Löffler is an MA in Philosophy, Politology and Communications Science (FU Berlin) and MD h.c. (University Basel) who has dedicated his efforts to the translation and the dissemination of science and technologies towards a better medicine and a sustainable society in Europe, complemented with activities to improve healthcare in the poorest countries. He has initiated or delivered,with his Company Löffler Associates Concept Engineering key contributions to a number of important activities in Basel and the trinational upper rhine valley that have evolved to international prominence. His most recent achievement is the building up of the non-profit CLINAM foundation which he co-founded to international prominence through strong interaction with keyplayers in Nanomedicine worldwide. CLINAM foundation has the goal to advance medicine through translation, application and dissemination of leading edge technologies, in particular nanoscience, and to explore the implications, to the benefit of patients and society. CLINAM activities are highly acclaimed worldwide.

Marko Loparic

Dr. med. Marko Loparic, MD-PhD
Chief Medical Officer
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 projects 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 AM and called ARTIDIS was designed and prototype was developed. The successes of the two KTI projects has lead 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

PD 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.

PUBLICATIONS

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.
Landfester, K. and V. Mailaender, Nanocapsules with specific targeting and release properties using miniemulsion polymerization. Expert Opinion on Drug Delivery, 2013. 10(5): p. 593-609.
Hofmann, D. and V. Mailander, Pharmacology of nanocarriers on the microscale: importance of uptake mechanisms and intracellular trafficking for efficient drug delivery. Nanomedicine, 2013. 8(3): p. 321-323.

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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Hofmann, D. and V. Mailander, Pharmacology of nanocarriers on the microscale: importance of uptake mechanisms and intracellular trafficking for efficient drug delivery. Nanomedicine, 2013. 8(3): p. 321-323.

Alessandro Maiocchi

Alessandro Maiocchi graduated in Industrial Chemistry in 1989 at the Science Faculty of the University of Milan. He is working in the Bracco Group companies since 20 years as a senior scientist covering several roles in the R&D organization. Currently he is the Research Projects Manager at the Bracco Research Centre in Italy. From 2004-2010 he served as contract professor at the Dept. of Biotechnology and Molecular Sciences at the University of Varese in Italy. Currently he is serving as chairman of the Nanodiagnostic working group at the European Technology Platform for Nanomedicine. His current research activity is
focused on the design and development of small and nanosized probes for molecular imaging applications in Magnetic Resonance, Ultrasound and Optical Imaging. He is member of several societies and author of more than 100 scientific publication on international journals and conference proceedings in the field of drug design, contrast agents characterization, pharmaceutical product development and imaging methods.

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). 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 MUG managing director of 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 (http://biotechmedgraz.at/de/).

Mira Marcus-Kalish

miram@post.tau.ac.ilv

Dr. Mira Marcus-Kalish is currently the director for international research affairs at Tel Aviv University and a Senior Research Fellow at the Interdisciplinary Center for Technological Analysis and Forecasting. 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 Institute of Technology, Haifa, developing a computerized system for E.C.G. diagnosis. Her post doctorate training was at Harvard University, at the MBCRR laboratory (Molecular Biology Computer Research and Resource) and the Dana Farber Cancer Institute. Her B.Sc. is in Statistics and Biology from the Hebrew University in Jerusalem.
Coming back to Israel, she joined the Weizmann Institute working with Prof. Ephraim Katzir mainly on protein interactions, then Tel Aviv University Business School, focusing on Medical informatics and the Biotechnology department taking active part in Converging Technologies and contributing to the recent EU-US Wtec-NBIC2.
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 was and is involved in many EU framework projects, such as: the Nano2Life NoE, SkinTreat, ReNaChip, EpoCan, NanoAthero and recently the Human Brain Project (HBP) flagship, GLAM and ENATRANS.
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, etc.

Massimo Masserini

Massimo Masserini is full Professor of Biochemistry and Molecular Biology at the Medical School, University of Milano-Bicocca , Italy, where he is Head of the Nanomedicine Center (NANOMIB), an interdepartmental Consortium devoted to the bio-medical applications of nanotechnology . His research interests have always been focused on neurochemistry and molecular mechanisms of neurodegeneration, biochemistry of cell membranes and on physicochemical studies on membrane models, in particular liposomes. He has authored or co-authored over 130 papers in national and international journals. He has Coordinated the NMP Project “Nanoparticles for therapy and diagnosis of Alzheimer Disease (NAD)”, involving 18 Partners of 13 European Countries.He is leading different nanomedicine projects, including a Marie Skłodowska-Curie innovative training networks (MSC-ITN – MULTI) “Design and development of advanced Nanomedicines to overcome Biological BArriers and to treat severe diseases” (NABBA).

Mariarosa Mazza

Mariarosa is Research Fellow in Nanomedicine within the Nanomedicine Lab, Faculty of Medical and Human Sciences, at the University of Manchester. She is a registered pharmacist with the General Pharmaceutical Council and a Member of the Royal Society of Chemistry. She obtained her PhD from the UCL School of Pharmacy in 2012 working on the development of peptide nanofibers for drug delivery. Her thesis work was endorsed with a patent application. She also worked at UCL as Post-Doc in the Nanomedicine Lab with Prof Kostas Kostarelos, focusing on the development of nanotube-mediated drug delivery systems. In 2013, following the relocation of the Nanomedicine Lab, she was appointed Research Fellow in Nanomedicine at the University of Manchester. Her research outputs stretch from the molecular design of nanomedicines based on soft materials (lipids, peptide amphiphiles) and hard materials (carbon based) to the development and pharmacological evaluation of these novel nanoparticles for applications that range from drug and gene delivery, to biomedical imaging.

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.

Marijana Mionic

Marijana Mionić 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.

Konstantinos Mitsakakis

Dr. Konstantinos Mitsakakis studied Physics at the University of Crete, Greece and did his MSc on Nanoscience & Nanotechnology at the Aristotle University of Thessaloniki, Greece. He acquired his doctoral degree (2009) from the Department of Materials Science & Technology, University of Crete, Greece, working at the Biosensors Technology Lab, Institute of Molecular Biology & Biotechnology, Foundation for Research & Technology Hellas (IMBB-FORTH), Greece. His PhD thesis was on developing a multi-analyte acoustic biosensor platform for cardiac marker detection. He joined the Lab-on-a-Chip Group at IMTEK, University of Freiburg, with a Humboldt Foundation fellowship and is currently the Coordinator of the EU FP7-ICT project "DiscoGnosis" and the new Horizon 2020 project "DIAGORAS". His research interests lie in the field of micro/nanotechnology for life sciences and diagnostics, biosensor technologies, lab-on-a-chip and microanalytical systems.

Moien Moghimi

(moien.moghimi@sund.ku.dk)

Moein Moghimi is based at the University of Copenhagen (Denmark) where he serves as Professor of Nanomedicine at the Department of Pharmacy, Professor of Pharmaceutical Nanotechology at the NanoScience Centre, and Director of the Centre for Pharmaceutical Nanotechnology and Nanotoxiocology. He is also a full member and professor at the Department of Translational Imaging, Houston Methodist Research Institute (Weill Cornell Medical College), Houston Methodist Hospital Systems, Houston, Texas (USA), an adjoint professor at the Department of Pharmaceutical Sciences, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of Colorado-Denver Medical Center (USA), visiting professor at University of Padua (Italy) and the elected Fellow of the Institute of Nanotechnology (UK). Earlier, he served as the Honorary Professor of Nanomedicine at the Multidisciplinary Research Center, Shantou University (China). Before joining Copenhagen, Moein was Senior Lecturer in Biopharmacy and Molecular Pharmaceutics at the School of Pharmacy, University of Brighton (UK) and The University Research Fellow in Advanced Drug Delivery Systems at the Department of Pharmaceutical Sciences, University of Nottingham (UK). His research activities are focused on pharmaceutical nanoscience, and fundamental nanomedicine/nanosafety, and renowned in 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 the molecular basis of nanomaterial/polymer immune toxicity and cytotoxicity. Since 2009, Moein has been the recipient of many research awards securing over €10 million in competitive research funds as the principal investigator and partnering two large-scale competitive European Commission FP-7 programmes in translational nanomedicine/drug delivery, addressing Alzheimer's disease and atherosclerosis with secured budgets of €14 million and €8.5 million, respectively.

Moein has over 170 peer-reviewed publications/patents to his credit with over 10700 citations and h-index of 46 (Jan 2015). His editorial activities have included the role of Theme Editor for Advanced Drug Delivery Reviews (Elsevier), Maturitas (Elsevier), Journal of Biomedical Nanotechnology (American Scientific Publishers) and Current Drug Delivery (Bentham) and Associate Editor of Nanomedicine: Nanotechnology, Biology and Medicine (Elsevier) and the Journal of Biomedical Nanotechnology. Moein further serves on the editorial board of several peer-reviewed scientific international journals, including Advanced Drug Delivery Reviews, Nanomedicine-UK (Future Medicine), Journal of Liposome Research (Informa Healthcare) and Molecular and Cellular Therapies (BioMed Central). He is currently completing two books in nanomedicine.

Over the past two decades, Moein has been practicing in the capacity of consultant to over 50 pharmaceutical, biotechnology, health and food industries as well as investment banks, management consultancy firms and other entrepreneurial enterprises worldwide. He is a regular invited assessor and expert evaluator in nanomedicine/nanotoxicology for governmental bodies, research councils and private organizations (over 50 establishments in 25 countries). As a frequent speaker, chair and organizer, Moein has given over 300 invited speeches, keynotes and plenaries at international scientific conferences, universities, and governmental and multi-national organizations. Recently delivered keynotes, plenaries and invited speeches have included those at the American Society for Gene & Cell Therapy 18th Annual Meeting 2015 (New Orleans, USA); Society for Brain Mapping and Therapeutics 2015 (Los Angeles, USA); University of Vienna 2015; Department of Neurosurgery, Cedars Sinai Medical Center 2014 (Los Angeles, USA); World Congress of Basic and Clinical Pharmacology 2014 (Cape Town, South Africa);

BioNanoMed 2014 (Krems, Austria); CLINAM 2013, 2014 and 2015 (Basel, Switzerland); Annual Meeting of the German Pharmaceutical Sciences 2013 (Freiburg); Stanford University 2013; University of California Santa Barbara 2013; New York Academy of Sciences 2013; Roche (Nutley, USA) 2013; Sanofi Research and Development 2013 (Paris); Nanotechnology Characterization Laboratory (Frederick National Laboratory for Cancer Research) 2013; TechConnect World 2013 (Washington DC); Houston Methodist Research Institute 2013; Volkswagen Foundation 1st Herrenhausen Conference 2012 (Hannover, Germany); NanoImpactNet-QNano Conference 2012 (Dublin); 5th International Liposome Conference 2011 (London); 8th European Biophysics Congress 2011 (Budapest); Novo Nordisk 2011 (Denmark); Annual Conference of the National Health Technologies Institute and College de France 2010 (Paris), and Tsinghua University (Beijing) 2010.

Following completion of secondary education at d'Overbroeck's College Oxford (UK), Moein read 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 post-doctoral training programme in Advanced Drug Delivery Research at the School of Pharmaceutical Sciences (University of Nottingham, UK) under the guidance of Prof. S.S. Davis.

Moein is listed in Marquis Who's Who in the World, USA, Marquis Who's Who in Science and Engineering, USA, and Marquis Who's Who in Medicine and Healthcare, USA (by invitation). His profile has also featured in APV (the International Association for Pharmaceutical Technology, Mainz, Germany) Drug Delivery Focus Group Newsletter (October 2014).

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.

Jan Mollenhauer

Prof. Dr. Jan Mollenhauer was born in Kiel, Germany, in 1968, studied biology from 1989-1994 at the University of 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 Editorial Board member of Nanomedicine NBM, and has been 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.

Ýrr Mørch

Ph.D, Research Scientist at SINTEF Materials and Chemistry
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Ýrr Mørch studied chemistry at the Norwegian University of Science and Technology (NTNU) and obtained her Master degree in chemical engineering in 1999. After various positions within research and teaching she obtained her Ph.D. degree in biotechnology from NTNU in 2008 entitled "Novel Alginate Microcapsules for Cell Therapy". From 2008 to 2010 she worked as a research scientist at Dept. of Physics and Dept. of Biotechnology (NTNU) within biopolymer research. During her post doc period (2011–2013) she developed nanoparticle-stabilized microbubbles for ultrasound-enhanced drug delivery. Since 2013 Ýrr Mørch has been working as a research scientist and project manager at SINTEF Materials and Chemistry in Trondheim on various national and international projects within nanomedicine. Her current activities are encapsulation of drugs and contrast agents in polymeric nanoparticles, surface modification and physico-chemical characterization of nanoparticles.

PROFESSIONAL SKILLS (KEYWORDS):

Microencapsulation, nanoencapsulation, cell encapsulation (cell therapy), miniemulsions, polymerization, surface modification and targeting of nanoparticles, physico-chemical characterization of micro- and nanoparticles, biopolymer gelling techniques (inter-

nal and external gelling, capsules, beads, films, foams, cylinders), surface modifications and chemical modification of biopolymers and synthetic polymers, enzymatic modification of biopolymers, cell handling and culturing (including staining and imaging and various cell function techniques), rheological measurements.

ACADEMIC AWARDS:

- Tekna's Young Scientist of the Year award 2009
- Poster award, International Hydrocolloid Conference, Trondheim July 2006
- Personal Ph.D Student Grant, awarded by the Norwegian Research Council 2001–2006
- Number one graduate student award, Reykjavik 1995

PUBLICATIONS:

20 peer reviewed publications (times cited: >660 ICI Web of Science, February 2015), 2 submitted, >12 public science publications, >10 (first author) presentations at international scientific conferences, invited (plenary) speaker at 2 international and 3 national conferences/meetings.

Jan Mous

PD Dr.
CEO of PharMida AG, Basel, a nanomedicine-focused, affiliated company of Midatech Ltd. in Abingdon/Oxford, UK (2009–present).

EDUCATION

- 1980: PhD in biochemistry at the University of Leuven (Belgium)
- 1981–1984: post-doctoral stays at the Rega Institute in Leuven (1981) and at the Institute of Molecular Biology II of the University of Zürich (Switzerland, 1982-1984)
- 1993: venia docendi of the University of Basel (Switzerland).

PROFESSIONAL CAREER

- 1985–2000: R&D manager at Hoffmann-La Roche's Pharma division in Basel (Switzerland), last position: senior VP, corporate Genomics
- 2000-2002: CSO of LION bioscience AG, a bio-IT company in Heidelberg (Germany) and non-executive director of LION bioscience Inc.(Boston, MA)
- 2003–2007: President & CEO of molecular diagnostics company IntegraGen SA in Evry (France); 2008: independent consultant, MRM Consulting GmbH, in Giebenach (Switzerland).

Stefan Mühlebach

Professor, PhD, Hospital Pharmacist FPH,
Chair of the NBCD WG c/o TI Pharma

POSITION

Stefan Mühlebach is Chair of the Non-Biological Complex Drugs (NBCDs) Working Group at Top Institute Pharma, a non for profit organisation in the Netherlands (http://www.tipharma.com/pharmaceutical-research-projects/regulatory-innovation/non-biological-complex-drugs-working-group.html).
He is Head of Regulatory Science in GRA at Vifor Pharma Ltd. in Switzerland, a company with a long-term expertise and experience in R&D, manufacturing, and world-wide marketing of colloidal iron complex-based therapeutics, representatives of NBCDs (http://www.viforpharma.com/en/About-Vifor/about-home.php.).

TRAINING, EDUCATION, UNIVERSITY ACTIVITIES

Stefan Mühlebach obtained at the University of Bern, Switzerland, a MSc Pharm and a federal diploma (1975), a PhD in pharmacology and toxicology in 1979.
In 1993: habilitation in pharmacology at the Medical Faculty of the University of Bern.
In 2000: "venia docendi" at the Medical Faculty of the University of Basel, teaching since mainly at the Department of Pharmaceutical Sciences.
In 2004 appointed Professor of Pharmacology and Hospital Pharmacy at the University of Basel. He is a member of the Clinical Pharmacy and Epidemiology unit. His research activities include topics in pharmacology and applied sciences also in clinical nutrition (https://pharma.unibas.ch/research-groups/people/profile/person/muehlebach/?tx_x4epersdb_pi1%5BoriginPageID%5D=44842&cHash=42bcfbe93a4ca4a20bc6e61d8e5cea1a).
Stefan Mühlebach published more than 60 peer-reviewed and indexed papers, is author of several book chapters, and of many scientific reports. He is regularly presenting in national and international conferences on topics of his expertise like regulatory science (NBCDs, nanosimilars etc.) clinical nutrition, and (hospital) pharmacy and pharmacology.
He was a founding member of the Swiss Academy of Pharmaceutical Sciences in 2014 and is elected Vice-President of the Senate's board (http://www.saphw.ch/en/portrait/structure-mission-tasks).

PROFESSIONAL CAREER

- Hospital Pharmacy: Chief hospital pharmacy in Switzerland 1980-2005 (Biel, Aarau); he established the Swiss curriculum (Foederatio Pharmaceutica Helvetiae) of the hospital pharmacy postgraduate specialisation and trained the first fellows. He served as president of the Swiss Association of Public Health Administration and Hospital Pharmacists and became a honorary member in 2012.
- Authorities (Swissmedic, the Swiss Agency for Therapeutic Products): Head of the Pharmacopoeia and Head of the Swiss Delegation at the EDQM in Strasbourg 2005-2008.
- Industry: Chief Scientific Officer in 2008 at Vifor Pharma, Switzerland; since 2009 in the international headquarter of Vifor Pharma Ltd in Zürich (Global Regulatory Department).
- Chair of the Non-Biological Complex Drugs Working Group at TI Pharma (Top Institute Pharma, The Netherlands) since 2010 aiming to provide science-based support for appropriate authorization of NBCD follow-on versions, some of them also being nanomedicines.

Bert Müller

Bert Müller is Thomas Straumann-Chair for materials science in medicine at the Medical Faculty, University of Basel. He is a physicist, but mainly gets the inspiration for his research from medical doctors. His research topics are diverse, as were the positions Bert Müller held before accepting the professorship at the University of Basel in 2006. During his career, he not only passed through various posts, he often hold positions simultaneously. Recently the International Society for Optics and Photonics awarded the grade of Fellow of the Society in recognition of his distinguished and valuable contributions to the field of optics and photonics again demonstrating that he masters this approach perfectly.
Bert Müller, who was born in Berlin 1962, initially completed an apprenticeship as a mechanical engineer. Simultaneously, he went to school and completed his high school diploma. After gaining some work experience as an electrical fitter in building big power transformers, he decided to study physics in Dresden University of Technology. As English has been important for natural scientists, it made sense for him to study English in parallel. So he finished

his education in 1989 not only with a university masters degree in physics but also in English translation specialized in physics and mathematics. Subsequently, he conducted research at the Paul Drude-Institute for Solid State Electronics in Berlin before joining the team of Professor Martin Henzler at the University of Hannover - first as visiting scientist dealing with scanning tunneling microscopy on semiconductor surfaces and later as doctoral student working on high-resolution reflection high-energy electron diffraction. His PhD-thesis was honored with the Morton M. Traum Award by the American Vacuum Society in 1994.
The next stop was a temporary replacement of Professor Klaus Lischka at the University of Paderborn. Here, Müller has dealt with research activities directed towards blue-light emission using III-V-, II-VI- and organic semiconductors. In 1995, Müller started to become acquainted with research institutions in Switzerland. First, he obtained a scholarship from the Humboldt Foundation at the Institute of Experimental Physics, EPF Lausanne. In the team of Professor Klaus Kern, Müller performed variable temperature scanning tunneling microscopy of the early stages of metal crystal growth. In 1997, he became team leader for thin organic layers in the physics department of the ETH Zurich. Within the nonlinear optics lab headed by Professor Peter Günter, his team has grown thin films for both non-linear optics and organic light emitting diodes. Later, he moved to the Swiss Institute for Materials Testing and Research (Empa) to study chiral molecules and understand micro computed tomography. He quickly realized that the work environment at the university suits him better, so in 1999, he returned to the ETH Zurich. In his role as leading research associate in the group of the surgeon Professor Erich Wintermantel for Biocompatible Materials Science and Engineering, he was more and more confronted with medical questions. This contact with scientists from other disciplines increased in the following years. In 2001, he became General Manager of the NCCR Co-Me (National Center of Competence in Research Computer-Aided and Image-Guided Medical Interventions). In this interdisciplinary program, medical specialists and engineers including computer scientists got together and had to learn to speak the same language. During this time as General Manager, Müller conducted his own research outside of the NCCR Co-Me and has taught as Privatdozent at the Physics Department, ETH Zurich.

Hanspeter Naegeli

Hanspeter Naegeli studied veterinary medicine and graduated 1985 at the University of Zurich. After postdoctoral trainings at the Stanford University Medical School (1990) and the University of Texas Southwestern Medical Center at Dallas (1991–1992), he returned to the Vetsuisse Faculty Zurich to initiate a new Division of Toxicology, which also comprises the www.clinitox.ch consulting service in clinical toxicology. Since 2002, Hanspeter Naegeli is Professor of Toxicology and 2015 he became Director of the Institute of Veterinary Pharmacology and Toxicology. He is member of the Cancer Biology Program Zurich and also member of Swissmedic expert committees as well as member of the EFSA (European Food Safety Authority) panel on genetically modified food. His research group has been active in multidisciplinary projects in both molecular and food toxicology. Hanspeter Naegeli uses advanced methods of cellular and molecular biology to identify and characterize adverse endpoint pathways and complex reaction patterns by which living organisms respond to endogenous or exogenous toxic insults.

Patrick Nef

Prof. Patrick Nef has experience in preclinical research and drug development from academia (Assistant Professor), big Pharma
(vice-Director R&D), biotech (CEO, CSO, CBO), and public-private partnerships. He has obtained a PhD degree from the University of Geneva, Switzerland in 1988. In 1992 he became Professor of Biochemistry at the University of Geneva, Switzerland.
In 1998 he moved to F. Hoffmann-La Roche Ltd. (Basel, Switzerland) in the preclinical Central Nervous System department and became Vice-Director in 1999 in charge of the preclinical CNS department. In 2002, he served as Director in the Roche Partnering & Licensing department and was in charge of the preclinical Research and Technology licensing activities and for early in-licensing of CNS, metabolic, genito-urinary and respiratory clinical candidates. In Jan. 2005, he became Chief Executive Officer of Faust Pharmaceuticals, Strasbourg, France, then in 2006 CSO/CBO at Xytis SA, Nyon, Switzerland both start-up biotech companies financed
by VC's from France. From 2006-2008 he was acting-CSO at Synosia Therapeutics Inc., San Francisco, USA, and Basel, Switzerland, now merged with BioTie Therapies Corp. In 2008, he co-founded BioXpress Therapeutics SA, Geneva, Switzerland, a service company for development of biosimilars/biogenerics. From 2008–Aug2011, he was the Executive VP Business Development at Medicine for Malaria Venture, a product-development partnership not-for-profit foundation based in Geneva, Switzerland. Since Sept 2011 he is co-founder and CEO of TransCure BioServices SAS, Archamps, France, a start-up providing access to humanized immune system
mouse models in cancer, inflammation, and infectious diseases. Prof. P. Nef has published more than 40 papers or reviews and is still an Adjunct Professor at Rockefeller University, NY, USA in the laboratory of Nobel Laureate Prof. Paul Greengard.

Detlef Niese

Priv.Doz.Dr.
Independent Consultant for Health Policy and Medicines Development

Detlef Niese is an independent policy and science consultant since March 2013. In this role, he offers scientific and educational services to private and public organizations active in health care and medicines development.
Since 1989, Dr.Niese is member of the Faculty of Medicine of the University of Bonn, Germany. He also serves as board member of the Novartis Foundation for Biological and Medical Research as well as of the European Forum for Good Clinical Practice.
Dr. Niese teaches drug development sciences at the universities of Basel (medicine, bachelor) and Budapest (master level).
Before his retirement in 2013, Dr Niese was Head External Affairs Global Development at Novartis Pharma AG responsible for science policy and ethical issues concerning drug development. In this role, he was responsible for developing and implementing policies and guidances both internally and with public institutions for research involving humans (adults and children) with a particular focus on implementation of new technologies. He also served on Expert Groups developing recommendations on biobanks and clinical research for the OECD.
Since joining industry in 1992 Dr.Niese held different roles of increasing responsibility in Clinical Research and Development at Sandoz and Novartis. He led and supervised development teams for immunuppressants in organ transplantation and auto-immune

disorders in adults and children. He was member of the Executive Board of the Pharma Center at the university of Basel, the board of Directors of EuropaBio, the European Biotech Association and the board of the European Platform for Patient Associations, Science and Industry. Dr Niese also served on the Program Committee of DIA Europe and was member of the Advisory Board to EuroGenTest, a European project for improving the quality of genetic testing. From 2002-2004 Dr.Niese was appointed by the European Commission to an expert group on Legal, Societal and Ethical Implications of Genetic Testing. The group published their report in May 2004. From 2008 -2013 Dr.Niese was member of the Training and Education consortia (PharmaTrain, EMTRAIN, and EUPATI) of the European Innovative Medicines Initiative IMI developing master and professional education programs in medicines development on a European and International Level.
He is a licensed pharmacist and physician with a research based doctoral degree in immunogenetics granted by the faculty of medicine, university of Bonn, Germany. Dr.Niese is a board certified internist with specialization in rheumatology and clinical immunology. In 1989 Dr.Niese was appointed head of the department of clinical immunology, and also became head of clinical pathology at the department of Internal medicine at the Bonn university hospital.

Wolfgang Parak

Born on 22. 2. 1970 in Dachau, Germany, German citizenship
Parents: Fritz and Edeltraud Parak (nee Unterstaller)
1989–1997: General Physics studies at the Technische Universität München, Germany; finished on 13. 1. 1997 with the final degree "Diplom Physiker" (equivalent to masters in physics; grade: 1,4)

1995–1997: Diploma thesis ("Diplomarbeit") at the Technische Universität München, Germany, at the Institute for Biophysics in the group of Prof. Dr. Hermann Gaub with the topic "set-up and characterisation of a LAPS-Sensor for non-invasive measurements of membrane potentials"
1997–1999: Graduate student at the Ludwig Maximilians Universität München, Germany, at the Institute of Applied Physics (chair: Prof. Dr. Hermann Gaub with the topic "cell-semiconductor-hybrids"; finished on 15.12.1999 with the PhD degree ("Dr. rer. nat") (grade: 0,7; with honours, "Auszeichnung")
2000–2002: Postdoc at the Department of Chemistry at the University of California at Berkeley, California, USA, in the group of Prof. Paul Alivisatos, whereby the first year was sponsored by a fellowship of the German Research Foundation (DFG) field of work: biological applications of colloidal nanoparticles
2003–2006: Leader of a Junior Research Group (Emmy-Noether fellowship of the German Research Foundation (DFG), equivalent to Assistant Professor), hosted at the Ludwig Maximilians Universität München, Germany, at the Institute for Applied Physics (chair: Prof. Dr. Hermann Gaub) and at the Center for Nanoscience
2005: Temporary position as Associate Professor for Physical Chemistry at the Department of Chemistry and Pharmacy at the Ludwig Maximilians Universität München, Germany for the Summer Semester
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); present h-index: 58

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: 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).

Dan Peer

Prof. Dan Peer is an Associate Professor that leads an NIH-funded lab in the Faculty of Life Science and the Faculty of Engineering 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 did all his training at Tel Aviv University (B.Sc., M.Sc. and Ph.D.) with two internships during his Ph.D. studies one with Cesar Milstein (Nobel Laureate, Cambridge University, UK) and one with Robert Langer at MIT (Cambridge, MA, USA), then moved to Harvard Medical School (Boston, MA, USA) for a postdoctoral training.
He was recruited back to Tel Aviv University from Harvard University in 2008 to establish the Laboratory of NanoMedicine.
Prof. Dan Peer's work was among the first to demonstrate systemic delivery of RNA molecules (new class of drugs) 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.
He generated an international recognition and collaboration in inflammatory bowel diseases (IBD) and oncology area (in blood cancers, brain and ovarian cancers). He received numerous awards; among them he was recognized by the American Association for the Advancement of Science (AAAS) excellence in Science program for young investigators and was recently awarded the innovator (2010) and the breakthrough (2011, 2013) awards from the Kenneth Rainin Foundation on his pioneering work in inflammatory bowel diseases (IBD). Recently, he was awarded the 1st Untold News Award together with Prof. Rimona Margalit also from Tel Aviv

University on the “Cancer Bullet” invention that might change the world.
He is an editor of several books in the field of nanomedicine, Editor of Molecular and Cellular Therapies (Springer); Editor of Biology and Medicine in Nanotechnology (IOP), an Associate Editor of the Journal of Controlled Release (Elsevier); Journal of Biomedical Nanotechnology, and of Biochemistry, and on the Editorial Boards of the Biomedical Microdevises (Springer), Cancer Letters (Elsevier), Nanomedicine: Nanotechnology, Biology and Medicine (Elsevier) and Bioconjugate Chemistry (ACS).
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 II clinical evaluation. In addition, based on his work, 3 spin-off companies were generated Leuko Biosciences, Quiet Therapeutics and ESPL Pharma, aiming to bring nanomedicine into clinical practice.
Dr. Peer is the President of the Israeli Chapter of the Controlled Release Society, and a Member of the Israel Young Academy of Sciences and Humanities.

Simona Pînzaru

Function: Associate professor
Academic degree: Doctor in Physics
Date of Birth: 30.09. 1965
Nationality: Romanian
Working location: Cluj-Napoca, Babes-Bolyai University, Biomolecular Physics Department

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Fax number: +40-264-550790
E-mail: simona.cinta@phys.ubbcluj.ro, simocris02@yahoo.com

EDUCATION:

1998: Doctor in Physics of Babes-Bolyai University, under the joint scientific coordination of Professor Onuc Cozar from Babes-Bolyai University and Professor Wolfgang Kiefer from the University of Würzburg, Germany.

EXPERTISE:

Optical nanosensing, SERS spectroscopy, lasers, optoelectronics, nanotechnology, experimental physics, particularly in 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, Coordination of research projects: PN_II_ID_2284/ 2008-2011; Grant Director; Member in other projects grants teams – 2005, 2004, 2003, 2002;
1999–2002: World Bank Grant for Young Researcher: BM-T Grant Director
2000–2002: Grant Director CNCSIS –AT
2003–now: Associate Professor Department of Biomedical Physics, Theoretical and Molecular Spectroscopy Department, Babes-Bolyai University, Cluj-Napoca, Romania.
2003–2005: Visiting scientist at Institute of Physical Chemistry, University of Wùrzburg, Germany;
1998–2003: Lecturer, Optics and Spectroscopy Department, Babes-Bolyai University;
1995–1998: Assistant professor, Optics and Spectroscopy Department, Babes-Bolyai University;

AWARD:

The Prize of Excellence for scientific research, Babes-Bolyai University, 2011.

PUBLICATIONS:

71 papers in ISI ranked journals (Hirsch Index 14); more than 200 contributions in conferences proceedings, 46 oral presentations in conferences, 10 invited lectures; 4 Books/book chapters
Complete list of publications: Researcher ID: A-4543-2011

Jai Prakash

Dr. Prakash 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 for Biomedical Technology and Technical Medicine. His research group is focused on the design of novel specific cell-targeted nanomedicine against myofibroblasts and macrophages in the tumor microenvironment and fibrosis.

Nadja S. Prang-Richard

PhD, MBA
Nadja has over 15 years’ experience in the development of therapeutics. After she received her PhD degree in immunology and virology she worked over 5 years in a Contract Research Laboratory in Munich developing bioassays for the pharmaceutical industry before she joined Amgen/ Micromet, as Head of Bioanalytical Development and QC in 2002. In 2005 she became Director of Global Bioanalytical Development of Serono in Rome and was appointed Head of Biotech Product Development for Oncology & Emerging Therapies in Merck-Serono in 2007. End of 2008 she joined LFB SA in Paris as Program Director for Monoclonal Antibodies. Today she is working as Chief Scientific Officer for TECObiosciences, a company within the TECOmedical Group that provides consultancy and regulatory services for the (bio)pharmaceutical industry.

Koen Raemdonck

Dr. Koen Raemdonck obtained his Master’s degree in Pharmaceutical Sciences in 2004 at Ghent University. In the same year he became a doctoral fellow of the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT). He initiated his PhD research at the Laboratory of General Biochemistry and Physical Pharmacy under the supervision of Prof. Stefaan De Smedt and Prof. Jo Demeester. His doctoral research was mainly focused on the application of nanogels for the controlled intracellular delivery of small interfering RNA (siRNA) for which he earned the title of doctor in pharmaceutical sciences in 2009. Since 2010, Koen Raemdonck is a postdoctoral fellow of the Research Foundation-Flanders (FWO). For his work, he received the biennial National Prize of the Belgian Society of Pharmaceutical Sciences in 2011 and the Prize of the Royal Academy of Medicine for Scientific Research in Pharmacy

in 2014. Currently, Dr. Raemdonck is working as a visiting postdoc in the Institute of Pharmaceutical Sciences of ETH Zürich. His research activities to date resulted in >30 peer reviewed publications, many of which in leading journals in the field (ACS Nano, Advanced Functional Materials, Biomaterials, Journal of Controlled Release, etc.). As a senior postdoc, Dr. Raemdonck supervises 4 PhD students, exploring novel bio-inspired approaches for small RNA delivery.

Euan Ramsay

Euan Ramsay, Ph.D. is the COO and co-founder of Precision NanoSystems Inc. He has 15 years experience in the science and commercialization of nanoparticle systems. Euan has an undergraduate degree in pharmacy from the U. of Strathclyde, Glasgow, Scotland and a Ph.D. in gene therapy from Cardiff U., Wales. Prior to Precision, Euan worked at the Centre for Drug Research and Development where he led a funding program that raised over $33 million for drug discovery and development projects. Additionally, Euan was Co-inventor of the nanoparticle drug, Irinophore C, taking it to Phase 1 clinical trials.

Christoph Rehbock

Dr. rer. nat.
Date of birth: March 12, 1979
University of Duisburg-Essen
Faculty of Chemistry, Technical Chemistry I and Center for Nanointegration Duisburg-Essen (CENIDE), Universitätsstraße 7
45141 Essen
Phone: +49 201 183 3040
E-Mail: Christoph.rehbock@uni-due.de

Christoph Rehbock studied chemistry in Hannover and made his PhD in the group of Prof. Scheper in Hannover in the field of biotechnology. Following a short period of postdoctoral work at the Laser Zentrum Hannover he went to the University of Duisburg-Essen and joined the group of Prof. Barcikowski in 2011. Since then he has been working as a postdoctoral researcher in the field of nanoparticle generation by pulsed laser ablation in liquids. His main research topics entailed the fundamental understanding of the particle formation process in the presence of different electrolytes as well as the bioconjugation of laser-fabricated nanoparticles. Since 2014, he has been appointed group leader of the newly-founded “NanoBio” subgroup supervising five projects in the field of nanotechnology for biological and medical applications. Since 2011 he authored 13 peer reviewed publications in the respective field.

Matthias Reumann

Matthias Reumann (1978) received the Masters of Engineering in Electronics with the Tripartite Diploma from the University of Southampton, UK, in 2003 and continued his PhD studies at the Karlsruhe Institute of Technology with Prof. Olaf Doessel at the Institute for Biomedical Engineering, Universitaet Karlsruhe (TH). Reumann focused on translational research in cardiac models and his PhD with summa cum laude in 2007. The research was awarded with two prestigious research awards by both clinical and biomedical professional societies. Reumann continued research in multi-scale systems biology at the IBM T. J. Watson Research Center, Yorktown Heights, NY. His work focused on creating high resolution heart models that scale on supercomputers that yielded several high profile publications in Science Translational Medicine, the Journal of the American College of Cardiology and Supercomputing. He expanded his research interest to Genomics in 2010 at the IBM Research Collaboartory for Lifesciences – Melbourne, investigating higher order interaction of single nucleotide polymorphisms in breast and prostate cancer in collaboration with Prof. John Hopper. In 2011, Reumann build up and the healthcare research team at the IBM Research – Australia laboratory with focus areas in healthcare analytics, medical image processing and genomics. The goal in genomics was to bring next generation sequencing into a production environment in a public health microbiology diagnostic unit. Reumann moved back to Europe in December 2013 and joined the IBM Research – Zurich laboratory where his research intents to leverage high performance computing in the field of Systems Biology. Reumann is associate editor of the IEEE Journal on Translational Engineering in Health and Medicine, Senior Member of the IEEE and has served on the Administrative Committee of the IEEE Engineering in Medicine and Biology Society from 2009 – 2013 as well as on the IEEE Technical Advisory Board form 2011 – 2012. His research is mentioned in editorials and reviews and has received numerous awards.

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.

Eder Lilia Romero

Eder Lilia Romero was educated at the Universidad Nacional 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), associate professor of Chemistry at the Science and Technology Department of the Universidad Nacional de Quilmes (UNQ, 2008). Since 2007 she is the Director of the Nanomedicine Research Program at the UNQ. She has co-supervised 2 and supervised 5 doctoral thesis, published more than 40 articles in peer reviewed international journals, 5 book chapters, and given nearly 100 national and international conferences and invited lectures. Since 2008 she joined the Advisory Committee of the Argentinean Foundation for Nanotechnology (FAN). She is a founding member of the Argentinean Association for Nanomedicines (Nanomed-ar) (2010-). She has received funding from the UNQ, CONICET, ANPCyT, EULANEST and from private sources (pharmaceutical companies). She is responsible for the Nanomedicine in Latino America Schools and Editorial Board Member of Nanomedicine: Nanotechnology, Biology and Medicine (Elsevier), between other peer reviewed journals. She acts as an academic consultant for different Latin American pharmaceutical companies. Her main research interests are: Natural nanomaterials from sustainable sources: Preclinical development of therapeutic and prophylactic strategies against a) protozoan parasite infections (Chagas disease, leishmaniasis); b) fungal (Candida and other) and bacterial (Pseudomonas and other) diseases; c) anti-inflammatory diseases, based on designing nanostructures (nanovesicles, solid lipid nanoparticles, nanostructured lipid carriers) prepared with lipids extracted from hyperhalophile archaea from Patagonia, Cuyo, center and north west Argentine salt ponds. Polymeric legos: Design of tecto-dendrimers (commercial dendrimers as units) as drug delivery systems 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.

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 BioNanomaterials 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. She is also contributing to the expert discussions in the review of drug applications, and the revision of the Japanese Pharmacopoeia.
She is responsible for the research on evaluation method for highly functionalized 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. 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. We also characterize exosomes from prostate cancer cells 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 around 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 68.
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.

Raymond Schiffelers

Raymond Schiffelers studied Bio-Pharmaceutical Sciences at Leiden University (1990-1995). After an industrial traineeship at SmithKline Beecham Pharmaceuticals (UK) he did his PhD in medical microbiology at Erasmus University Rotterdam on liposomal targeting of antimicrobial agents (1996-2001). Subsequently, he became post-doc at Utrecht University working on liposomes targeting tumor vasculature. In 2002-2003, at Intradigm Co (USA), he expanded his tumor vasculature-targeting work with polymers for delivery of siRNA. After his return to Utrecht University he became assistant and then associate professor. In 2011, he moved to the Laboratory for Clinical Chemistry & Hematology of the University Medical Center Utrecht, where he became professor of nanomedicine, both for diagnosis and therapy. In particularly, he focuses on extracellular vesicles in the circulation as inspiration for new drug delivery systems and diagnostic readouts. He is founding member of the International Society for Extracellular Vesicles (ISEV) founding member and Associate Editor of the Journal Extracellular Vesicles, and Founder of EXCYTEX-an extracellular vesicle-based company.

Louis Schlapbach

Dr. sc. nat.
ETH Zurich, Prof.em. Physics ETH
Director Empa 2001-2009, guest at National Institute for Materials Science NIMS, Tsukuba

Louis Schlapbach, born 1944 (Belp-Berne), Swiss, was Director of Empa (Swiss Federal Lab for Materials Science and Technology) and Full Professor of Physics at ETH 2001-2009. He graduated from the Swiss Federal Institute of Technology Zurich (ETH) in Experimental Physics and got his PhD in Solid State Physics – Magnetism also at ETH. As a postdoc at a CNRS laboratory in Paris, he studied hydrogen storage in intermetallic compounds. Back at ETH, he developed the surface science aspects of the hydrogen interaction with metals and alloys.
From 1988 to 2001, Louis Schlapbach was Full Professor for Experimental Physics at the University of Fribourg. As such he built up a research team of 20-25 people working on the topic „New Materials and their Surfaces“ resulting in about 40 PhD, 280 scientific papers and several patents. A strong collaboration with industry was established.
From 2001-2009, he was Director of Empa, a Materials Science and Technology Institution of the ETH domain with 800 coworkers in Dübendorf-Zurich, St. Gallen and Thun. He successfully transformed the former materials testing institution into a modern materials research and technology laboratory.
His research interests concern mainly nanoscopic properties of new materials and surfaces/interfaces, hydrogen interaction with solids, functional surfaces and coatings, materials for energy technology as well as new analytical tools. His NATURE-paper “Hydrogen-storage materials for mobile applications“ (414, p. 353, 2001) was cited more than 1900 times, and the Springer books “Hydrogen in Intermetallic Compounds I, II” were quickly sold out. He is co-editor of “Hydrogen as a future energy carrier”, Wiley 2008. (ISI Web of Knowledge 2013: 380 publications, average citations 30, h-Index above 50)
Louis Schlapbach was member oft he Research Council of the Swiss National Science Foundation SNF from 1997 to 2004; 2009-2015 he presides the steering committee of the National Research Programme NRP „Smart Materials“. He works as an expert of the Swiss Innovation Promotion Agency (KTI/CTI) and of the Swiss Academy for Technical Sciences. He is honorary member oft he Swiss Physical Society.
In 2009/10 he joins the new „Global Research Center for Environment & Energy based on Nanomaterials Science“ (GREEN) of the National Institute for Materials Science (NIMS), Tsukuba,Japan, part time, and participates at the hydrogen technology work of I2CNER at Kyushu University. He is on the Scientific Boards of NIMS and its nanoscience programme MANA, of the Hasler Foundation, of the Fonds National de la Recherche Luxembourg FNR, of the Agence Nationale de la Recherche ANR, France, and of the Dr. h.c. Robert Mathys Foundation (RMS) and became member of the Helmholtz Senat in 2010.
He spent sabbaticals at NIMS Tsukuba, at IBM Research Center San José, at Hebrew University in Jerusalem, at Stanford University, at Osaka National Laboratory, at CNRS Paris, and attended an INSEAD Executive Management Training.

Ruth Baumberger Schmid

Year of birth	1952
Nationality	Swiss
Position	Vice President Marketing
Institute	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 poly-

mers, 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

1. H. Johnsen & R. Schmid, J. Microencapsulation 24, 731-742 (2007). "Preparation of polyurethane nanocapsules by miniemulsion polyaddition."

2. R. Schmid & H. Johnsen, 34th Annual Meeting & Exposition of the Controlled Release Society, 7.-11.7.2007, Long Beach, CA, USA. "Biodegradable and biocompatible nanocapsules and particles prepared by the miniemulsion polymerisasjon."

3. A. Dessy, S. Kubowicz, M. Alderighi, C. Baroli, A. Piras, R. Schmid & F. Chiellini, Colloids and Surfaces B: Biointerfaces 87 (2), 236-242 (2011). "Dead Sea Minerals Loaded Polymeric Nanoparticles."

4. S. Kubowicz, P. Stenstad, H. Johnsen & R. Schmid, 38th Annual Meeting & Exposition of the Controlled Release Society, 31.7.-3.8.2011, National Harbour, Maryland, USA. "Protein-Coated Biodegradable Poly(butyl-2-cyanoacrylate) Nanoparticles with Stealth Surface Properties."

5. R. Schmid, P.M. Stenstad & Y. Mørch, "Ultrasound bubbles stabilized with multifunctional nanoparticles for combined diagnostics and therapy", International Workshop COST Action TD1004, 17.-18.2.2012, Torino, Italy.

6. S. Armada, R. Schmid, W. Equey, I. Fogoaga & N. Espallargas, J Thermal Spray Techn. 22, 10-17 (2013). "Liquid-Solid Self-Lubricated Coatings."

7. R. Schmid, Y.A. Mørch, P. Stenstad, R. Hansen, Y. Hansen, M. Afaczi, S. Eggen & C. de Lange Davies, BioNanoMed 2013, 13.-15.3.2013, Krems, Austria (invited speaker). "Multifunctional Nanoparticles for Ultrasound-mediated Diagnosis and Therapy."

8. Y.A. Mørch, P.M. Stenstad, R. Schmid, C. de Lange Davies, S. Eggen, A. Åslund & S. Snipstad, Nanobiotechnology Int. Workshop, EC JRC, 3.-5.12.2013, Ispra, Italy. "Characterization of nanoparticles and microbubbles."

9. R. Schmid, Y.A. Mørch, P. Stenstad, R. Hansen, S. Berg, Y. Hansen, M. Afaczi, S. Eggen, H. Blom & C. de Lange Davies, 40th Annual Meeting & Exposition of the Controlled Release Society, 21.-24.7.2013, Honolulu, Hawaii, USA. "Gas Bubbles Stabilized by Multifunctional Nanoparticles for Ultrasound-Mediated Durg-Delivery."

10. 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 (2015), accepted. "Nanoparticle-Stabilized Microbubbles for Multimodal Imaging and Drug Delivery."

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 received 20 awards, including being a Horev Fellow – Leaders in Science and Technology, an Alon Fellow; a former recipient of the Intel Nanotechnology-, TEVA Pharmaceuticals-, and the Wolf Foundation PhD-student Awards. Avi is the author of many research papers and inventor on 12 patents.

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 Advisory Board of Oryzon Genomics, a Spanish leading biotech company. He helds 12 patents, most transfered to leading companies of the biotech and pharma sectors. He also leads the "drug delivery and targeting group" at the CIBBIM-Nanomedicine. In this context, 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" where he co-authored the 2006 Research Strategic Agenda intended to the European Commission. 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 50 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 has been recently appointed as Deputy Director and technology transfer coordinator. Dr Schwartz is also a Co-founder and Science Advisor of ARGON Pharma SL (2008), 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 acting as Science Advisor of ORYZON GENOMICS, 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 Soci- ety 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.

Christopher Scott

Chris Scott's background is in the development of novel antibody-based therapeutic strategies and more recently in nano medicine. Chris's work is focused on the development and translation of nano medicine for treatment of cancer and inflammation and is supported by the Medical Research Council. He is currently a Royal Society In dustrial Fellow with GSK.

Michael Sela

Date and place of birth: 6 March 1924
Tomaszow, Poland

Scientific discipline: Immunologist and protein chemist
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THE MOST IMPORTANT AWARDS, PRIZES AND ACADEMY MEMBERSHIPS INCLUDE:

Israel Prize in Natural Sciences (1959); Rothschild Prize in Chemistry (1968); Otto Warburg Medal, German Society of Biological Chemistry (1968); Emil von Behring Prize of the Phillips University (1973); Gairdner Foundation International Award, Toronto (1980); The Prize of the Institut de la Vie, Fondation Electricite de France (1984); Commander's Cross of the Order of Merit of the Federal Republic of Germany (1986); Officer of l'Ordre de la Legion d'Honneur France (1987), Albert Einstein Golden Medal (UNESCO) (1995); Harnack Medal of Max-Planck Society (1996); Interbrew-Latour Health Prize, Belgium (1997); Caballero, Order de San Carlos, Colombia (1997); Wolf Prize in Medicine (1998), Commandeur; l'Ordre de le

Legion d'Honneur, France (2011), Gran Ufficiale of the Italian Solidarity Star (2007).

HONORARY DOCTORATES:

Universite de Bordeaux II (1985); National Autonomous University of Mexico (1985); Tufts University, Medford MA (1989); Colby College, Maine (1989); Universite Louis Pasteur, Strasbourg (1990); Hebrew University, Jerusalem (1995); Tel Aviv University (1999); Ben-Gurion University of the Negev (2001) Honorary Fellow, the Open University of Israel (2004),. Honorary Degree Honoris Causa from the College of Management Academic Studies, Rishon Le Zion (2009), Honorary Fellow, Interdiciplinary Center Herzlia.

MEMBERSHIP IN:

Israel Academy of Sciences and Humanities; American Academy of Arts and Sciences; Pontifical Academy of Sciences; U.S. National Academy of Sciences; Deutsche Akademie der Naturforscher Leopoldina; Russian Academy of Sciences; French Academy of Sciences; Italian Academy of Sciences; American Philosophical Society; Romanian Academy; Polish Academy of Arts and Sciences. European Academy of Sciences and Arts. Honorary Member: American Society of Immunologists; Gesellschaft fur Immunologie; Scandinavian, French, Chilean and Colombian Societies of Immunology.

ACTIVITIES:

Michael Sela obtained his Ph.D. from the Hebrew University in Jerusalem for research carried out at the Weizmann Institute of Science. He continues until this day at this Institute, as a Professor from 1963, as Head of the newly created Department of Chemical Immunology from 1963 to 1975, as Dean of the Faculty of Biology from 1970 to 1973, as Vice-President in 1970–71, as President from 1975 to 1985 and since then until now, as Institute Professor and Deputy Chairman of the Board of Governors. He has been a visiting scientist or professor at the NIH (1960–61, 1973–74), University of California in Berkeley (1967–68), College de France in 1973 and in 1986–87 he was at the Tufts University, MIT and Harvard University. Since 1967 he has been a Foreign Member of the Max-Planck-Institute for Immunobiology in Freiburg. Between 1970 and 1974 he served as Vice-Chairman and Chairman of the Basel Institute of Immunology, between 1975 and 1979 as Chairman of the EMBO Council, between 1978 and 1981 as Chairman of the Scientific Advisory Committee of EMBL, between 1977 and 1980 as President of the International Union of Immunological Societies, from 1989–1996 as President of the Pasteur-Weizmann Scientific Council, and from 1998 Honorary President, Pasteur-Weizmann Council.

Served in various capacities as a consultant to the World Health Organization, including between 1979 and 1982 as a Member of its Global Advisory Committee. Served between 1984 and 1993 as a Member of the Executive Committee of the International Council of Scientific Unions. In 1996 he became the first President of the newly created FISEB (Federation of Israeli Societies of Experimental Biology).

Published more than 800 articles, chapters, books, in the fields of immunology, biochemistry and molecular biology.

Among his many activities outside of science can be mentioned that he serves as Honorary President of the Public Council of the Batsheva Company for Modern Dance, Honorary Vice-Chairman of the Arthur Rubinstein International Master Piano Competition, Chairman of the Committee of the Marcus Sieff Prize for Outstanding Initiative in Improving Relations between Jews and Arabs, Chairman of the Presidium of the Movement for Quality Government in Israel, and Founding Member of the Itzhak Rabin Memorial Center for Israeli Studies.

Hripsime Shahbazian

Mrs. Hripsime Shahbazian holds a MSc. in Medical Physics and a BSc in Molecular Physics. She worked as a Research Assistant in Physics Department at Carleton University while working towards her Master of Science degree. She joined Health Canada in 1988 as a Technology Assessor at the Medical Devices Bureau (MDB) and from 1991 to 1998 she was acting in different managerial roles within the Bureau. While at the MDB she contributed to the development and finalization of the new Medical Devices Regulations. Mrs. Shahbazian chaired and participated in various working groups involved in planning and implementation of the new regulations.

In 1998 Mrs. Shahbazian joined the Office of Science within the Therapeutic Products Directorate (TPD), at the Health Products and Food Branch (HPFB) 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 Committees, Scientific/Expert Advisory Panels, Reconsiderations and Second Level of Appeals. Mrs. Shahbazian is one of the key members working on the development and implementation of nanotechnology related activities at Health Canada. She chairs the Directorate (TPD) and Branch (HPFB) Working Groups 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. Mrs. Shahbazian was a member of the Health Portfolio team that developed the Policy Statement on Health Canada's Working Definition for Nanomaterial.

Martin Shaw

Martin Shaw has had many years experience in the development and application of novel laboratory tests and biomarkers in the laboratory medicine and pharmaceutical research industries. He has participated in industry consortia on biomarker qualification, lectured at many congresses and published peer-reviewed articles on the application of biomarkers in pharmaceutical research. Martin combines knowledge of the basic science of biomarkers and their practical application.

Marco Siccardi

Department of Molecular and Clinical Pharmacology, University of Liverpool, Liverpool, United Kingdom
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Marco Siccardi graduated with an MSc in Clinical Biology (2006) at the University of Turin, Italy. He obtained his PhD at the University of Liverpool, Liverpool, UK (2011) focusing his research on molecular mechanisms influencing drug distribution and predictors of exposure in patients. During his post-doctoral research he developed physiologically-based mathematical models to investigate the pharmacokinetics of traditional formulation and nanoparticles in virtual patients and simulate the outcome of various clinical scenarios. He was recently appointed as a Lecturer in Nanomedicine across the faculties of Health & Life Sciences and Science & Engineering at the University of Liverpool. He has authored >50 peer

reviewed publications, presenting his findings at several national and international congresses. In 2013, he was awarded with the "Most Informative Scientific Report 2013" at the Simcyp Academic in vitro – in vivo extrapolation (IVIVE) Awards, which recognise innovative teaching methods and cutting-edge published research in the fields of IVIVE, pharmaceutics, modelling and simulation. His research interests focus on the optimization of novel nanomedicine and traditional formulation for drug delivery based on experimental pharmacological data from in vitro and in vivo models, aiming to improve pharmacokinetics, efficacy and side effects. Moreover he is interested in the clarification of the ADME processes involved in drug disposition and the identification of nanoformulation characteristics influencing drug exposure, through the application of physiologically based pharmacokinetic models.

Dmitri Simberg

Received his Ph. D. in Biochemistry, The Hebrew University of Jerusalem, Israel. Thesis: "Cationic lipid-mediated transfection in vitro and in vivo: the main obstacles and means to overcome them". Did the postdoctorate in the laboratory of Prof. Erkki Ruoslahti, Burnham Institute, La Jolla. His research theme was: Amplified targeting of nanoparticles for tumor imaging and treatment. Second postdoctoral fellowship was in the laboratory of Prof. Robert Mattrey, Department of Radiology, UCSD, on perfluorocarbon-based molecular imaging reagents. After the postdoc became an assistant project scientist at NanoTumor Center, Moores Cancer Center, UCSD and in 2013 he joined the Department of Pharmaceutical Sciences, The Skaggs School of Pharmacy, UC Denver as an assistant professor,.

Dr. Simberg's work in focused on immune interaction between nanoparticles and body milieu. Dr. Simberg is a member of controlled release society, American association for cancer research, NIH review panels, and scientific advisory committee for Journal of Pharmaceutical Sciences. He is the author of over 30 peer reviewed papers and reviews, 3 book chapters, and made over 30 presentations at national and international meetings.

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 more than 90 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.

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, 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 University of Buenos Aires in 1994 and M.Sc. (equivalency) and Ph.D. degrees in applied chemistry from the Casali Institute of Applied Chemistry (The Hebrew University of Jerusalem, Israel, 2003) under the supervision of Prof. Daniel Cohn. Between 2003 and 2006, 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). In 2006, he was appointed Adjunct Professor (tenure) of Pharmaceutical Technology at the Faculty of Pharmacy and Biochemistry (University of Buenos Aires) and Investigator (National Science Research Council). In Argentina, he established a research group that works at the interface of biomaterials science, nanotechnology, and therapeutics. His research interests are focused on the exploration of micro and nanotechnologies for the encapsulation, delivery, and targeting of drugs involved in the pharmacotherapy of HIV, tuberculosis, cancer and other diseases. In this context, he has directed several competitive research grants and supervised 3 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 is co-author of over 100 peer-reviewed articles, reviews, editorials and book chapters and co-inventor in three patents and patent applications. He has been Visiting Professor and Scientist at the National University of Colombia (Colombia), the University of Santiago de Compostela (Spain), Council for Scientific and Industrial Research (South Africa), North-West University (South Africa), the National Autonomous University of Mexico (Mexico), and the Hospital Sant Joan de Déu (Spain) where he taught graduate courses and presented invited conferences and seminars. Prof. Sosnik has 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 gathers eleven research groups and companies of Spain, Portugal, Mexico, Cuba, Colombia, Brazil and Argentina and over 75 scientists and coordinated it in the period 2011-2013. He joined the Department of Materials Science and Engineering of Technion as Associate Professor in 2013 where he established the Laboratory of Pharmaceutical Nanomaterials Science. He was awarded the Marie Reintegration Grant of the European Commission for the period 2014-2018. His

main research lines are polymer and macromolecular chemistry, biomaterials science, microwave-assisted polymer synthesis, drug crystallization and nanocrystals, colloidal chemistry (drug and polymer self-assembly), mucoadhesive drug delivery systems, nanomedicine (drug encapsulation, release and targeting), therapy of poverty-related diseases (HIV, tuberculosis), cancer, intestinal diseases and pharmacokinetics (oral, inhalatory and intranasal administration routes).

Silvia Staniscuaski Guterres

Sílvia Stanisçuaski Guterres is a Full Professor of Pharmaceutical Technology in the College of Pharmacy at the Federal University of Rio Grande do Sul, Brazil, since 1989. She received her PhD in Pharmaceutical Nanotechnology from the University of Paris XI, France, in 1995. She was advisor of more than 45 graduate students (master and Ph.D. levels) from 1997 to 2015 at the Programa de Pós-Graduação em Ciências Farmacêuticas (PPGCF/UFRGS – UFRGS Pharmaceutical Sciences Graduate Program). She is a National Council for Scientific and Technological Development (CNPq/Brazil) researcher and leader of the research group entitled Nanostrucutured Systems for Drug Administration. Guterres's research interests are focused on the development, physicochemical characterization and biological applications of innovative nanocarriers aiming at drug delivery via oral, cutaneous and parenteral routes. She is the Director of one of the Brazilian National Nanotechnology Network, supported by the Brazilian Ministry of Science and Technology. She is the Coordinator of collaborative projects on Nanotechnology between France (Université de Paris-Sud, Professor Elias Fattal), Germany (Saarland University, Professor CM Lehr) and Italy (Università degli Studi di Parma, Professor Fabio Sonvico) and Brazil, supported by the Brazilian Ministry of Education and National Council for Scientific and Technological Development (CNPq/Brazil). She has published more than 200 peer-reviewed manuscripts and 8 book chapters. She filed 28 patents relating to nanotechnology products and processes. She is a member of the Brazilian Chemical Society (SBQ), the Centro de Nanociência e Nanotecnologia (CNANO/UFRGS) and the Brazilian Association of Pharmaceutical Sciences (ABCF). She is member of the Editorial Board of the Journal of Drug Science and Technology. She is a member of the Scientific Council of ITEHPEC (Brazilian Institute of Technology and Studies of personal Hygiene, Perfumery and Cosmetics).

Gert Storm

Professor Gert Storm studied biology at the Utrecht University, The Netherlands. He graduated in 1983. He obtained his Ph.D. degree in 1987 at the Dept. of Pharmaceutics of the same university. His research interests are in the fields of biopharmaceutics and drug targeting. In 1988-1989 he was a visiting scientist at Liposome Technology Inc. in Menlo Park, USA, and visiting assistant professor at the School of Pharmacy, UCSF, San Francisco. In 1990-1991 he was senior research scientist at Pharma Bio-Research Consultancy B.V. in Zuidlaren, The Netherlands. During this period he contributed to the design, co-ordination and evaluation of clinical pharmacological studies. In September 1991 he took up his position at the Utrecht University. In 1999, he was appointed adjunct professor at the Royal School of Pharmacy, Copenhagen. From July 2009 on, he is Honorary Professor in Biomacromolecular Drug Delivery at the University of Copenhagen. In 2000, he was appointed as professor (Targeted Drug Delivery) at Utrecht University. From 2012 on, he is also professor (Targeted Therapeutics) at the MIRA institute of the University of Twente. Furthermore, he also keeps a position at the University Medical Center Utrecht (UMCU) within the CBOI institute (Center for Image-Guided Oncological Interventions).
He is author/co-author of >450 original articles, reviews and book chapters, in the field of advanced drug delivery/drug targeting, and theme (co-)editor of Advanced Drug Delivery Reviews and the book 'Long Circulating Liposomes. Old Drug, New Therapeutics'. His H-index is >70 (Google Scholar), and he is included in the 2014 list of The World's Most Influential Scientific Minds of Thomson Reuters (Highly Cited Researchers 2014, period 2002-2012). Also recognized in Europe as 'Most Cited Author' in the field of Pharmacology & Pharmacy (Publication Analysis 2005-2011, Labtimes 05/2013). He was co-ordinator of an Integrated Project (FP6) on targeted nanomedicines (MediTrans) based on the collaboration of 30 european partners and funded by the EC and industry. He is program director of the program Drug Delivery embedded within the recently approved New Nano Initiative (NanoNextNL) strongly sponsored by the dutch government and industry. He is also principal investigator of a national industry-academia partnership (HIFU-CHEM) studying the clinical application of MRI-guided high-intensity focused ultrasound (HIFU) to improve cancer chemotherapy with temperature-sensitive targeted nanomedicines. He is course director of the GUIDE/UIPS/LACDR Course on Advanced Drug Delivery & Drug Targeting, co-sponsored and accredited by EUFEPS and the GALENOS Network, and held in The Netherlands. He is on the Board of Scientific Advisors (BSA) of the Controlled Release Society (CRS). He is on the Scientific Board of the spin-off company Enceladus Pharmaceuticals BV (Amsterdam). He is involved in organizing conferences in the field of advanced drug delivery, e.g. chairman of the ESF-UB Conference "Nanomedicine: Reality Now and Soon", held 23-28 October 2010 in San Feliu de Guixols, Spain. He is member of the editorial (advisory) board of a variety of scientific journals. He was involved in the foundation and is currently on the board of the European Foundation for Clinical Nanomedicine (CLINAM) and The Netherlands Platform for Targeted Nanomedicine (NPTN). He has received awards for his activities as translational pharmaceutical scientist.
contact: g.storm@uu.nl

Rudolf W. Strohmeier

Deputy Director-General of the European Commission's Directorate-General for Research and Innovation since 2010. In this capacity, he oversees the collaborative research activities of the Directorate-General. He acts as European Co-chair of the Executive Committee of the International Group on Earth Observations (GEO), alternating Chairman of the GB of the Innovative Medicines Initiative (IMI) and Member of the GBs of the Joint Undertakings Fuel Cells and Hydrogen (FCH) and BioBased Industries (BBI, in formation). He also chairs the European Research Area and Innovation Committee. After studies of Law and Economics at the University of his home town Würzburg and that of Bonn, he started his career as teaching assistant at the Department of Civil and Commercial Law at the University of Würzburg in 1979. In 1980, he joined the Bavarian Liaison Office to the Federal Govern-ment in Bonn. Following a 2-years detachment to the Development Policy department of the European Commission, he established the Bavarian Information Office in Brussels and became its first Director.
From 1987, he served in the three Cabinets of Commissioner Peter Schmidhuber (Macroeconomics and Regional Policies, later Budget and Budget Control). In 1995, Agriculture and Rural Development Commissioner Franz Fischler appointed him Deputy Head of his Cabinet.
Nominated Head of Division in DG Agriculture and Rural Development in 2000, he joined the Cabinet of Commission President Prof. Romano Prodi in 2003 as Adviser i.a. for industrial, agricultural and

environmental policies. From 2005-2010, he served as Head of Commissioner Viviane Reding's 2nd Cabinet (Telecommunication and Media).
He is alumni of the German Marshall Fund of the US, founding member of the Belgian-Bavarian Society, member of the Scientific Society for Middle Classes (Munich), editor of two books and author of various articles dealing with European policies.

Janos Szebeni

Janos Szebeni, M.D., Ph.D., D.Sc., Med. Habil., immunologist, director of the Nanomedicine Research and Education Center at Semmelweis University, co-sponsored by the Bay Zoltán Applied Research Nonprofit Ltd. in Budapest, Hungary. He also has teaching or guest professor affiliations at the following institutions: Institute of Pathophysiology, Semmelweis University; Department of Nanobiotechnology and Regenerative Medicine, Faculty of Health Science, Miskolc University; and Faculty of Pharmaceutical Sciences and NanoScience Center, University of Copenhagen, Denmark. He regularly teaches biology, immune biology and nanomedicine. He obtained M.D. in 1978 at Semmelweis University, and then held various scientific positions in Hungary and abroad, including the Institute of Hematology in Budapest, Christchurch University (Christchurch, New Zealand), ETH (Zurich, Switzerland), University of Arizona (Tucson, Arizona), Harvard University (Boston, MA), National Cancer Institute at NIH and the Walter Reed Army Institute of Research (Bethesda, MD, USA). His research over 34 years on various themes in hematology, membrane biology and immunology resulted some 90 scientific papers, 12 book chapters, 2 patents, a book "The Complement System: Novel Roles in Health and Disease" (Kluwer Academic Press, 2004) and a topical issue of "Critical Reviews in Therapeutic Drug Carrier Systems". Two fields stand out where he has been most active: liposomes and the complement system. He is best known for spearheading the concept that complement activation underlies numerous liposomal- and other nanodrug-induced hypersensitivity (anaphylactoid) reactions, called complement activation-related pseudoallergy (CARPA). Along with numerous social commitments in Hungary and abroad, he is a founder and scientific director of an immune toxicity CRO in Hungary (SeroScience Ltd).

Birgit Teubl

Birgit Teubl received her diploma degree in Pharmacy in 2010 from the University of Graz. Her diploma thesis was focused on buccal permeability studies of polystyrene- and silver nanoparticles. After graduation she undertook her PhD at the University of Graz, Institute of Pharmaceutical Sciences, Department of Pharmaceutical Technology with a project focusing on nano-safety and transport studies of nanostructured materials into/across the oral mucosa. During her PhD, she joined the working group of Prof. Lehr at the Helmholtz-Institute for Pharmaceutical Research Saarland for one year. Since 2014, she has been at the University of Graz as a postdoc.
Her research field includes the interactions of nanoparticles with human oral biological barriers and possible adverse effects.
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 nano-periodic property patterns/relationships normally associated with traditional atoms. Tomalia is now applying many of this nano-periodic paradigm and these principles to nanomedicine (J. Internal Medicine, in press (2014)).

Irmgard Thorey

Dr. Irmgard Thorey (PhD) is a member of Roche's Large Molecule Research organization within Pharma Research & Early Development at the Roche Innovation Center Penzberg, Germany. Her current work focuses on antibody engineering, bi-specifics, and on delivery platforms for targeted payload delivery. Dr. Thorey combines extensive experience in transgenic animal systems with applications in the field of large molecules. She has thereby been instrumental in the development of a proprietary platform within Roche that enables the generation of human antibodies in rabbits. Prior to Roche, Dr. Thorey applied her experiences in the Biotech industry (Switch Biotech & Therapeutic Human Polyclonals/acquired by Roche), as well as in clinical institutions (University of Frankfurt Medical School).

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 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 (2011), 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 Board, Nanomedicine (Elsevier); Ed. Board of Bioconjugate Chemistry and is a founding member of the Ed. Board for NanoLetters (2000-4).
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 > 2,680 citations. Tomalia was inducted into the Thomas Reuters Hall of Citation Laureates in Chemistry (2011) (i.e., top 40 most highly cited scientists in the field of chemistry).

Susumu Tonegawa

Susumu Tonegawa received his B. Sc. from Kyoto University and his Ph.D. from University of California, San Diego (UCSD). He then undertook postdoctoral work at the Salk Institute in San Diego, before working at the Basel Institute for Immunology in Basel, Switzerland, where he performed his landmark immunology research. Tonegawa won the Nobel Prize for Physiology or Medicine in 1987 for "his discovery of the genetic principle for generation of antibody diversity." Using advanced techniques of gene manipulation, Tonegawa is now making seminal contributions in the field of neuroscience by unraveling the molecular, cellular and neural circuit mechanisms that underlie learning and memory. His studies have broad implications for psychiatric and neurologic diseases. Tonegawa is currently the Director of the RIKEN-MIT Center for Neural Circuit Genetics at MIT, as well as the Director of RIKEN Brain Science Institute.

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). He is also the Founder / Owner / Managing Director of the CosmoPHOS Ltd which is an Innovative Translational Nanomedicine SME (small-medium enterprise) established in Thessaloniki, Greece (Ellas) since 2012. CosmoPHOS Ltd is focused on the Translational Research & Development of Novel Nanomedicine Products for the Early Diagnosis, Targeted Therapy, and Therapy Monitoring of Cardiovascular Diseases, and especially of Atherosclerotic Heart Disease which causes the myocardial infarctions (heart attacks), and of Atherosclerosis in general. Additionally, Dr med Trohopoulos is the Founder and the Scientific / Exploitation / Strategic Coordinator of the CosmoPHOS-nano Project 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 Participants with 4,5 Million Euros, having a total project budget of 13 Million Euros. The CosmoPHOS-nano is a Five-year R&D Project started on March 1, 2013 and will be concluded on February 28, 2018, and it is a Multidisciplinary R&D Project consisting 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 CosmoPHOS-nano Project 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) Molecular Imaging by using Near-Infrared Fluorescence (NIRF), b) Targeted Therapy by using Photodynamic Therapy (PDT), 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 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 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 Panagiotis (Panos) N. 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 Industry and set up together with the European Commission, addressing the application of nanotechnology to achieve breakthroughs in healthcare. Additionally, Dr med Panagiotis (Panos) N. Trohopoulos is and Advisory Board Member of CLINAM (European Foundation for Clinical Nanomedicine) which is a non-profit institution aiming at advancing medicine to the benefit of individuals and society through the application of nanoscience. Finally, Dr med Panagiotis (Panos) N. Trohopoulos is Founding Member and Steering Board Member of the International Society for Nanomedicine, 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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Arnold von Eckardstein

Arnold von Eckardstein studied medicine and then specialized in laboratory medicine and clinical chemistry. Since 2001 he is professor at the medical faculty of the University of Zurich and the director of the Institute of Clinical Chemistry of the University Hospital of Zurich (Switzerland) (www.ikc.unispital.ch). His main research interests include risk factors and biomarkers of cardiovascular and metabolic diseases as well as structure, function, and metabolism of high density lipoproteins (HDL) and sphingolipids. He has published more than 290 original and review papers in international peer reviewed scientific journals.

Arnold von Eckardstein is Associate Editor of Atherosclerosis and member of the Editorial Boards of Arteriosclerosis, Thrombosis and Vascular Biology, European Heart Journal, Journal of Lipid Research, and Journal of Clinical Chemistry and Laboratory Medicine. He is Fellow of the American Heart Association (Council on Arteriosclerosis, Thrombosis and Vascular Biology), Distinguished Fellow of the International Atherosclerosis Society, the president of the Swiss Working Group on Atherosclerosis and Lipids, and member of the executive board of the European Atherosclerosis Society. He is also Past Chairman of the European Lipoprotein Club, The German Atherosclerosis Society, and the Swiss Society of Clinical Chemistry.

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.

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. Hans was one of the founders of Izon in 2005 and became its fulltime 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 application to nanomedicine development and for extracellular vesicle research.

Viola Vogel

Viola Vogel is a Professor in the Department of Health Sciences and Technology (D-HEST) at the ETH Zürich, where she heads the Laboratory of Applied Mechanobiology. She received her doctorate from Frankfurt University after completing her graduate research at the Max-Planck Institute for Biophysical Chemistry, and was then a postdoctoral fellow at the University of California, Berkeley. Professor Vogel started her academic career in the Department of Bioengineering at the University of Washington, Seattle (1991-2004), and was there the Founding Director of the Center for Nanotechnology (1997-2003) before she moved to the ETH in 2004. She discovered many nanoscale mechanisms how forces switch molecular and cellular functions, and illustrated how to exploit such information for biomedical and technical applications. Major awards include the Otto-Hahn Medal from the Max-Planck Society (1988), Feodor-Lynen Fellowship Humboldt Foundation (1989-1990), "FIRST" Award from the National Institutes of Health (General Medicine) (1993), Research Award from the Philip Morris Foundation (2005), Julius Springer Award for Applied Physics (2006), the ERC Advanced Grant from the European Research Council (2007), the International Solvay Chair in Chemistry Brussels (2012), and an Honorary Doctorate from the University of Tampere Finland (2012). Her various advisory board functions include serving on the Jury of the Queen of Elizabeth Prize for Engineering (2015) and on the World Economic Forum Global Agenda Council.

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).

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

Peter Wick

Dr. Peter Wick
Head of Particles-Biology Interactions Lab
Empa
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9014 St. Gallen
Switzerland

Peter Wick heads since 2014 the research laboratory for Particles-Biology Interactions at the Federal Laboratories on Materials Science and Technologies Empa in St. Gallen. He studied and received his PhD in cell- and molecular biology at the University in Fribourg (Switzerland). Thereafter (2002) he moved to Empa and began his research in nanotoxicology among others with the national project NanoRisk, and is now active in further projects of the 7th Framework program of the EC, for example, NANOHOUSE, MARINA, NanoSolutions & Flagship Graphene and is also founded by the SNF NFP64 research program.
His general research interest is to study the interactions of nanomaterials with human barrier tissues in vitro and ex vivo with the purpose to obtain detailed mechanistic information about their uptake, accumulation, transport and effect on different types of cells or entire tissue. In addition he is interested in improvement of currently used acute toxicological test methods to obtain reliable and robust results. He is author of around 100 publications, including over 60 peer-reviewed papers, in the field of Nanosafety.
He is a member of the advisory board of the Swiss Action Plan on Nanomaterials and Editorial Board Member of Nanotoxicology.

Andreas Wicki

Andreas Wicki studied medicine at the University of Basel. He trained in medical oncology both in Switzerland and in Germany. He completed his PhD in the lab of Gerhard Christofori with a focus on mechanisms of cancer invasion and targeted delivery of anti-cancer compounds.
Currently, he is the head of the phase 1 oncology unit of the University Hospital Basel, a consultant in clinical oncology, and a project leader at the Dept. of Biomedicine, University of Basel. He also chairs the specialty training and continuing medical education committee of the Swiss Society of Medical Oncology.

Marieluise Wippermann

CEO, TECOmedical AG, Sissach, Switzerland
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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 is pursuing a Ph.D. in Nanoscience and Technology at 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 more than 20 scientific articles and has received 17 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 in which the drugs are introduced to. She also aspires to generate fruitful inter-institutional research collaborations around the world.

Yuliang Zhao

Professor
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Prof Zhao is well-known for his studies on toxicological properties of manufactured nanomaterials and cancer nanomedicines. He proposed the nanotoxicology research in 2001 in China and was one of the pioneer scientists worldwide initiated the nanotoxicology and nanosafety study. His research interests span both basic and translational research including Nanotoxicology, Cancer Nanomedicine, Nanochemistry for lowering the toxicity of nanomaterials or nanomedicines, and Molecular Dynamics simulation on nano-bio interface. He developed a novel nanomedicine (without delivery systems) for low-toxic cancer chemotherapy, especially, proposed the use of non-killing cell mechanism (prison cell not poison cells by low-toxic nanostructure materials) to tenderly inhibit cancer growth, rather than cancer cells killing which results in innocently normal cells being slaughtered. He is the author of over 320 publications, 10 books, and 17 book chapters. He has delivered over 200 invited talks worldwide.
Yuliang Zhao holds a BS degree from Sichuan University in Radiochemistry (1985) and a PhD degree in Radiochemistry from Tokyo Metropolitan University (1999). He was a JSPS Postdoctoral Fellow at TMU and a researcher at RIKEN prior to becoming a Hundred Elite professor at Institute of High Energy Physics, Chinese Academy of Sciences (CAS) in 2001. Prof. Zhao is the founder and director of CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety.

Dejian Zhou

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www.chem.leeds.ac.uk/People/Zhou.html

EDUCATION & EMPLOYMENT HISTORY

2007–now University of Leeds, Leeds, UK (Senior Lecturer & PI, with tenure from Oct 2009)
2000–2007 University of Cambridge, Cambridge, UK (Senior/Research Associate)
1997–2000 Cranfield University, Cranfield, UK (Postdoc)
1995–1997 Peking University, Beijing, China (Lecturer in Inorganic Chemistry)
1990–1995 Peking University, College of Chemistry, Beijing, China; Ph.D. in Inorganic Chemistry (Supervisor: Prof. Chun-Hui Huang)
1986–1990 Peking University, College of Chemistry, Beijing, China; B.Sc. in Chemistry (UK 1st Class honours equivalent).

HONORS AND AWARDS

- National 100 Excellent PhD Thesis Award (Ministry of Education, China, 1999)
- Excellent PhD Thesis Award (1st Class Prize, Peking University, Beijing, China, 1998)
- Young Chemist Award (Chinese Chemical Society, China, 1996)

PROFESSIONAL MEMBERSHIP & ACTIVITIES

Editorial Board Membership

Associate Editor: Computational and Structural Biotechnology Journal, RNCSB Academic Publishing-Sweden
Editorial Board Member: Advances in Chemistry, Hindawi Publishing Corporation (2013-)

Professional Membership

Member of Royal Society of Chemistry (2005–); Member of American Chemical Society (2005–).
Member of EPSRC Peer-review College (2012–)

Conference Presentations

30 Invited Plenary, Keynote and Oral presentations at international/UK conferences
24 Invited research talks and seminar at some key UK and international academic institutions

REPRENTATIVE RECENT PUBLICATIONS (TOTAL=120)

(Total citation counts: >2800; NC: number of citations; H-index = 30)

1. Y. Zhang, Y. Guo*, P. Quirke & D. Zhou*, “Ultrasensitive single-nucleotide polymorphism detection using target-recycled ligation, strand displacement and enzymatic amplification”. Nanoscale 2013, 5, 5027 (IF = 6.74, NC = 14).
2. Y. Zhang, C. Pilapong, Y. Guo,* Z. Ling, P. Quirke & Dejian Zhou*, “Sensitive, Simultaneous Quantitation of Two Unlabeled DNA Targets Using a Magnetic Nanoparticle-Enzyme Sandwich Assay” Anal. Chem., 2013, 85, 9238 (IF = 5.83, NC = 6).
3. H. Zhang, G. Feng, Yuan Guo* & D. Zhou*, “Robust, specific ratiometric biosensing using a copper-free clicked quantum dot-DNA aptamer sensor” Nanoscale, 2013, 5, 10307 (IF = 6.74, NC = 5).
4. L. Song, V. V.B. Ho, C. Chen, Z. Yang, D. Liu, R. Chen* & D. Zhou*, “Efficient, pH-triggered drug delivery using a pH-responsive DNA conjugated gold nanoparticle” Advanced Healthcare Materials, 2013, 2, 275. (IF = 4.88, NC = 14) Journal back cover.
5. Y. Kong, J. Chen, F. Gao, R. Brydson, B. Johnson, G. Heath, Y. Zhang, L. Wu & D. Zhou*, “Near-Infrared Fluorescent Ribonuclease-A-Encapsulated Gold Nanoclusters: Preparation, Characterization, Cancer Cell Targeting and Imaging”. Nanoscale, 2013, 5, 1009 (IF = 6.74, NC = 22).
6. J. Garcia, Y. Zhang, H. Taylor, O. Cespedes, M. E. Webb & D. Zhou*, “Multilayer enzyme-coupled magnetic nanoparticles as efficient, reusable biocatalysts and biosensors”; Nanoscale, 2011, 3, 3721. (IF = 6.74, NC = 31)
7. E. Cheng, Y. Xing, P. Chen, Y. Yang, Y. Sun, D. Zhou,* L. Xu, Q. Fan & D. Liu*, “A pH-trigged, fast-responding DNA hydrogel” Angew. Chem. Int. Ed. 2009, 48, 7660. (IF = 11.34; NC = 77)
Highlighted in NPG Asian Materials: http://www.natureasia.com/asia-materials/highlight.php?id=587
8. D. Liu,* A. Bruckbauer, C. Abell, D.-J. Kang, S. Balasubramanian, D. Klenerman & D. Zhou*, “A Reversible pH-Driven DNA Nanoswitch Array”, J. Am. Chem. Soc. 2006, 128, 2067. (IF =11.44: NC = 118)

Andreas Zumbuehl

Andreas Zumbuehl was born in Interlaken, Switzerland. He graduated from ETH Zürich 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.

CURRICULA VITAE OF POSTER PRESENTERS

Seyedeh Hoda Alavizadeh

Name: Seyedeh Hoda
Surname: Alavizadeh
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EDUCATION

- 2010-present: Ph.D. student in Pharmaceutical Nanotechnology of School of Pharmacy, Mashhad Medical University, Mashhad, Iran (Average Grade up to now: 19.66 /20).
- Thesis title: Formulation and characterization of temperature-sensitive nanoliposomal cisplatin targeted with anti-Her-2/neu affibody and evaluation of their antitumor effects with local hyperthermia in vitro and in vivo in mice model bearing tumor.
- 2004-2010: Pharm.D, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran (Total grade: 17.85 /20).
- Pharm.D. thesis title: Evaluation of lesion development and type of immune response generated in mice inoculated with L. major mixed with LPD nanoparticles containing CpG ODN (Written and available in English) (Score: 19.75/20)

RESEARCH EXPERIENCES

- Application of liposome in cancer treatment
- Application of liposome in vaccine delivery
- Application of LPD nanoparticles in vaccine delivery

INTERNATIONAL PUBLICATIONS

- Alavizadeh SH, Badiee A, Khamesipour A, Jalali SA, Firouzmand H, Abbasi A, Jaafari MR., The role of liposome-protamine-DNA nanoparticles containing CpG oligodeoxynucleotides in the course of infection induced by Leishmania major in BALB/c mice. Exp Parasitol. 2012 Nov; 132(3):313-9
- Rastgoo M, Hosseinzadeh H, Alavizadeh SH, Abbasi A, Ayati Z, Jaafari MR., Antitumor activity of PEGylated nanoliposomes containing crocin in mice bearing C26 colon carcinoma. Planta Med. 2013 Apr; 79(6):447-51.
- Firouzmand H, Badiee A, Khamesipour A, Heravi Shargh V, Alavizadeh SH, Abbasi A, Jaafari MR., Induction of protection against leishmaniasis in susceptible BALB/c mice using simple DOTAP cationic nanoliposomes containing soluble Leishmania antigen (SLA). Acta Trop. 2013 Dec; 128(3):528-35.
- Alavizadeh SH, Hosseinzadeh H., Bioactivity assessment and toxicity of crocin: a comprehensive review. Food Chem Toxicol. 2014 Feb; 64: 65-80.
- Alavizadeh SH, Badiee A, Golmohammadzadeh SH, Jaafari MR., The influence of phospholipid on the physicochemical properties and antitumor efficacy of liposome encapsulating cisplatin in mice bearing C26 colon carcinoma. Int J Pharm. 2014 Oct, 473; 326-333

REFEREE

Professor Mahmoud Reza Jaafari, School of Pharmacy, Mashhad University of Medical Sciences (MUMS), Mashhad – Iran, E-mail: jafarimr@mums.ac.ir

Patrick Vingadas Almeida

Mr. Patrick V. Almeida is a pharmacist specialized in pharmaceutical nanotechonology, tumour targeting and drug delivery. In 2012, he obtained his Integrated Master's degree (MSc) in Pharmaceutical Sciences from the Faculty of Pharmacy, University of Coimbra, Portugal. In the same year of 2012, Mr. Almeida joined the Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, as a research assistant, collaborating in different research projects in the research group he integrated.

Currently and since the beginning of 2013, Mr. Almeida is a doctoral researcher in the Unit of Pharmaceutical Nanotechnology and Chemical Microsystems (NAMI), which is part of the Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki. In 2013, Patrick Almeida received a grant from the Finnish Cultural Foundation for the period of one year, which was renovated for three more years in the subsequent term. Mr. Almeida's research is focused on pharmaceutical nanotechnology and nanomaterials, including nanoporous silicon, self-assembly chemistry and multifunctional nanosystems, particularly for tumour targeting, drug delivery and diagnostics. He is author/co-author of 5 original research articles, 3 book chapters and 1 MSc thesis, as well as 5 conference abstracts, in a total of 14 publications. He has also been involved in the co-supervision of three MSc students and in teaching activities at the Faculty of Pharmacy, University of Helsinki. In 2015, he was a member of the winning team of a commercialization opportunity pitching competition, as part of the PhDs to Business Life project of the Think Company, University of Helsinki.

Additionally, Mr. Almeida has been a board member of the Finnish Society of Physical Pharmacy committee, as well as a member of the Finnish Pharmaceutical Society since the year of 2013.

Patrizia Andreozzi

Ph.D.
Born 10 April 1977, Ceprano (FR), Italy
Present address: IFOM-IEO Campus, via Adamello 16, Milan, 20139, Italy.
Permanent Address: Via Campo Uccelli 1, Ceprano (FR), 03024, Italy.
Tel.: +393393578030 (mobile)
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EDUCATION/REASEARCH

• August 2011 – Present: Carlo Besta Neurological Insitute Fundation, IRCCS, in Milan. Post Doc (with Prof. F. Stellacci and Dr. S. Krol). Experimental work on synthesis and characterization of nanomaterial and their interaction with biological systems, such as human cell lines (cancer and normal cells) and viruses (adenovirus, rotavirus, adeno-associated virus). I'm involved in the synthesis and characterization of multifunctional nanoparticles by layer-wise deposition of biodegradable polymers and the different functional units (target, drug, fluorophore) on solid cores of iron oxide nanoparticles (SPIONs).

January 2010 – July 2011: National Nanotechnology Laboratory (NNL) - Nanochemistry Division Istituto di Nanoscienze del CNR, in Lecce. Post Doc (with Dr. A. Athanassiou, and Dr. D. Cozzoli). Experimental work on synthesis and design of multifunctional nanocomposites-based epoxy resins loaded with colloidal inorganic nanocrystals.

• March 2009 – December 2009: Department of Chemical Sciences and Technologies of University of Rome Tor Vergata. Post Doc (with Prof. G. Paradossi and Prof. A. Palleschi). Experimental work on development synthesis and physical-chemical characterization of

biocompatible polymers functionalized with enzyme.
• January 2009: Chemistry Department of Chemistry at the Sapienza University of Rome. Post Doc (with Prof. C. La Mesa). Experimental work on preparation and physical-chemical characterization of cat-anionic vesicles.
• November 2005 – December 2008: The Sapienza University of Rome. PhD, Physical Chemistry of Colloid Systems (with Prof. C. La Mesa). Title of the dissertation: "Synthesis and Physical-Chemical Characterization of Functionalized Spherical Nanoparticles".
Experimental work on physical-chemical properties of colloidal systems: soft and hard nanoparticles, vesicles, micelles stabilized by electrostatic and steric interaction.
• October 1996 – February 2005: The Sapienza University of Rome. Master of Science. Main field: Physical Chemistry of Colloid Systems. Relators: Prof. Camillo La Mesa and Dr. Roberto Di Leonardo. M.Sc. Thesis: "Colloid Crystals and Colloid Glasses Based on Stabilized PMMA Nanoparticles". (score 110/110).
Experimental work on study of the phase diagram of hard spheres.
• September 1991 – July 1996: Higher school diploma at "Nicola Parravano" Technical Institute of Arpino, (FR), Italy. Field: Industrial Chemistry.

LIST OF PUBLICATIONS

• Epoxy Resin Reinforced with Oleate-Capped Iron Oxide Nanocrystal Fillers. P. Andreozzi, F. Pignatelli, L. Ceseracciu, G. C. Anyfantis, L. Marini, M. Malerba, A. Barone, R. Cingolani, P. D. Cozzoli, A. Athanassiou, in preparation.
• Gelling Kinetics of Protein-Surfactant-Lipid Adducts. M. Calabresi, G. Gente, P. Andreozzi, C. La Mesa, submitted.
• Blood Protein coating of gold nanoparticles as potential tool for organ targeting. M. Schäffler, F. Sousa, A. Wenk, L. Sita, S. Hirn, C. Schleh, N. Haberl, M.Violatto, M. Canovi, P. Andreozzi, M. Salmona, P. Bigini , W. G. Kreyling, and S. Krol. Accepted in Biomaterials Journal, 2014.
• Erythrocyte Incubation as a Method for Free-Dye Presence Determination in Fluorescently Labeled Nanoparticles. P. Andreozzi, C. Martinelli, R. P. Carney, T. M. Carney, F. Stellacci. Mol. Pharmaceutics, 2013, 10, 875.
• Size and Charge Modulation of Surfactant-Based Vesicles". A. Barbetta, C. Pucci, F. Tardani, P. Andreozzi, C. La Mesa. J. Phys. Chem. B, 2011, 10, 115.
• Multi- to Uni-lamellar Transitions in Cat-anionic Vesicles. P. Andreozzi, S. S. Funari, C. La Mesa, P. Mariani, M. G. Ortore, R. Sinibaldi, F. Spinozzi. J. Phys. Chem. B, 2010, 114, 8056.
• Biological activity of SDS-CTAB cat-anionic vesicles in cultured cells and assessment of their cytotoxicity ending in apoptosis. C. Aiello, P. Andreozzi, C. La Mesa, G. Risuleo. Colloids and Surfaces B: Biointerfaces, 2010, 78, 149.
• Stable TiO2 dispersions for nanocoatings preparation. N. Veronovski, P. Andreozzi, C. La Mesa, M. Sfiligoj-Smole. Surface & Coatings Technology, 2010, 204, 1445.
• Use of Gemini Surfactants to Stabilize TiO2 P25 Colloidal Dispersions. N. Veronovski, P. Andreozzi, C. La Mesa, M. Sfiligoj-Smole, V. Ribitisch. Colloid Polym. Sci, 2009, DOI 10.1007/s00396-009-2133-x.
• Polymer-Assisted Synthesis of Two-Dimensional Silver Meso-Structures. L. Suber, G. Campi, A. Pifferi, P. Andreozzi, C. La Mesa, H. Amenitsch, R. Cocco, W. R. Plunkett. J. Phys. Chem. C, 2009, 113, 11198.
• Biocompatible catanionic vesicles formed in arginine glycerol-based surfactants - DPPA systems. N. Lozano, A. Pinazo, C. La Mesa, L. Perez, P. Andreozzi, and R. Pons. J. Phys. Chem. B, 2009, 113, 6321.
• Assembly Kinetics in Binary Mixture of Strongly Attractive Colloids. N. Ghofraniha, P. Andreozzi, J. Russo, C. La Mesa and F. Sciortino. J. Phys. Chem. B, 2009, 113, 6775.
• Solution Behaviour of Alkyl-Glycosides and Structurally Related Compounds. P. Andreozzi, G. Gente, C. La Mesa, Surfactant Sci. Ser., in "Sugar Based Surfactants" (C. Carnero Ruiz) 21 – 60, Marcel Dekker, 2008.
• Gemini Surfactant Binding onto Hydrophobically Modified Silica Nanoparticles. P. Andreozzi, R. Pons, L. Pérez, M. R. Infante, R. Muzzalupo, L. Suber, C. La Mesa. J. Phys. Chem. C, 2008; 112; 12142.
• Electrostatic Interactions between a Protein and Oppositely Charged Micelles. P. Andreozzi, A. Bonincontro and C. La Mesa. J. Phys. Chem. B, 2008, 112, 3339.
• Polymorphic Behaviour and Supra-molecular Association in Systems Containing Bile Acid Salts. M. Calabresi, P. Andreozzi, and C. La Mesa. Molecules, 2007, 12, 1731.
• Formation and Physical-Chemical Characterization of Silica-Based Blackberry-like Nanoparticles Capped by Polysaccharides. P. Andreozzi, C. La Mesa, G. Masci, L. Suber. J. Phys. Chem. C, 2007, 111, 18004.
• Protein Binding onto Surfactant-Based Synthetic Vesicles. C. Letizia, P. Andreozzi, A. Scipioni, C. La Mesa, A. Bonincontro, E. Spigone. J. Phys. Chem. B, 2007, 111, 898.

CONFERENCE TALKS/POSTERS

• June 2013. Workshop on Modern Light Scattering Technologies, by LS Instruments. University of Leeds, Leeds.
• July 2012. Unconventional Strategy to Explore Nanoparticles Cellular Uptake. NanoBio Seattle, 2012. (Poster).
• October 2010. Studies and Applications of Magnetic Nanocrystals. MEETING ISTITUTO NANOSCIENZE-CNRNano, Matraia Lucca, Italia. (Poster).
• October 2010. Size, Shape and Compositional Control of Colloidal Magnetic Nanostructures. MEETING ISTITUTO NANOSCIENZE-CNRNano, Matraia Lucca, Italia. (Poster).
• August 2008. Gemini Surfactant Binding onto Hydrophobically Modified Silica Nanoparticles. ECIS-2008, Kracow, Poland. (Poster).
• February 2008. Formation and Physical-Chemical Characterization of Silica Based Blackberry-like Nanoparticles Capped by Polysaccharides. 5th International Meeting on Photodynamics, La Habana, Cuba. (Poster).
• July 2007. European Student Colloid Conference 2007, Ven, Sweden. (Talk).
• June 2006. Aggregazione di Nanoparticelle con Formazione di Cristalli e Vetri Colloidali. Convegno Giovani Chimici, Roma, Italy. (Poster).
• June 2006. 8th European Summer School on Scattering Methods Applied To Soft Condensed Matter. Bombannes, Gironde, France. (Talk).
• February 2006. Colloid Crystals and Colloidal Glasses Based on Stabilized PMMA Nanoparticles. 4th International Meeting on Photodynamics, La Habana, Cuba. (Poster).
• June 2005. Sperimental Phase Diagram of Hard Spheres Based on Stabilized PMMA Nanoparticles. 34th Congresso Nazionale Società Chimica Italiana, Divisione di Chimica Fisica, Siena, Italy. (Poster).
• February 2005. Closing Remarks on Polymer-Surfactant Interactions: from Modeling to Applications. COST D15, Maribor, Slovenia. (Poster).

TEACHING

• 2006 – 2007. Teaching Assistant for Laboratory Courses of Thermodynamics and Molecular Spectroscopy.

METHODS TECHNIQUES/SKILLS

• Physical-Chemical Characterization of Colloid Systems: Dynamic and Static Light Scattering (DLS, SLS); Zeta Potential, Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES); Densimetry, Refractometry, Surface Activity, Conductivity.
Morphological Characterization: TEM, SEM.
• Spectroscopy Characterization: UV-vis Spectroscopy, FT-IR Spectroscopy, Fluorescence Spectroscopy, NMR and PFG-SE NMR, Confocal Microscopy.
• Mechanical and Thermal Characterization: Rheology, DMA, DSC, TGA.
• Culture of mammalian cells, Immunofluorescence Assay, Cell Viability Assay, Flow Cytometry Analysis, Western Immuno Blot.
• Computer Skills: OS Windows, MAC, Word, Excel, Power Point, Adobe Photoshop CS4, Light Room, Origin, KaleidaGraph, SigmaPlot, WSxM4.0, MestReC, ImageJ, Gatan Digital Microscopy, SciFinder, EndNote.

Mohammed Anwar

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ABOUT MYSELF

- Hardworking, diligent individual with good communications and interpersonal skills.
- Ability to meet multiple deadlines and perform well under pressure.
- A demonstrated talent for analyzing problems, finding and implementing innovative solutions.

PERSONAL DETAILS

Date of Birth: 1st Dec 1983
Sex: Male
Nationality: Indian
Marital status: Married

Currently Persuing Ph.D in Pharmaceutics (Thesis title: - Development of targeted nanocarriers of paclitaxel for effective management of solid tumors) from Jamia Hamdard, (Hamdard University), New Delhi, India. In the year 2010, I have completed Master degree in Quality Assurance (Thesis title: - Nanosized drug polymer conjugates for bioavailability enhancement) from Jamia Hamdard, (Hamdard University), New Delhi, India.
My research areas of Interest are Nano science & Technologies, targeted drug delivery systems in oncology, Supercritical fluid technology, Formulation development on Controlled drug delivery system, Quality control & Quality assurance.
International Travel Grant was awarded by organising committee to attend First international translational nanomedicine conference (IT Nano), 26th to 28th July 2013.Boston, MA, USA and by DST, India to attend Colloids and Nanomedicine 2012, 15-17th July 2012, Amsterdam, Netherlands. Best poster was award in 13th International Conference on Magnetic Fluids, Jan 7-11, 2013, NPL, New Delhi, India.

I have contributed in three book chapters and published 22 international publications in peer reviewed journals. Some of them are as follows:

1. M. Anwar, M. Asfer, A.P. Prajapati, S. Mohapatra, S. Akhter, A. Ali, F.J. Ahmad, Synthesis and in vitro localization study of curcumin-loaded SPIONs in a micro capillary for simulating a targeted drug delivery system, International journal of pharmaceutics, 468 (2014) 158-164.
2. M. Anwar, M. Asfer, S. Akhter, S. Mohapatra, M. Warsi, N. Jain, N. Mallick, G. Jain, A. Ali, P. Panigrahi, Aqueous phase transfer of oleic acid coated iron oxide nanoparticles: influence of solvents and surfactants on stability and pharmaceutical applications of ferrofluid, Magnetohydrodynamics, 49 (2013) 339-343.
3. M.Z. Ahmad, S. Akhter, M. Anwar, F.J. Ahmad, Assam Bora rice starch based biocompatible mucoadhesive microsphere for targeted delivery of 5-fluorouracil in colorectal cancer, Molecular pharmaceutics, 9 (2012) 2986-2994.
4. M. Anwar, M.H. Warsi, N. Mallick, S. Akhter, S. Gahoi, G.K. Jain, S. Talegaonkar, F.J. Ahmad, R.K. Khar, Enhanced bioavailability of nano-sized chitosan–atorvastatin conjugate after oral administration to rats, European Journal of Pharmaceutical Sciences, 44 (2011) 241-249.

Leila Arabi

Pharm.D.,Ph.D. candidate
Current Address: School of pharmacy, Mashhad University of Medical Sciences, University Campus- Mashhad-Iran,
+98(0)5118823255(office)
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RESEARCH INTERESTS

Establishing of targeted drug and gene delivery systems using nanotechnology methods and materials, Isolation and characterization of cancer stem cells, Targeting tumor cells, Cancer stem cells and tumor microenvironment, Nanomedicine, Gene delivery using non-viral vectors (liposomal and cationic polymer (polyethyleneimine: PEI) based gene transfer system, Therapeutic implications of non-coding RNA, Primary culture of patient derived malignant effusions as specific models for in vitro assays and personalized medicine

EDUCATION

• August 2012-August 2013
1-year internship at the Molecular Pathology department of the Institute for Pathology (University Hospital Basel). involved in the projects such as the “Molecular regulation of cancer stem cells in gastrointestinal neoplasia”, “Anticancer-treatment effects in vitro using real-time impedance measurements on patient-derived samples”, “Prognostic and therapeutic implications of the oncogenic miR-17-92 cluster in Osteosarcoma”, “ Evaluation of putative amplification target genes (Vinculin and KCNMA1) in prostate and breast cancer”

• since 2009: Ph.D. candidate of Pharmaceutical Nanotechnology: School of Pharmacy, Mashhad University of Medical Sciences (MUMS), Mashhad, Iran. Ranks first in Ph.D. based on overall scores (18.90 out of 20) Ph.D. Theses Title: “in vitro and in vivo targeted drug delivery to cancer stem cells isolated from murine colon carcinoma by doxorubicin nanoliposomes conjugated with CD44, CD133, EpCAM monoclonal antibodies”.under the supervision of Dr Mahmoud Reza Jaafari.

• 2003-2009: Pharm.D. School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran. Ranks second in Pharm.D. based on overall scores (18.05 out of 20), Pharm.D. Theses Title: “Preparation and characterization of nanoliposmes modified with lipopolymers constructed from polyethyleneimine (10 kDa) and bromoalkane derivatives, as a non-viral gene transfer system”.under the supervision of Dr Malaeke Nikuei, Dr Mohammad Ramezani.

PUBLICATIONS AND PRESENTATIONS

• L.Arabi, J.Gsponer, J.Smida, M.Nathrath, G.Jundt, C.Ruiz, L.Quagliata and D.Baumhoer,”Upregulation of the miR-17-92 cluster and its two paraloga in osteosarcoma – reasons and consequences”, Accepted in GENES&CANCER, The original submission date remains March 11, 2014

• L.Quagliata, M.Matter, S.Piscuoglio,**L.Arabi**, C.Ruiz, A.Procino, M.Kovac, F.Moretti, Z.Makowska, L.Tornillo, S.Diederich, M.H. Heim, C.Cillo and L.Terracciano,”lncRNA HOTTIP/HOXA13 expression is associated with disease progression and predicts clinical outcome in HCC patients”, HEPATOLOGY, Vol. 59, No. 3,MARCH 2014.

• **L.Arabi**, A.Badiee,MR.Jafari. “Isolation and Identification of Putative Cancer Stem Cells in C26 Murine Colon Carcinoma Cell Line”, The 1st Middle East Controlled Release Conference (MECRC 2014) and The 6th Iranian Controlled Release Society Conference (ICRC 2014), 25-27th February 2014, Tehran University of Medical Sciences, Iran.

• **L.Arabi**., B.Malaekeh Nikuei, R.Kazemi Oskuee, A.Dehshahri,M. Ramezani, “Polycationic Nanoliposomes as DNA Nanocarriers with Low Toxicity: A Promising Gene Transfer System for Further in Vivo Study”, The European Summit for Clinical Nanomedicine (6th CLINAM 2013) , June 23-26,2013Basel- Switzerland

• **L.Arabi**, B.Baschiera, S.Wyttenbach, S.Kustermann, C.Ruiz, A. Zippelius, A.Roth, L.Bubendorf. "The use of patient derived malignant effusions as specific models for in-vitro assays", 5th Freiburg Symposium on Anticancer Drug Discovery, April 24 – 27, 2013, Freiburg, Germany

• **L.Arabi**, L.Quagliata, A.Rufle, L.Terracciano, C. Ruiz and D.Baumhoer. "Prognostic and therapeutic implications of the oncogenic miR-17-92 cluster in osteosarcoma",Non-coding RNA, epigenetics and transgenerational inheritance April 11-12, 2013,Cambridge,UK

• L. Quagliata, M. Matter, **L.Arabi**, S. Piscuglio, Z. Makowska, M.Kovac, L. Tornillo1, M.H. Heim, C.Cillo, L. Terracciano"HOTTIP Expression Levels Predict Patients' Survival and Metastasis Formation In Hepatocellular Carcinoma" Charles Rodolphe Brupbacher Symposium, January 30 - February 1, 2013, University Hospital Zurich, Switzerland

• **L.Arabi**., B.Malaekeh Nikuei, R.Kazemi Oskuee, A.Dehshahri,M. Ramezani "Preparation, characterization and transfection efficiency of polycationic nanoliposomes as DNA nanocarriers with low toxicity"Biovalley Life Science Week- 25th September-2012- Basel-Switzerland

HONORS AND AWARDS

• Ranks third among all Ph.D. candidate in research field in school of pharmacy (MUMS-Iran) during 2012-2014

• Fellowship of CLINAM foundation for The European Summit for Clinical Nanomedicine 2014 (7th CLINAM 2014) Basel- Switzerland

• Award for poster presentation in Biovalley Life Science Week-25th September- 2012- Basel- Switzerland

• Ranks first among candidates, Iranian National "pharmaceutical Nanotechnology" Ph.D. comprehensive Exam based on examination and interview 2011

• Ranks first among candidates, Iranian National "pharmaceutical Nanotechnology" Ph.D. Entrance Exam based on entrance examination and interview 2008

• Ranks first in Ph.D. based on overall scores (18.90 out of 20)

• Ranks second in Pharm.D. Based on overall scores (18.05 out of 20)

• 5% upper first in national comprehensive exam of basic sciences.

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.

Lidia Ciobanu

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Nationalité romana

POSTE VISE/PROFESSION/ EMPLOI RECHERCHE/ETUDES RECHERCHEES:

Chirurgien orale a temps plain dans l'hôpital

EXPERIENCE PROFESSIONNELLE

01.01.2012–15.07.2013	chirurgien orale dans l'hôpital de Chirurgie OMF «Dan Teodorescu»
01.02.2012–01.02.2013	chirurgien dentiste dans un pratique privée « Eurodent Bianchi »
02.02.2013–présent	chirurgien dentiste dans un pratique privée «Veisa Dental Care»

EDUCATION ET FORMATION

2012–2014	la diplôme de spécialiste de chirurgien orale libere par le Ministère de la Santé,Bucharest
2005–2011	la diplôme de chirurgien dentiste UMF «Carol Davila», Bucharest
2001–2005	la diplôme d'études secondaires aux CN «Elena Cuza»,Craiova

CONGRÈS, CONFÉRENCES :

1. 2014– CLINAM Basel, Swiss
   - poster 'Investigation regarding quality of dental enamel. A new approach and explanation by using RAMAN and AFM technique', E. Gatin, R Sfeatcu, C. Luculescu, A. Balan, L. Ciobanu and I. Patrascu ;
   - Oral presentation ' Investigation regarding dental enamel. A new approach and explanation by using RAMAN and AFM technique', E. Gatin and L. Ciobanu ;
2. 2012-EMRS Strasbourg, France
   - poster "AFM Nanomicrography with application to dental ceramics". E. Gatin, E.Barna, C.R. Luculescu, R.R. Cara, L. Ciobanu, I. Patrascu, C. Berlic, S.Iordache,A.Balan
3. 2011-Microscopy Conference Kiel, Germany
   - poster ''Study of the thermal influences during manufacturing process of Zirconia dental ceramic core', E. Gatin, R. Birjega, C. R. Luculescu, R.R. Cara, L. Ciobanu, I. Patrascu, C. Berlic.
4. 2010-Congrès national "Les jours de chirurgie dentaire Craiova" Etude comparative des matériaux de restauration dentaire sur les propriétés physico-chimiques et mécaniques. G.Ciobanu, E.Gatin, L. Ciobanu.
5. 2009- Congrès national "Les jours de chirurgie dentaire Craiova" Le traitement complexe de l'allergie provoquée par chrome-nickel -G.Ciobanu, L. Ciobanu,E.Gatin.

ARTICLES

1. Particulate Science and Technology, Vol. 31, Issue 2, 2013, 'Alumina Versus Zirconia Comparative Survey of Thermic Influence during Dental Ceramic Core Manufacturing Process', E. Gatin, C. R. Luculescu, R. Birjega, L. Ciobanu & I. Patrascu, http://www.tandfonline.com/doi/abs/10.1080/02726351.2012.675016 ;
2. Journal of Advanced Microscopy Research Vol. 5, 1–6, 2010 Scanning Electron Microscopy for Testing the Quality of Direct and Indirect Dental Restorations B. Iordache, E. Gatin, R. R. Ilici, C. Luculescu, L. Ciobanu, and I. Patrascu ;
3. Journal of Optoelectronics and Advanced Materials,vol.11,nr.4, 2009- Investigation of Ni-Cr alloys in order to asses the quality of cast dental restorations.E.Gatin,C.Berlic,B.Iordache, L.Ciobanu.

Hadar Cohen

EDUCATION:

2009-2011, Tel Aviv University, Masters degree studies in the faculty of life sciences, Dept. of molecular microbiology & biotechnology, Prof. Ehud Gazit as supervisor.
2006-2009, Bar-Ilan University, faculty of life sciences, Bachelor's degree graduate in the biotechnology program.
2000-2003, Amal – Highschool for Sciences and arts, Hadera, graduated in the biological-chemical program with honors.

WORK EXPERIENCE

Currently, Ph.D student at the Dept. of Chemical Biology, Weizmann institute of science.
2009-2011, instructor in the undergraduate molecular biology lab course. Graduate student in the Dept. of molecular microbiology and biotechnology under Prof. Ehud Gazit.
Technoda dorset – Center for science & technology education
Teaching electronics, science, chemistry and physics courses.
2008-2009, Medical cadets health organization
10th grade guide for the "Health Ambassadors" project, a joint project by the MCHO and the "Super Pharm" Chain.
2006-2007, Bank Hapoalim campus
Instructor in the "Tellers course" for new consultants joining the bank.
2005-2007, Bank Hapoalim
Teller in the Business and private departments at a number of Bank Hapoalim branches.
2003-2005, Israeli Air Force
Operations Sergeant (Sambatzit). In charge of computing systems in the technical support center.
Later in military service – in charge of training.

SKILLS & KNOWLEDGE

- High Level writing
- Independent and fast comprehension and learning
- Knowledge in computer operation and Office programs.
- Basic Knowledge in "C++" language programming
- Works well under pressure

*** Research project (bachelor's degree) –** Performed under the supervision of Prof. Igal Cohen at the Bar Ilan University (Plant sciences).
Field of research- characterization of proteins involved in resistance to the fungus Phytophthora Infestans in the "currant tomato" Lycopersicon Pimpinellifolium.
*** Research project (master's degree) –** Performed under the supervision of Prof. Ehud Gazit at the Tel Aviv University.
Field of research- Formulation and Characterization of Aromatic Di-peptide Nanospheres : Application for Targeted Drug Delivery.

Herve Courthion

Apidel SA
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EDUCATION

2011–2013 : Master of Sciences (MSc with honors) in Bioengineering and Biomedical Engineering, EPFL, Switzerland
2008–2011: Bachelor of Sciences (BSc) in Life Sciences, EPFL, Switzerland

PROFESSIONAL EXPERIENCE

2013–now: Apidel SA, Switzerland. Development Scientist: Formulation and analytical development and optimization for topical applications. Implementation of the analytics for polymer specifications. Evaluation of technical solutions for upscale production
2012–2013: Laboratory for Biomaterials and Drug Delivery, MIT/ Boston Children's Hospital, USA. Visiting student: research on non-invasive oral drug delivery systems

AWARD:

2013: Best Master's project in Bioengineering, EPFL, Switzerland

Domokos Csukás

Domokos Csukás graduated as a veterinarian in 2005 at the Faculty of Veterinary Science, Szent István University Budapest, Hungary. After 7 years practice in small animal oriented veterinary hospitals he joined the team of the Department of Surgical Research and Techniques in the Faculty of Medicine of Semmelweis University, Budapest. He has special interests in laboratory animal care, animal model development and immune toxicological tests in the field of nanomedicine. He is working on his PhD thesis focusing on the side effects of nanodrugs, and the role of PIM cells in complement activation related pseudoallergy.

Monika Dabrzalska

Monika Dabrzalska, MSc, is a PhD Student at the Department of General Biophysics at the University of Lodz. She received her BSc degree in Biology from the University of Lodz in 2010. In 2012, she received MSc degree in biology, in speciality Medical Biophysics and Bioinformatics, at the University of Lodz. During the master studies she was a member of the TEAM programme, supported by the Foundation of Polish Science and financed by the European funds, entitled "Biological properties and biomedical applications of dendrimers". In the TEAM programme, her research was focused on the interactions of PAMAM dendrimers with proteins. Her master thesis was entitled "PAMAM dendrimers and pepsin – the influence of surface groups on the interaction between nanoparticles and protein". After receiving her MSc she became a PhD student at the Department of General Biophysics at the University of Lodz. She was granted a scholarship from the City Council of Lodz for students studying in the fields of priority for the development of Lodz.
She was a participant of the COST Action "Dendrimers in biomedical applications". She participated in the Short Term Scientific Mission within the COST Action, for two months in the Biophysics Unit at the Autonomous University of Barcelona, Spain. She is a coordinator of the VENTURES project supported by the Foundation of Polish Science, co-financed with European Union funds from the European Regional Development Fund. The project is entitled "Phosphorus dendrimers as carriers of photosensitizers in photodynamic therapy and its combination with hyperthermia in in vitro studies". Monika Dabrzalska is also a participant of the HARMONIA grant "Studying phosphorus dendrimers as systems transporting photosensitizers" supported by the National Science Centre. She is a co-author of three publications. Additional two publications focusing in the area of dendrimers in photodynamic therapy have been sent to international journals recently.

László Dézsi

Function: Senior Research Associate, in vivo lab.
Degree: Ph.D., Dr. habil.
Date of Birth: 29th of Dec. 1955
Semmelweis University, Budapest, Nanomedicine Research and Education Center & Seroscience Ltd., Hungary
Phone +36206663502
E-mail: dr.dezsi.laszlo@gmail.com

László Dézsi PhD, DrHabil., works as Senior Research Associate in the in vivo laboratory of Nanomedicine Research and Education Center of the Semmelweis University since 2012 in Budapest, Hungary.

He obtained his MSc degree in biology at Eötvös Loránd University and his PhD in physiology at Semmelweis University Medical School, Budapest, Hungary. He conducted teaching and research activities at Semmelweis University (1981-1999), while he also 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 participated in curriculum development and had been Secretary of Biomedical Engineering Course Committee at Technical University, Budapest (1994-2000). He made his habilitation at Semmelweis University in 2005 and became Adjunct Professor in 2006. He established his own teaching course (2008) “Cardiorespiratoric and neurophysiological measuring techniques” at the Department of Human Physiology (Prof. Z. Benyó).

Currently he is working in the field of nanomedicine investigating cardiopulmonary and immunological effects of nanoparticles in in vivo models of complement activation related pseudoallergy (CARPA) under the supervision of Prof. J. Szebeni.

Tiziana Di Francesco

Tiziana Di Francesco is a 2nd 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.

Simona Dostálová

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E-mail: simona1dostalova@gmail.com
Nationality: Czech
Date of birth: 2. 5. 1990
Gender: Female

EDUCATION AND TRAINING

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

- 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
- 05/2012 2nd instead, Poster section, The Conference XIII. Pracovní setkání fyzikálních chemiků a elektrochemiků. S. Dostalova, K. Smerkova, M. Ryvolova, J. Hubalek, L. Trnkova, V. Adam, R. Kizek, Optimization of DNA isolation using magnetic microparticles.

RESEARCH

Author or coauthor of 18 conference papers and 12 original scientific papers in ISI-indexed journals with a total of 12 citations, H=2 according to Web of Science.

Stella-Saphira Ehrenberger

Stella-Saphira Ehrenberger started her Ph.D. in 2013 at the Department of Biopharmacy within the School of Pharmaceutical Sciences at the University of Geneva and the University of Lausanne, Switzerland. Her research is focused on active targeting of superparamagnetic iron oxide nanoparticles for detection of early prostate cancer metastases in lymph nodes. She is also working on the development of an injectable, in-situ forming nanocomposite for application of local hyperthermia. Her work is part of the Nano-Tera project MagnetoTheranostics and supervised by Prof. Gerrit Borchard and Dr. Olivier Jordan. Stella-Saphira graduated in Pharmacy at the Albert Ludwigs University of Freiburg, Germany. During her studies she was intern at the pharmacy department of the Khoo Teck Puat Hospital in Singapore as well as at the Novartis sterile production of biopharmaceuticals in Stein, Switzerland.

Elisabet Fernández-Rosas

Dr. Elisabet Fernández got her PhD degree in 2011 in Cell Biology at the Universitat Autònoma de Barcelona (UAB) for the project entitled "Microtools for the study of living cells," in conjunction with the Cell Biology Unit and the group of Microsystems in the Microelectronics Institute of Barcelona - Centro Nacional de Microelectrónica (IMB-CSIC), CSIC. She has a degree in Biology from the UAB as a specializing in Sanitary Biology, holds a DEA in Biological Anthropology from the same university, for his work on genetic analysis of human populations, and a Master degree on anthropology and forensic genetics (Universidad de Granada). She has also been part of the Department of Pathology and Experimental Therapeutics at the Universitat de Barcelona (UB), where worked on the cytotoxicological analysis of new drugs. She has participated in various national and international conferences, and his works have been published in international scope indexed journals with high impact factor. She has collaborated in the analysis and identification of individuals with the analytical laboratory of the regional Catalan police "Mossos d'Esquadra" and in the dissemination of science with the Catalan Foundation for Research and Innovation (FCRI). Today she leads the nanomedicine and nanobiosensors group of the Safety and Sustainability division of LEITAT Technological Centre, centered on the production of organic and inorganic nanoparticles and nanocapsules for biomedical applications: as drug delivery systems in systemic or focused diseases, for in vivo imaging, theranostics for cancer or biomolecules sensing.

Ralf P. Friedrich

Dr. rer. nat.

Position: Research associate
Section of Experimental Oncology and Nanomedicine
Department of Otorhinolaryngology
University Hospital Erlangen

ACADEMIC BACKGROUND AND PROFESSIONAL RECORD

2013 - Research associate in the Section for Experimental Oncology and Nanomedicine, Department of Otorhinolaryngology, University Hospital Erlangen, Friedrich Alexander University of Erlangen-Nuremberg, Germany.
Topic: "Magnetic cell levitation for tissue engineering".

2008 - 2013 Research associate in the research group Neuroproteomics, Max-Delbrueck-Centrum for Molecular Medicine (MDC), Berlin, Germany
Topic: "Modulators of Amyloid Formation".

2006 - 2008 Postdoc in the research group Protein folding and Aggregation, Leibniz Institute for Age Research, Fritz-Lipmann-Institute, Jena, Germany.
Topic: "Mechanism of Plaque Formation in Alzheimer's Disease".

2001 - 2005 PhD thesis at the Chair for Biochemistry and Pathobiochemistry, Medical faculty of the Friedrich-Alexander-University of Erlangen-Nuremberg, Germany.
Topic: "Functional redundancy of POU domain transcription factors during Schwann cell development".

2001 Diploma thesis at the Institute for Microbiology, Biochemistry and Genetics, Scientific faculty of the Friedrich-Alexander-University of Erlangen-Nuremberg, Germany.
Topic: "Functional analysis of a gene from Saccharomyces cerevisiae with homology to lipoic acid transferases"

1996 - 2001 Biology studies at the Friedrich-Alexander-University of Erlangen-Nuremberg, Germany

Eduard Gatin

Address(es): Al. Plenita 4 , Bl. Y 8, Ap.29, Sect.3 Bucharest, Romania
Telephone(s): +40 21340.12.24
Mobile: + 40-742.896.270
E-mail: edgatin@netscape.net, masterdent2009@yahoo.coom
Nationality: Romanian
Date of birth: 02/05/1960

Occupational field: Polymers Physics Department; Advanced Materials Applied to Dental Restorations; Biophysics and Physiology.

WORK EXPERIENCE

• 2000–present: Occupation or position held Lecturer Ph. D., Chief of Department; Main activities and responsibilities: Chief professor for advanced courses in "Polymers Membranes and Filters" and "Modelling molecules and polymers technology" – classes with students year IV and master students; Cooperation/Part time on same position: Laboratory of Biophysics, students year I; Dental Materials Laboratory, students year III – Faculty of Dentistry. (details: www.fizica.unibuc.ro, www.univermed-cdgm.ro, www.aracis.ro).Investigation of dental materials for dental restorations (dental resin composites, modelling biomaterials, corrosion studies, bacteria plaque). 2010, Member of Japan Academy of Dental Materials and Equipments; 1 st Name and address of employer: University of Bucharest, Faculty of Physics , Polymers Physics Department
ype of business or sector: Education/research

• 1990–2000: 2001 – present: Occupation or position held: Assistant professor; Lecturer – Chief of Department; Main activities and responsibilities: Teaching classes:Seminars, classes and Biophysics Laboratories, Dental Materials Lab. Research activity: Materials structure, physical / chemical properties, dental enamel, bacteria activity, polymer resin composites, dental ceramics, metal alloys and corrosion studies. Techniques: RAMAN spectroscopy (improved by SERS), SEM, EDX.

• 2010 – present: Lecturer – Biophyscs Department; 2 nd Name and address of employer: University of Medicine "Carol Davila", Faculty of Dentistry; Type of business or sector: Education/research

• 1985–1989: Occupation or position held: High school Teaching Physics; Main activities and responsibilities: Teaching, Physics Classes; Name and address of employer: 1985-1989: teacher, Liceul

" Alexandru Sahia" – Oltenita; 1988–1990 : pregatirea lotului pe Bucuresti pentru Olimpiada Nationala de Fizica, in colaborare cu Inspectoratul Municipiului Bucuresti; 1989 – 1990: Teacher physics classes , High School " Horia Hulubei " – Bucuresti; Type of business or sector: Teaching

EDUCATION AND TRAINING

• 1994–1999:Title of qualification awarded: Doctor in Biology & Physiology (Ph D degree); Principal subjects/occupational skills covered: Research in material science- polymers, advanced nano materials, ceramics, metal alloys, corrosion, dental materials and tissue regeneration. Name and type of organization providing education and training: University of Bucharest, Faculty of Physics; Level in national or international classification: Post university studies

• 1980-1985: Title of qualification awarded: Physicist; Principal subjects/occupational skills covered: Education, research in polymer and materials science, dental materials. Name and type of organization providing education and training: University of Bucharest, Faculty of Physics

SHORT LIST OF REPRESENTATIVE PUBLICATIONS:

1) E. Gatin, C. Ciucu, G. Ciobanu, C. Berlic, "Investigation and comparative survey of some dental restorative materials", Optoelectronics and advanced materials, vol. 2/no.5. pg. 284-290, 2008.

2) E. Gatin, D. Alexandreanu, C. Berlic, A. Popescu, E. Barna, Dana Moja "Modifications on Polysulfone Membranes Surface Obtained by RF Plasma Treatment", Physica Medica, XV / 4 / 1999.

3) E. Gatin, D. Alexandreanu, C. Berlic, Maria – Luiza Flonta "Comparative Survey for Microporous Material Used for Contact Lenses", Physica Medica, XV / 3 / 1999.

4) R. Ilici, C. Nicola, E. Gatin, C. Prejmerean, I. Pătraşcu, abstract presentation on "Evaluation of polymerisation shrinkage stress in maxillary premolars restored with experimental composites based on Bis-GMA derivatives", Congresul International de Stomatologie, Napoca Biodent 2009, 5-7 Noiembrie 2009, Cluj Napoca .

5) E. Gâtin, C. Berlic, B. Iordache, Paula Prioteasa, "Study of the corrosion process for some dental metal alloys under artificial saliva enriched with yeast and Streptococcus Mutans bacteria", Journal of Optoelectronics and advanced materials, vol. 11/ 11, pg. 1870–1873, 2009.

6) R.R. Cara, E. Gatin, A. Didilescu, R. Sfeatcu, C. Nicola, I. Patrascu, abstract presentation on "Cuspal Deformation during Light-Curing of Low-Shrinking Posterior Composite Restorations", IADR General Session, ref 137450, Congress July 2010, Barcelona.

7) E. Gatin, D. Alexandreanu, A. Popescu, C. Berlic, Ionela Alexandreanu "Correlation between permeability properties and pore-size distribution of the porous media "hydron" useful for contact lenses, Physica Medica, XVI/ 1 / 2000.

8) G. Ciobanu , E. Gatin, L. Ciobanu, abstract presentation on "Comprative survey on dental restoration materials regarding mechanical and physic – chemical properties", Congresul International de Endodontie al Asociatiei Romane de Endodontie 2010", pg. 148 – 149, ISBN 978 – 973 – 106 – 153 – 5, Editura Medicala Universitara Craiova 2010 (24 – 28 martie 2010, Craiova)

9) B. Iordache, E. Gatin, R.R. Ilici, C. Luculescu, L. Ciobanu, I. Patrascu, "Scanning electron microscopy for testing the quality of direct and indirect dental restorations", Journal of advanced electron microscopy research, vol. 5 / 1, 2010.

10) R.R. Ilici, E. Gatin, E. Matei, A. Didilescu, C. Nicola, S. Sava, I. Patrascu, "Cuspal deflection and adhesive interface integrity of low-shrinking posterior composite restorations", Acta Stomatologica Croata, vol. 44 / 3, ASCRO 2010.

11) R.R. Cara, E. Gatin, A. Didilescu, R. Sfeatcu, C. Nicola, I. Patrascu, "Cuspal Deformation during Light-Curing of Low-Shrinking Posterior Composite Restorations", Journal of Dental Research, Vol. 89 (Spec Issue B, Letter); abstract # 3066, 2010 (www.dentalresearch.org).

12) E. Gatin, R. Birjega, C.R. Luculescu, R.R. Cara, L. Ciobanu, I. Patrascu, C. Berlic, 'Study of the thermal influences during the manufacturing process of Zirconia dental ceramic core', Proceedings MC2011 Kiel, Materials Science, vol. 3, ISBN 978-3-00-033910-3, 2011 (www.dge-homepage.de).

13) E. Gatin, E. Matei, D. A. Pirvu, B. M. Galbinasu, S. Iordache, 'Comparative survey of the most used self-adhesive dental cements based on resin composites', Digest Journal of Nanomaterials and Biostructures, vol. 7 (1), pg. 207 – 215, 2012.

14)E. Gatin, C. Luculescu, R. Birjega, L, Ciobanu, I. Patrascu, 'Alumina versus Zirconia comparative survey of thermic influence during dental ceramic core manufacturing process', Particulate Science and Technology, Vol. 31, Issue 2, 2013; http://www.tandfonline.com/doi/abs/10.1080/02726351.2012.675016 .

ADDITIONAL INFORMATION AND RESUME:

• 16 scientific papers ISI indexed (available on Scopus and ISI Web of knowledge);

• 1 book chapter;

• 1 patent under registration (pending), regarding investigation of dental enamel quality by RAMAN method;

• Period 2010 – 2013, postgraduate study on EU program POSDRU 2007 – 2013, topic on "Dental Materials";

• Participations on conferences, congress with topics as dentistry, dental materials, biomaterials (IADR; Electronic Microscopy Conference – Germany; EMRS Conferences – Strasbourg, Lille; CLINAM 2014 - Basel);

• Invited lesson, Faculty of Dentistry – University of Zagreb, Croatia – November 2012;

• Invited lesson, Faculty of Dentistry – University Semmelweis, Budapest, Hungary – February 2015.

Christina Giannakou

Christina Giannakou was born in 1988, in Greece. In 2006, she started her Diploma in Biology at Aristotle University of Thessaloniki, Greece. During the diploma studies she studied as an ERASMUS exchange student at the University of Hamburg for 5 months in 2009. She conducted a 4 month diploma internship in LEITAT on Industrial Microbiology Laboratory in 2011. She started her master education on the MSc program of Toxicology and Environmental Health at Utrecht University in 2012. During the two years of her master studies she worked on food allergy at the Immunotoxicology group of IRAS under the supervision of and on Nanotoxicology at the Centre for Health Protection of the National Institute for Public Health and Environment (RIVM). From September 2014, she is started her PhD at Toxicogenomics department of Maastricht University on a project in collaboration with RIVM. Her project is focusing on the methods and criteria for evaluating potential immunotoxicity of nanomedicinal products.

Marianna Giannini

Marianna Giannini is a PhD student in Innovative strategies in biomedical research at the Institute of Life Science, Scuola Superiore Sant'Anna, in the Nanomedicine lab, a jointed laboratory between the Scuola Superiore Sant'Anna and the University of Pisa. In 2012 she graduated at the University of Pisa in Molecular and Industrial Biotechnologies Master's degree, final score 110/100 with honours. Coming from a Life Sciences background, she is currently working in a multidisciplinary environment, with clinical doctors, biologists and engineers.

PUBLICATIONS

Giannini M., Giannaccini M., Sibillano T., Giannini C., Liu D., Wang Z., Baù A., Dente L., Cuschieri A., Raffa V. Sheets of vertically aligned BaTiO3 nanotubes reduce cell proliferation but not viability of NIH-3T3 cells. PLOSONE 2014. doi: 10.1371/journal.pone.0115183.

Giannaccini M., Giannini M., Calatayud M.P., Goya G.F., Cuschieri A., Dente L., Raffa V. Magnetic nanoparticles as intraocular drug delivery system to target Retinal Pigmented Epithelium (RPE). Int J Mol Sci. 2014; Jan 22; 15(1):1590-605. doi: 10.3390/ijms15011590.Int. J.Mol. Sci. 2014; 15, 1-x manuscripts; doi: 10.3390/ijms150x000x.
Marracci S., Giannini M., Vitiello M., Andreazzoli M., Dente L. Kidins220/ARMS is dynamically expressed during Xenopus laevis development. Int J Dev Biol. 2013; 57(9-10):787-92. doi: 10.1387/ijdb.130080sm.

COMMUNICATIONS

Giannini M., Raffa V., Dente L., Cuschieri A., Pellegrino M. A new platform for in vivo monitoring of zebrafish embryo cardiovascular system. Presented at the Heart of Europe zebrafish meeting, Warsaw, organized by the International Institute of Molecular and Cell Biology in Warsaw (IIMCB), 17–19 September 2014.
Giannaccini M., Giannini M., Calatayud M.P., Goya G.F., Cuschieri A., Dente L., Raffa V. Magnetic nanoparticles as intraocular drug delivery system to target Retinal Pigmented Epithelium (RPE). Presented at the 10th SIBBM seminar Frontiers in Molecular Biology, organized by the SIBBM (Società Italiana di Biofisica e Biologia Molecolare) in Trento (Italy), 11–13 June 2014.

Maria Gregori

Date of birth: 20 dicembre 1981
Studies: 2008 PhD in Chemistry (University of Milano-Bicocca)
2005: Masters Degree in Industrial Biotechnology (University of Milano-Bicocca) (110/110 cum laude).

ESPERIENCES:

From 2014 she is Research Fellow for the project "Development of nanoparticles for the diagnosis of neurodegenerative diseases", under the supervision of Prof. M. Masserini, at the University of Milano-Bicocca. She is also partner in the project ""mApoE-Functionalized Lipidic -and Polymeric- Nanocomposite for Human Glioblastoma Imaging and Treatment" co-funded by Regione Lombardia, within the "Start-up Packages and PhD Program" project of the European Centre for Nanomedicine (CEN) (duration of the project: march 2014–september 2015). From 2011 to 2013 she was Research Fellow for the project "Nanoparticles for therapy and diagnosis of Alzheimer Disease", under the supervision of Prof. M. Masserini, at the University of Milano-Bicocca. From 2009 to 2011 she was Research Fellow in the FP7 European Project NAD (Nanoparticles for therapy and diagnosis of Alzheimer disease), that involved 19 partners around Europe, under the supervision of Prof. M. Masserini, at the University of Milano-Bicocca.

SCIENTIFIC COMPETENCES:

She has experience in Organic Chemistry and in the field of Bio-nanotechnologies. During her PhD in Chemistry, she built up experience in organic synthesis and also in peptide synthesis on solid phase, using chromatographic separation techniques, UV/Vis Spectroscopy, IR, NMR. She acquired also competences of Mass Spectrometry (MALDI-Tof, ESI), staying 3 month in the laboratory of Prof. T. Jorgensen at the University of Southern Denmark, Odense (Denmark). Since 2009 she moved to the field of bio-nanotechnology. Her scientific research concerns the preparation and funzionalization of nanoparticles, in particular liposomes, solid lipid nanoparticles and polymeric nanoparticles, for biomedical applications. With the competencies in organic chemistry she carried out different functionalization of nanoparticles with drugs, peptides and antibodies. She got also competences about biochemistry techniques, cell cultures, Atomic Force Microscopy and Surface Plasmon Resonance. The results of her research have led to the publication of 21 scientific articles in peer-reviewed journals.
Schools attended in nanotechnology field:
September 2009. First training course about nanoparticles for delivery to brain, Patrasso, Greece. September 2010. Pharmacokinetics of nanoparticles and their passage through the blood-brain barrier, Milano, Italy October 2011. School on Dendrimers as composites of advanced drug delivery nanosystems, Athens, Greece.

RECENT PUBLICATIONS:

Ordóñez-Gutiérrez L, Re F, Bereczki E, Ioja E, Gregori M, Andersen AJ, Antón M, Moghimi SM, Pei JJ, Masserini M, Wandosell F. (2015) Repeated intraperitoneal injection of liposomes containing phosphatidic acid and cardiolipin reduce amyloid-ß levels in APP/PS1 transgenic mice. Nanomedicine. 11:421–430.
E. Salvati, F. Re, S. Sesana, I. Cambianica, G. Sancini, M. Masserini, M. Gregori (2013) Liposomes functionalized to overcome the blood-brain barrier and to target amyloid-β peptide: the chemical design affects the permeability across an in vitro model. Int J Nanomedicine, 8:1749-58.
G. Sancini*, M. Gregori*, E. Salvati, I. Cambianica, F. Re, F. Ornaghi, M. Canovi, C. Fracasso, A. Cagnotto, M. Colombo, C. Zona, M. Gobbi, M. Salmona, B. La Ferla, F. Nicotra, M. Masserini (2013) Functionalization with TAT-Peptide Enhances Blood-Brain Barrier Crossing In vitro of Nanoliposomes Carrying a Curcumin-Derivative to Bind Amyloid-b Peptide. J Nanomed Nanotechnol, 4:171.
*These authors contributed equally to this work

Jennifer Grossman

Ph.D.
Dr. Jennifer Grossman is a 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 nano-bioinformatics, nanomedicine, and other nanotechnology issues.
www.linkedin.com/in/grossmanjennifer/

Sonja Hartl

Sonja Hartl, MSc. started her professional career as technical expert and employee of the BioNanoNet Forschungsgesellschaft mbH in 2011. She holds a master degree (MSc.) in applied biomedical science from FH JOANNEUM, Graz. Ms. Hartl has started working on the CE Project "NANOFORCE - Nanotechnology for Chemical Enterprises - how to link scientific knowledge to the business in the Central Europe space" (this project is implemented through the CENTRAL EUROPE Programme co-financed by the ERDF) working on "How to foster the responsible use of nanotech and manage associated risks". Since 2013, Ms. Hartl is actively involved in the FP7 Project NANoREG focusing on regulatory testing of nanomaterials. Furthermore Ms. Hartl is responsible for communication and outreach activities in the EU FP7 project NanoDiode and coordinates the work package on "EDUCATE – Professionalise Nanotechnology Education and Training". Additionally Ms. Hartl is supporting the communication and dissemination activities and providing knowledge and expertise on regulatory aspects of nanotechnology, nanosafety and standardization for the medical, health and nanotox main focus areas of BioNanoNet.

Bin Mohamed Suffian Izzat Fahimuddin

Izzat Fahimuddin Bin Mohamed Suffian was awarded the Malaysia National Scholarship from Public Service Department, Government of Malaysia to complete his Bachelor of Engineering in Chemical Science and Engineering (B. Eng) degree at the Department of Chemical Science and Engineering, Faculty of Engineering, Kobe University, Japan (2008-2012). He was then awarded the Excellence Student Programme Scholarship from Public Service Department, Government of Malaysia to pursue his full-time PhD student at the Institute of Pharmaceutical Science, Faculty Life Sciences and Medicine, King's College London, United Kingdom

During his undergraduate degree, Izzat Fahimuddin was involved in a research project under supervision of Professor Akihiko Kondo and Associate Professor Chiaki Ogino, working on engineering Hepatitis B Virus core particles for drug delivery. He worked briefly as a research assistant at the Biochemical Engineering Laboratory, Kobe University where he focussed on studying the role of arginine in the arginine-rich domain of Hepatitis B Virus core particles on cell uptake using in vitro models. During his undergraduate studies, he developed experience in techniques such as genetic engineering, protein purification, cell culture, confocal laser scanning microscopy, and flow cytometry.

Izzat's current work involves on engineering targeted Hepatitis B Virus core particles for cancer therapy. He was awarded a number of biomedical imaging awards. On March 2014, he was selected as one of the winners of Wellcome Image Awards 2014 organised by Wellcome Trust. On April 2014, Izzat has won first place in the 'innovation' category of a national science photography competition organised by the Engineering and Physical Sciences Research Council (EPSRC).

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.

Ranit Kedmi

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EDUCATION

2014–2015: Post-doc, Cell biology & Nanotechnology, Tel Aviv University, Tel Aviv
2008–2014: Ph.D , Cell biology & Nanotechnology, Tel Aviv University, Tel Aviv
2005–2007: M.Sc, Life Science, The Weizmann Institute, Rehovot
2001–2004: Additional Undergraduate Program, Life Science, Tel Aviv University, Tel Aviv, With honor
1998 – 2003: LL.B, law, B.A, business (Finance), The Interdisciplinary Center, Herzelya, With honor

HONORS AND AWARDS

2010: Best flash presentation, The center for Nanoscience & Nanothechnology, 6th workshop
2008–2009: Ph.D, on the Dean's excellence list, Tel-Aviv University.
2008–2009: Legacy Heritage, Stem cell fellowship for graduate students.
2005: B.Sc.: cum Laude , Biology, Tel Aviv university.
2003: LL.B. & B.A: cum Laude Law & Business, The Interdisciplinary Center.

PUBLICATIONS AND PATENTS

1. Srinivas Ramishetti*, Ranit Kedmi*, Meir Goldsmith, Fransisca Leonard, Andrew G. Spraguen, Biana Godin, Michael Gozin, Pieter R. Cullis, Derek M. Dykxhoorn and Dan Peer. Systemic Gene Silencing in Primary T lymphocytes using Targeted Lipid Nanoparticles. ACS nano, submitted.
* denoted equal contribution.
2. Kedmi R., and Peer D., Inventors: Immuno-nanoparticles as a universal platform for targeted drug delivery. PCT filling (2013), pending.
3. Kedmi R, Benhar I, Peer D. The development of a universal delivery platform for personal therapy. In preparation
4. Dahan L, Huang L, Kedmi R, Behlke MA, Peer D. SNP Detection in mRNA in Living Cells Using Allele Specific FRET Probes. PLoS One. Sep 2013
5. Bahat A*, Kedmi R*, Gazit K, Richardo-Lax I, Ainbinder E, Dikstein R. TAF4b and TAF4 differentially regulate mouse embryonic stem cells maintenance and proliferation. Genes Cells. Mar 2013.
* denoted equal contribution.
6. Ben-Arie N*, Kedmi R*, and Peer D. Integrin-targeted nanoparticles for siRNA delivery. Methods Mol Biol. 2012
* denoted equal contribution.
7. Kedmi R*, Ben-Arie N*, Peer D. The systemic toxicity of positively charged lipid nanoparticles and the role of Toll-like receptor 4 in immune activation. Biomaterials. Sep 2010. * denoted equal contribution.
8. Kedmi R, and Peer D. RNAi nanoparticles in the service of personalized medicine. Nanomedicine. Nanomedicine (Lond). 2009

Marcelo J. Kogan

Prof. Dr. Marcelo J. Kogan is Professor at the Department of Pharmacology and Toxicology of the School of Pharmacy at the University of Chile and Principal Investigator at the Advanced Center for Chronic diseases (ACCDiS). He is the Director of the Laboratory of NANOMEDICINE AND NANOTHERANOSTICS at this center. In 2006 was Visitant Professor at the University of Texas Medical Branch and in 2002 Visitant professor at University of Barcelona. Biochemist and Pharmacist at the University of Buenos Aires and PhD in Organic Chemistry from the same University. He awarded two postdoctoral fellowships for government of Spain. His interest is centered on applications of nanobiomaterials in biomedicine for diagnosis and treatment of conformational diseases including drug delivery, Alzheimer, Cancer and cardiovascular diseases. He is a pioneer in the field of use nanoparticles for disaggregation of amyloids (Kogan et al, Nanoletters, 1,110, 2006). He has published 60 articles in international ISI Journals, 10 reviews, and four chapter books. He has been invited to present around 30 conferences in international meetings. He belonged to different organizing committees of international meetings.

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.

Anna A. Korchagina

Date of Birth: March 25, 1987
Present position: Research Scientist
Office Address: Department of Fundamental and Applied Neurobiology,
Serbsky Federal Medical Research Centre for Psychiatry and Narcology
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EDUCATION

2005-2010 M.S., Biophysics, Department of Biology, Lomonosov Moscow State University, Russia.
2010-2013 PhD, Biochemistry (Biological Sciences), Department of Medicinal Nanobiotechnologies, N.I. Pirogov Russian National Research Medical University, Moscow, Russia.

RESEARCH INTERESTS:

Brain tumors, targeted delivery, recombinant technology, monoclonal antibodies

PEER-REVIEWED PUBLICATIONS

1. Korchagina AA, Shein SA, Leopol'd AV, Volgina NE, Gurina OI, Lazarenko IP, Antonova OM, Baklaushev VP, Chekhonin VP. Generation of recombinant extracellular fragment of vascular endothelial growth factor receptor 2 and specific monoclonal antibodies to this receptor \\ Bull Exp Biol Med \\ 2014; 156(3): 357-362 2. Shein SA, Nukolova NV, Korchagina AA, Abakumova TO, Kuznetsov II, Abakumov MA, Baklaushev VP, Gurina OI, Chekhonin VP. Site-directed delivery of VEGF-targeted liposomes into C6 intracranial glioma // Bull Exp Biol Med // 2015; 158(3): 371-376
3. Chekhonin VP, Shein SA, Korchagina AA, Gurina OI. VEGF in tumor progression and targeted therapy // Curr Cancer Drug Targets // 2012; 13(4): 423-443 4. Nekrasova OV, Sharonov GV, Tikhonov RV, Kolosov PM, Astapova MV, Yakimov SA, Tagvey AI, Korchagina AA, Bocharova OV, Wulfson AN, Feofanov AV, Kirpichnikov MP. Receptor-binding domain of ephrin-A1: production in bacterial expression system and activity // Biochemistry // 2012; 77(12): 1387-1394

SELECTED PRESENTATIONS

1. Korchagina A.A., Nukolova N.V., Shein S.A., Abakumova T.O., Gurina O.I., Chekhonin V.P. Anti-VEGFR2 nanogels as a delivery system of therapeutic agents to the brain tumor cells. Nanocon 2014. Czech Republic. Abstracts. P. 132
2. Sergey A. Shein, Natalia V. Nukolova, Anna A. Korchagina, Vladimir P. Baklaushev, Olga I. Gurina and Vladimir P. Chekhonin. Anti-VEGF-coated immunoliposomes for targeted delivery therapeutics in brain tumors. 5th International Conference on Drug Discovery and Therapy. Dubai 2013. Abstracts.P. 136.
3. Korchagina AA, Shein SA. Production of monoclonal antibodies against vascular endothelial growth factor receptor 2. VIII International Scientific Medical Conference of Students and Young Scientists named after N.I. Pirogov, Moscow, Russia, 2013.

Melanie Kucki

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

Dr. Melanie Kucki was born in 1977 in Germany. After completion of her undergraduate study in biology at the University of Kassel, Germany she started her PhD thesis at the Institute of Chemistry at the University of Kassel in the field of biological photonic crystals/diatom research. She completed her PhD in 2009. In 2010 she started a three-year Post-Doc position in the field of nanoparticle cell interactions at the INM –Leibniz Institute for new materials, Saarbruecken, Germany. Within the BMBF project NanoKon her focus was the investigation of the impact of nanoscale contrast agents on human intestinal cells in vitro, as well as the determination of endotoxin contaminations in nanoparticle dispersions. In 2013 she joined Empa, St.Gallen as research associate in frame of the EU-Flagship Graphene. Her research focus is the investigation of the interaction of graphene related materials (GRM) with human epithelial barrier models in vitro.

Johanna Lempp

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Johanna was born in Bietigheim, Germany in 1987 and studied pharmacy at Freiburg University, from where she graduated in 2010. Subsequently she did an internship at the Department for Pharmaceutical Formulation at Dr. Kade in Konstanz. In 2012 she started her PhD course at the Department of Pharmaceutical Technology at Regensburg University. Since then she has been working on the diffusion of nanoparticles into cartilage. Furthermore, her work includes the investigation of the effect of platelet-derived microvesicles on cartilage.

Stefan Lyer

Dr. rer. nat. STEFAN LYER studied Biology at the Friedrich-Alexander University Erlangen/Nuremberg. After finishing his PhD theses at the German Cancer Research Center (DKFZ)/Ruprecht-Karls-University Heidelberg he stayed as a Post Doc at the Department of Molecular Genome Analysis at the DKFZ for another 2 years working

on basic genomic mechanisms of cancer development, molecular target search and standardizing in vitro cancer research.
In 2008 he moved back to Erlangen starting a post doc position at the group of Prof. Dr. med. Christoph Alexiou at the ENT-Department of the University Hospital Erlangen. Here, he focussed on the application of nanoparticles in cancer therapy and the preclinical animal model in rabbits as well as imaging and angiographic intervention in this model. In 2011 he was assigned as assistant group leader and laboratory manager. In 2009 the group moved in an own building and was renamed Section for Experimental Oncology and Nanomedicine (SEON). Since that time the SEON-team grew from 4 persons to 20 scientists, technical assistants, PhD-, and master/bachelor students. In the same time the cell culture was standardized and nanotoxicology as well as cardiovascular and regenerative nanomedicine were implemented and the synthesis infrastructure was mirrored in the GMP-laboratories of the pharmacy of the University Hospital Erlangen.

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.

Arnaud Millet

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RESEARCH INTERESTS

My actual research is focused on the nanomechanical characterization of tumors and the study of the impact of the mechanical tumoral microenvironment on the activation states of tumor associated macrophages.

PROFESSIONAL POSITION

Present: Inserm Post-doctoral Researcher at CLINATEC, Grenoble, France

EDUCATIONNAL BACKGROUND

2014	PhD , University Paris-Descartes, Cochin Institute, Paris, France
2010	MD, University of Rennes I, France
2000	Master Degree in History and Philosophy of Science (University Paris I-Sorbonne)
	Master Degree in Physics (Ecole Normale Supérieure Cachan -University Paris VII-XI)

SELECTED PUBLICATIONS

1: De Chiara A, Pederzoli-Ribeil M, Mocek J, Candalh C, Mayeux P, Millet A, Witko-Sarsat V. Characterization of cytosolic proliferating cell nuclear antigen (PCNA) in neutrophils: antiapoptotic role of the monomer. J Leukoc Biol. 2013 Oct;94(4):723-31.
2: Millet A, Pederzoli-Ribeil M, Guillevin L, Witko-Sarsat V, Mouthon L. Anti-neutrophil cytoplasmic antibody associated vasculitides: Is it time to split up the group? Ann Rheum Dis. 2013 Aug;72(8):1273-9.
3: Mouthon L, Millet A, Régent A, Pederzoli-Ribeil M, Witko-Sarsat V.Pathophysiology of ANCA-associated vasculitides. Presse Med. 2012 Oct;41(10):996-1003.
4: Millet A, Gabillet J, Pederzoli-Ribeil M, Tacnet-Delorme P, Guillevin L,Mouthon L, Frachet P, Witko-Sarsat V. Proteinase 3, the autoantigen ingranulomatosis with polyangiitis, associates with calreticulin on apoptoticneutrophils, impairs macrophage phagocytosis, and promotes inflammation. J Immunol. 2012 Sep 1;189(5):2574-83.
5: Kantari C, Millet A, Gabillet J, Hajjar E, Broemstrup T, Pluta P, Reuter N,Witko-Sarsat V. Molecular analysis of the membrane insertion domain of proteinase 3, the Wegener's autoantigen, in RBL cells: implication for its pathogenic activity. J Leukoc Biol. 2011 Nov;90(5):941-50.

Gergely Milosevits

Dr. med.
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Date of birth: 01/01/1988

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.

Sharmistha Mohapatra

Date of Birth: 10th July'1986
Nationality: Indian
Marital status: Married

I have completed M. Pharm (Pharmaceutical Analysis & Quality Assurance) in the year 2010 from Roland Institute of Pharmaceutical Sciences, Berhampur, Odissa, India under project "New analytical method development, validation, and forced degradation studies of linezolid & olmesartan medoxomil in bulk and pharmaceutical dosage forms" as a part of my M-pharm thesis dissertation. After that I was engaged as assistant professor in Sunderdeep pharmacy college, NCR Delhi, India from 2010 to 2013. Currently I am pursuing my doctoral study in the department of pharmaceutical chemistry, Jamia Hamdard, New Delhi under the project entitled Synthesis and development of anticancer drug loaded SPIONs for the effective management of solid tumor.
I was awarded with Gold Medal during the PG level from my Institution. International Travel Grant was awarded by the organising committee to attend First international translational nanomedicine conference (IT Nano), 26th to 28th July 2013.Boston, MA, USA.
My research areas of Interest are Analytical Method Development and validation, Nano science & Technologies, targeted drug delivery systems in oncology, Supercritical fluid technology, Formulation development on Controlled drug delivery system, Quality control & Quality assurance.
I have published 11 national and international publications in peer reviewed journals. Some of them are as follows

- Anwar Mohammed, Asfer Mohammed, Prajapati Ayodhya, Mohapatra Sharmistha, Akhter Sohail, Ali Asgar, Ahmad Farhan J. Synthesis and in vitro localization study of curcumin-loaded SPIONs in a micro capillary for simulating a targeted drug delivery system.International Journal of Pharmaceutics; vol. 468: pp. 158-164, 2014.

- Mohapatra Sharmistha, Annapurna Mathrusri M, Ravi Kumar B.V.V., Mohammed Anwar, Warsi Musarrat Husain, Akhter Sohail. Validated Stability Indicating RP-HPLC Method for the Estimation of Linezolid in a pharmaceutical Dosage Form. Journal of Liquid Chromatography and related technology; vol. 34: pp. 2185-2195, 2011.
- Annapurna Mathrusri M, Mohapatra Sharmistha, Ravi Kumar B.V.V. Development and validation of RP-HPLC method for the determination of Lamotrigine and its degradation products in tablets. Journal of Pharma Education Research; vol.1 (2): pp. 82-87, 2010.

Francisco Morales Zavala

PhD candidate, Biochemistry

Francisco Morales Zavala is PhD candidate in the Biochemistry Doctoral program at Universidad de Chile. He is working on his PhD thesis proyect entitled "Gold nanoparticles modified with peptides: Biodistribution and effects on β amyloid aggregation", under the direction of Dr. Marcelo Kogan, PhD of the Universidad de Buenos Aires.

Francisco Morales Zavala received his degree in Biochemistry in 2011 from the Universidad de Santiago de Chile, and the next year he got the professional title in Biochemistry, after completing his undergraduate thesis proyect entitled: "Development of PHBV nanoparticles loaded with paclitaxel", under the direction of Dr. Luis Velasquez, PhD of the Universidad Católica de Chile.

SELECTED SCIENTIFIC PUBLICATIONS

- Cristian Vilos, Francisco A. Morales, Paula A. Solar, Natalia S. Herrera, et al. Paclitaxel-PHBV nanoparticles and their toxicity to endometrial and primary ovarian cancer cells, Biomaterials, 2013.
- Cristian Vilos, Marlen Gutiérrez, Roberto A. Escobar, Francisco Morales, Juliano C. Denardin, Luis Velásquez, Dora Altbir. Superparamagnetic Poly (3-hydroxybutyrate-co-3 hydroxyvalerate) (PHBV) nanoparticles for biomedical applications, Electronic Journal of Biotechnology, 2013.

CONFERENCE PRESENTATIONS

- Morales F., Vilos C., Orihuela., Bravo M.L., Owen G., Vélasquez L, Desarrollo de nanoparticulas de PHBV cargadas con paclitaxel, XXII Reunión Anual Sociedad Chilena de Reproducción y Desarrollo, Chile, 2011.
- Francisco Morales, Carolina Velasco, Ana Riveros, Natalia Hassan, Luis Velasquez and Marcelo Kogan. Nanovarillas de Oro mulimodificadas con potenciales aplicaciones en terapia de la Enfermedad de Alzheimer. Escuela de nanoestructuras, Universidad Federico Santa María, Valparaíso, 2013, Chile.
- Francisco Morales-Zavala, C. Velasco1, A. L. Riveros, E. Salas, M. Kogan. Evaluación de la incorporación celular de nanovarillas de oro mediante espectrofotometría. III Congreso Nacional de Nanotecnología, Hotel Cumbres, Puerto Varas, Chile 2014.
- Francisco Morales-Zavala, C. Velasco, A. L. Riveros, E. Salas, C. Vetterlein, M. Kogan. Nanovarillas de oro para el tratamiento de la enfermedad de Alzheimer. IV SIMPOSIO; LATINOAMERICANO DE NANOMEDICINAS, Buenos Aires, 2014.

Maria Jose Morilla

Maria Jose Morilla serves as Adjunct Researcher of the National Science Research Council (CONICET) and Adjunct Professor of Chemistry at the National University of Quilmes. Following her first degree in Biotechnology, Morilla completed a Ph.D. degree in Basic and Applied Sciences at National University of Quilmes focused on the design of anti-chagasic liposomes. She is member of the Nanomedicine Research Programe where she supervises projects on development of oral and mucose drug and adjuvant delivery systems, including dendrimers and related structures. To date, Maria Jose has supervised 5 Ph.D. students and on-going research programes that focus on topical delivery of anti-leishmania drugs and mucosal delivery of anti-inflammatory drugs. Her main contributions in the last five years have been published in the following journals: International Journal of Nanomedicine, Journal of Controlled Release, Expert Opinion in Drug Delivery, Advanced Drug Delivery Reviews, International Journal of Pharmaceutics. She is Founding Member of the Argentinean Association for Nanomedicines (Nanomedar).

Lubna Noorani

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SUMMARY

A highly motivated and enthusiastic scientist with a sound knowledge of Tumor biology combined with Pharmaceutical drug development; proven expertise in Pharmaceutical quality compliance/quality control, formulation development and manufacturing in national and global companies; Throughout my career I have developed extensive knowledge of quality systems, GMP/GLP, team leadership, ability to meet deadlines and handle stressful situations, superior attention to details and strong personal organizational skills.

CAREER OBJECTIVE

As a scientist my career objective is to save the lives of millions of patients suffering from life threatening diseases by involvement with the drug development research, especially clinical development of new therapies

RESEARCH EXPERIENCE

My project focused on 'Formulation and biological evaluation of drug loaded albumin nanoparticles for the treatment of ovarian cancer' a comparative study between the conventional drug and the nanotechnology based targeted drug delivery system for cancer. The key objective of this study is to increase solubility and bioavailability of the anticancer drugs as well as to increase efficacy and reduce toxicity using nab-technology.

- Involved in new drug development (anti-angiogenic therapy) for cancer and detailed understanding of drug efficacy and safety at early development stage.
- Good knowledge and understanding of key therapeutic targets in association with human diseases.
- Biomedical research design, development & execution. Developed a versatile drug delivery system to increase efficacy and reduce toxicity of nano formulation.
- Project management skills, implemented a large project successfully.
- Translation of research findings into preclinical and clinical practices.
- Knowledge of local regulations and compliance requirement
- Good understanding of clinical trials concepts and study design.
- Experienced in animal handling and ethics requirements. Expertise of working with mice model of ovarian and breast cancer to determine the in-vivo efficacy and safety of the drug.
- Experienced of producing high quality research reports or peer-reviewed journal articles.

PHARMA EXPERIENCE

Good understanding of implementing, improving and auditing Quality Systems of prescription, OTC medicines and complementary products, interest about new legislation and regulatory requirements and pharmacovigilance of new therapies.

Prior to involve in research, I spent more than 10 years in pharmaceutical quality assurance, quality control, regulatory affairs, research and development and production in different national and global companies. I Worked as a Quality Assurance Officer in Lipa Pharmaceuticals Ltd from March-2007 to Feb- 2011 and GMP Pharmaceuticals Ltd, Australia from March- 2005 to Feb-2007. I also involved with the quality development of Roche brand prescription products in Healthcare Pharmaceuticals Ltd (Licensee of Hoffmann-L Roche, Basel, Switzerland) from Jan-2002 to Jan-2005. I was dealt with product complaints and participated in validation team for the technology transfer from Basel, Switzerland. I have developed my cGMP concept working in Novartis Pharma, Bangladesh. I provided extensive efforts to qualify the EU-GMP audit in Novartis Bangladesh Ltd. to commence export of medicine in Europe.

EDUCATIONAL QUALIFICATION

Doctor of Philosophy (PhD in Medicine): Completed and thesis submitted in March, 2015
Master of Pharmacy (M. Pharm), Jahangirnagar University, Savar, Dhaka, Bangladesh in 1999
Bachelor of Pharmacy (B. Pharm), Jahangirnagar University, Savar, Dhaka, Bangladesh in 1997

CONFERENCE/WORKSHOP ATTENDED

- Lorne Cancer Conference 2014, Lorne, Victoria, Australia. 13-15 Feb, 2014 (Poster presentation)
- International Nanomedicine Conference in Sydney, Australia. 1-3 July, 2013 (Oral presentation)
- CLINAM 2013 (The European Summit for Clinical Nanomedicine and targeted medicine, Basel , Switzerland) 23-26 Jun, 2013 (Poster presentation and selected for oral session.
- Drug Delivery Australia (DDA, 2012) Melbourne, Australia, Nov, 2012
- A workshop on GMP and GLP organized by Novartis Bangladesh Ltd in Oct, 1999.
- A 7 day full time basic training course on 'Pharmaceutical Marketing and Regulatory Affairs' organized by Bangladesh Drug Manufacturers association in June, 1999.

Paolo Oliva

My name is Paolo Oliva and I'm a scientific researcher. I've joined Bracco Imaging S.p.A in the middle of 2013 working in the imaging team of research group. I'm involved in an European-funded project (NanoAthero) focalizing my activity in preclinical studies on atherosclerotic plaque exploiting "in vivo imaging" techniques, specifically Magnetic Resonance Imaging and Optical Imaging (Fluorescence).
I graduated with a Bachelor Degree in Molecular Biotechnology at University of Milan (2005) also joining an employment period in Isagro Bioframing S.p.A. working on a thesis concerning the growth and development in bioreactor of a bacterial strain expressing an enzymes of industrial interest. Then I graduated with a Master Degree in Industrial Biotechnology at University of Milan (2008) joining an employment period in Cell Therapeutics Inc. working on a thesis about the development of in vitro methods for screening of potential anticancer molecules that inhibit cellular pathways related to hypoxia and tumor angiogenesis.
I have completed my postgraduation in Pharmacological Research at the Oncology Department of "Mario Negri" Institute for Pharmacological Research (2011) working on preclinical evaluation of angiogenesis inhibitors and combination therapies, phisiologic regulation of angiogenesis, linfangiogenesis in the ovarian cancer, genetic expression in tumor associated endothelium, VEGF dependent modifications of the tumor microenvironment. In this period I started working with Magnetic Resonance Imaging and Optical Imaging.
In 2011 I joined Transgenic Operative Products S.r.L. a company specialized in the generation of reporter mice (transgenic animals that produce genetically encoded biomarkers to be detected through in vivo imaging). The main activities as research scientist were the maintenance of transgenic murine lines, the in vivo characterization and validation of newly generated reporter mice strains and the in vivo pharmacological and toxicological studies with reporter mice using bioluminescence based Optical Imaging (preliminary studies, experimental activity, data collection and elaboration).
My skills for the in vivo laboratory activity are 1) main surgical techniques, necroscopy on different mouse models (syngeneic, transgenic, immunodeficient) in "SPF" animal facility; 2) main pharmacology techniques and drugs administration; 3) tumor injection in ectopic and orthotopic sites (mammary fat pad, intra ovary, intra-kidneyetc); 4) response to therapy and efficacy determination and toxicity monitoring (following NCI guidelines); 5) molecular imaging (Optical Imaging and Magnetic Resonance). Skills concerning in vitro laboratory activity are: 1) principal cellular and molecular biology techniques; 2) basilar biochemical techniques.

Ildiko Papp

My name is Ildikó Papp and I graduated as material science engineer at University of Miskolc. I started my PhD studies at the University of Szeged in environmental science in 2009. In 2010, I started working for Bay Zoltán Nonprofit Ltd at the Department of Medical Informatics and since then I have been working on nanomedicine including liposome development and production with the management of Prof János Szebeni. As a research engineer, I am dealing with experiments on liposome development in GMP laboratories.

Yogita Patil

Postdoctoral Student
Hebrew University of Jerusalem
Shaare Zedek Medical Center
Jerusalem, Israel.
yogita.udps@gmail.com

RESEARCH EXPERIENCE

Postdoctoral Research Fellow at the Hebrew University of Jerusalem, Israel.
October 2013 - Present
Postdoc. Supervisor: Prof. Alberto Gabizon; Project: Folate-mediated tumor cell targeting of liposomal mitomycin-C prodrug (MLP).
Doctoral Student (PhD) at Indian Institute of Technology, Bombay, India
July 2007 - August 2013: PhD Supervisor: Prof. Sameer Jadhav; Project: Synthesis of Small Unilamellar vesicles (SUVs) for drug delivery by extrusion and cellular uptake of SUVs under shear stress conditions using cone and plate rheometer.
M. Pharm student at R.T.M. Nagpur University, Nagpur, India
July 2005 - July 2007: Supervisor: J. G. Avari;Project: Development of gastroretentive dosage form using ion exchange resin.

PUBLICATIONS

Esther Tahover, Yogita Patil, and Alberto Gabizon, Emerging delivery systems to reduce doxorubicin cardiotoxicity and improve therapeutic index: focus on liposomes, Anticancer Drugs, 26 (3), 241-58, 2015
Yogita Patil and Sameer Jadhav, Novel methods for liposome preparation, Chemistry and physics of lipids 177, 8-18, 2014.
Yogita Patil, Amanpreet Ahluwalia and Sameer Jadhav, Isolation of giant unilamellar vesicles from electroformed vesicle suspensions and their extrusion through nano-pores, Chemistry and physics of lipids 167, 1-8, 2013.
Yogita Patil, Mrunmayi Kumbhalkar and Sameer Jadhav, Extrusion of electroformed giant unilamellar vesicles through track-etched membranes, Chemistry and physics of lipids, 165 (4), 475-481, 2012.

AWARDS AND GRANTS

Outstanding Poster Award
9th Meeting of Israeli Chapter of Controlled Release Society 2014

Viorica Patrulea

Institution: University of Geneva, Switzerland and West University of Timisoara, Romania.

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 fellowship 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.
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.

Grisha Pirianov

Name: Dr Grisha Pirianov
Tel: 08451962745 (office)
E-mail: grisha.pirianov@anglia.ac.uk

CURRENT POSITION

2015: Senior Lecturer in Clinical Sciences, Department of Biomedical and Forensic Sciences, Anglia Ruskin University, Cambridge.

QUALIFICATIONS

1986: MSc, Molecular Biology, Sofia University, Sofia.
1992: PhD, Biomedical Sciences, Bulgarian Academy of Sciences, Sofia.
2012: PgCertHE, Health Care and Biomedical Education, St Georges University

INVITED REVIEWER

ATVB, Atherosclerosis, Cell Death and Differentiation, Archives in Medical Research, Journal of Vascular Surgery, British Journal of Pharmacology, Wellcome Trust, MRC, British Heart Foundation

RESEARCH INTERESTS

I possess long-term record of achievements in broad fields of biomedical sciences, with particularly strong relevance in translational medicine: discovering novel drugs and therapeutic targets for pharmacological intervention of vascular inflammatory based diseases. My peer-reviewed research publications (41) are cited over 2000 times and my H index is 16.
Academic and industrial collaborations are essential drivers of development of my research portfolio. My interests are focused on the efficacy of novel compounds for modulation of TLR4 signalling as a strategy for pharmacological intervention of aneurysm. In addition to their effect on aneurysm I anticipate that these molecules have the potential to intervene on atherosclerosis and inflammatory brain damage diseases. In this regard, I am starting my collaborative academic and industrial work on the effect of novel TLR4 modulators on mediators (oxidised LDL) of atherosclerosis development. In the meantime a couple of collaborative projects address the role of novel TLR4 modulators on neonatal brain damage in preterm labour and discovery of new inflammatory therapeutic targets in depression during pregnancy.

Johanna Poecheim

PhD Student
University of Geneva-University of Lausanne
School of Pharmaceutical Sciences – Biopharmacy ,30, Quai Ernest-Ansermet, CH-1211 Geneva 4, Switzerland
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Johanna Poecheim enrolled at the University of Innsbruck, Austria, in 2004 to study Pharmacy. During her Master thesis she focused on strategies to improve storage stability of drugs in aqueous parenteral formulations. The studies were performed in accordance with GLP standards within the pharmaceutical company ThioMatrix that specializes on the development of non-invasive drug delivery systems. Johanna graduated in 2009 and obtained the Austrian federal diploma in pharmacy in 2010. In 2010 she started her PhD in the Biopharmacy group of Prof. Gerrit Borchard at the University of Geneva, Switzerland. Within her thesis she has been working in the field of the development and testing of DNA vaccine formulations. The project included formulation development, physicochemical characterization, as well as in vitro testing. In vivo studies with mice were performed in collaboration with Dr. Nicolas Collin of the WHO Vaccine Formulation Laboratory (VFL) at the University of Lausanne. The results show the immunoactivity of three for

Anna Polomska

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Anna Polomska is working since 2012 in the group of prof. J.-C. Leroux on her PhD project concerning polymer coated drug nanocrystals for targeted tumor delivery. She studied Chemistry (specialty: Biological Chemistry) at the Jagiellonian University in Krakow, Poland, where she obtained her Master in Science Degree. She has also spent one year at the University of the West of Scotland in Paisley studying Medicinal Chemistry where she has obtained her Bachelor with Honours degree. Following her Master studies, she performed 8 months internship at the University of Geneva in the Laboratory of Colloid and Surface Chemistry working on adsorption of multivalent ions on charged latex particles and influence of stability of ionic liquids on colloidal suspensions. She is a co-author of 2 publications:

- Kathrin Fuhrmann, Anna Połomska, Carmen Aeberli, Bastien Castagner, Marc A. Gauthier, Jean-Christophe Leroux. Modular design of redox-responsive stabilizers for nanocrystals. ACS Nano 7, (2013), 8243–8250
- Istvan Szilagyi, Anna Połomska, Damien Citherlet, Amin Sadeghpour, Michal Borkovec. Charging and aggregation of negatively charged colloidal latex particles in the presence of multivalent oligoamine cations. Journal of Colloid and Interface Science 392 (2012) 34-41

Marina Pöttler

University Hospital Erlangen
Department of Otorhinolaryngology,
Head and Neck Surgery
Section of Experimental Oncology and Nanomedicine (SEON) , Glückstraße 10a, 91054 Erlangen
Phone: 09131-85 43985
E-Mail: marina@poettler@uk-erlangen.de

Dr. Marina Pöttler is a biologist specialized in nano- toxicology, 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 extensively strived on toxicological evaluations of superparamagentic iron oxide nanoparticles for the use in cancer therapy and diagnosis on different cell entities.

Srinivas Ramishetti

I am Ramishetti Srinivas, born in Andhra Pradesh, south part of India. I obtained master's degree in chemistry at Central University of Hyderabad in 2003. I qualified for research fellowship from Council of Scientific and Industrial Research (CSIR), government of India to carry out doctoral study in India. I started my doctoral research in the field of gene therapy under supervision of Dr. Arabinda Chaudhuri at Indian Institute of Chemical Technology, Hyderabad in 2004. My research involves an interdisciplinary approach, designing and synthesis of novel cationic lipids to target immune cells. I have synthesized novel cationic lipids containing mannose mimicking ligands for targeting dendritic cells to develop a DNA based vaccine against cancer. These results published in two scientific papers and granted one patent.

I moved to University of North Carolina at Chapel Hill, USA to pursue my post-doctoral study in October, 2009. I joined Prof Leaf Huang's lab to continue my research on developing novel drug delivery formulations for cancer therapy. I developed a metal based nanoparticle formulation to carry anti-cancer drug Etoposide phosphate and involved in several other projects. I have worked for more than 3 years at UNC and published several scientific papers including one patent.

I selected for post-doctoral fellowship form Center for Nano science and Nano technology, Tel Aviv University, Israel in 2013. Currently I am working with Prof Dan Peer and my research involves, developing lipid nanoparticles for targeted delivery of therapeutic RNAi to treat cancer and HIV therapy. We have a developed siRNA loaded targeted lipid nanoparticle system for treating Glioblastoma and the results were reported in ACS nano. In another targeted approach, we have successfully delivered the RNAi to primary T lymphocytes using antibody modified lipid nanoparticles. These results were submitted.

Elisa Salvati

Elisa Salvati obtained her M.S. degree in Medical Biotechnology in 2009 and her PhD in Translational and Molecular Medicine in 2013 from the University of Milano-Bicocca (Italy). During her PhD, under the supervision of Prof. Massimo Masserini, she developed lipid-based nanoparticles for the treatment and the diagnosis of Alzheimer's disease and she had the opportunity to present her work at International Conferences as well as to publish peer-reviewed journal papers. She is currently working as postdoctoral researcher at the FIRC Institute of Molecular Oncology Foundation (IFOM) in Milan. Her current research interests include the design and synthesis of functional gold nanoparticles and stimuli-responsive biodegradable nanoparticles with controlled properties for application in sensing and delivery of anticancer drugs.

Barbara Sanavio

PhD

Dr Barbara Sanavio is working as a postdoctoral researcher in the Nanomedicine Laboratory of Fondazione Istituto Neurologico Carlo Besta in Milan since 2012.

After a Master degree in Biotechnology in pharmacy in 2006 at the University of Bologna (Italy), she obtained a PhD in Statistical and Biological Physics (2010) at the International School for Advanced Study (SISSA/ISAS) in Trieste (Italy) under the supervision of prof. Giacinto Scoles and Dr. Loredana Casalis in the NanoInnovation Lab located at the ELETTRA Synchrotron Light Source. During her PhD and first postdoc, she pursued research in surface functionalization and nanopatterning of self-assembled monolayer for the oriented immobilization of intrinsically disordered proteins at the nanoscale and their characterization with Atomic Force Microscopy. In 2012 she jouned the group of Dr. Silke Krol and Prof. Francesco Stellacci at Fondazione Istituto Neurologico Carlo Besta in Milan (Italy), located at the IFOM- the Firc Institute of Molecular oncology. She is currently involved in the synthesis, physicochemical and in vitro characterization of gold and iron oxide magnetic nanoparticles and their functionalization for targeted delivery, imaging and biosensor applications.

Sergey A. Shein

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Present Position Senior Research Scientist
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EDUCATION:

2004–2009: M.S., Mycology and Algology Department, Biology Faculty, Lomonosov Moscow State University, Russia.

2009–2012: Ph.D., Chair of Medicinal Nanobiotechnologies, N.I. Pirogov Russian National Research Medical University, Russia.

RESEARCH INTERESTS:

Targeted therapy, drug delivery, nanocarriers, angiogenesis, immunotherapy, dendritic cells

Peer-reviewed publications:
1. Abakumov MA, Nukolova NV, Sokolsky-Papkov M, Shein SA, Sandalova TO, Vishwasrao H, Grinenko NF, Gubsky IL, Abakumov AM, Kabanov AV, Chekhonin VP. VEGF-targeted magnetic nanoparticles for MRI visualization of brain tumor. Nanomedicine. 2015 Jan 31. pii: S1549-9634(15)00017-9. doi: 10.1016/j.nano.2014.12.011. [Epub ahead of print]
2. Shein SA, Nukolova NV, Korchagina AA, Abakumova TO, Kuznetsov II, Abakumov MA, Baklaushev VP, Gurina OI, Chekhonin VP. Site-directed delivery of VEGF-targeted liposomes into C6 intracranial glioma. Bull Exp Biol Med. 2015 Jan;158(3):371-6.
3. Korchagina AA, Shein SA, Gurina OI, Chekhonin VP. VEGFRs in neoplastic angiogenesis and prospects for therapy of brain tumors. Vestn Ross Akad Med Nauk. 2013;(11):104-114.
4. Korchagina AA, Shein SA, Leopol'd AV, Volgina NE, Gurina OI, Lazarenko IP, Antonova OM, Baklaushev VP, Chekhonin VP. Generation of recombinant extracellular fragment of vascular endothelial growth factor receptor 2 and specific monoclonal antibodies to this receptor Bull Exp Biol Med. 2014;156(3):357-62.
5. Chekhonin VP, Shein SA, Korchagina AA, Gurina OI. VEGF in tumor progression and targeted therapy. Curr Cancer Drug Targets. 2013;13(4): 423-443.
6. Chekhonin VP, Shein SA, Korchagina AA, Gurina OI. VEGF in neoplastic angiogenesis. Vestn Ross Akad Med Nauk. 2012;(2):23-33.
7. Shein SA, Gurina OI, Leopol'd AV, Baklaushev VP, Korchagina AA, Grinenko NF, Ivanova NV, Volgina NE, Ryabukhin IA, Chekhonin VP. Generation of monoclonal antibodies to recombinant vascular endothelial growth factor. Bull Exp Biol Med. 2012 May;153(1):139-42.

Catarina Oliveira Silva

Catarina Oliveira Silva has a MD in Pharmaceutical Sciences since 2012 granted by Universidade Lusófona (ULHT) in Lisboa (Portugal). Her master thesis was a result of collaboration between University of Santiago de Compostela (USC) in Spain and ULHT on the development and characterization of nanocapsules as transdermal drug delivery systems. Since 2013 she is dedicated to a PhD Program in Biomedical Sciences, organized in cooperation of the University of Alcalá Henares (UAH) in Spain and ULHT, directed by Dr. Jesús Molpeceres García del Pozo and Dr. Catarina Pinto Reis. She also works as a researcher for Fundação para a Ciência e Tecnologia (FCT) with the project "A new approach to phototherapy tumor targeting: focusing the light through diffusion" (Ref: PTDC/888 BMD/0611/2012), in partnership with Aalborg University (AAU) in Denmark. Catarina has also collaborated as a teaching assistant at ULHT and has published 6 papers, including 3 book chapters, 1 review paper and 1 proceeding paper, three oral communications (one in an international conference and two in national scientific journeys) and several posters presentations in national and international congresses. Her main interests include nanomedicine, cancer treatment, drug delivery and phytotherapy.

Amnon Sintov

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Amnon Sintov, PhD, is a Professor of pharmaceutics and biomedical engineering at Ben Gurion University of the Negev, Be'er Sheva, Israel. Prof. Sintov earned his PhD from the Hebrew University of Jerusalem at the School of Pharmacy. His academic credentials include degrees from Bar-Ilan University, Ramat-Gan, Israel, the Hebrew University of Jerusalem, Jerusalem, Israel, and post-doctoral experience at the University of Michigan, An Arbor, MI. He has also been cooperating with the Laboratory for Drug Delivery as a visiting professor at the Jew Jersey Center for Biomaterials, Rutgers-The State University of New Jersey, Piscataway, NJ.
Since 1996, Prof. Sintov is specializing in biomedical/ pharmaceutical research, industrial pharmacy, R&D of new delivery systems for therapeutic agents, topical and transdermal routes of administration as well as intranasal drug delivery and controlled release technologies. His studies also involve novel carrier systems such as polymeric nanoparticles, nano-sized emulsions and nano-micellar systems. During years of experience, he has specialized in a variety of areas related to skin physiology, and he has managed various research projects in the development of new active compounds for treatment of cancer and skin disorders. He has also established a network of fruitful collaboration between industry and academia. Prof. Sintov holds multiple patents, scientific peer-reviewed articles, reviews and study reports.

Arieh S Solomon

MD, PhD
Associate Professor in Ophthalmology
Head Experimental Ophthalmology Laboratory Goldschleger Eye Research Institute
Faculty of Medicine , Tel Aviv University

EDUCATION

1975 - MD -Hadassah Medical School, Hebrew University, Jerusalem
1978 - Diploma in Ophthalmology, Post Graduate School in Medicine , TAU
1981 - Certified in Ophthalmology , Scientific Committee Board , Israel Med Association
2003 - PhD, Faculty of Medicine , Tel Aviv University

PUBLICATIONS

78 peer reviewed articles, collaborator in 11 book chapters

INVITED LECTURES

Sheye Eye Institute, Philadelphia, Maimonides Medical Center, New York,
Doheney Eye Institute, USC, Los Angeles,
Louisville Eye Center, Kentucky, Eye Department,
University Hospital, Geneve .

PATENTS

New Method for Visual Field Evaluation (US , Australia, EU , Israel)
New medication for treatment of anterior segment of the eye (US, EU)
New Glaucoma Implant (US, Israel)

INTERNATIONAL ACADEMIC ACTIVITY

1995-1998 National Standards Institute, USA
FDA Committee for Glaucoma Implants, Member
2004-2007 ARVO Committee for Regulation and Ethics in Human Research, Member
2005-2010 Nano2Life Scientific Program, European Committee, Member
More than 70 scientific presentations national and world wide

REVIEWER:

American Journal of Ophthalmology, Archives of Ophthalmology, British Journal of Ophthalmology, Indian Journal of Ophthalmology, TSVT - ARVO Journal, IOVS

Lenka Stefancikova

Marie Curie postdoc in CNRS, France

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Born: 3rd June 1983, Olomouc, Czech Republic

ACADEMIC QUALIFICATIONS AND PROFESSIONAL ACTIVITIES:

Postdoc (05/2014-present)
Institute of Molecular Sciences of Orsay (ISMO), University Paris-Sud, CNRS, France (Dr. S. Lacombe)
Project: Nanomedecine and Hadrontherapy; Marie Curie action
Postdoc (06/2011-12/2013)
Institut of Biophysics, Academy of Sciences of the Czech R. (Dr. M. Falk)
Project: DNA double strand break induction and repair and their consequences to cancerogenesis.
Research visits: (02 – 07/2013, 12/2012 and 06 – 08/2012) ISMO, UPSUD, France (Dr. S. Lacombe)
PhD in Molecular and Cell Biology (08/2007 – 05/2011)
Masaryk University, Brno and University Hospital Brno, Czech R., (Prof. J. Smardova)
PhD thesis title: Analysis of molecular markers in lymphomas, Ph.D. awarded 27th May 2011
Research internship: (09 - 12/2009) Hospital Clinic, Barcelona, Spain (Prof. E. Campo)
MSc. and BSc. in Molecular Biology and Genetics (09/2002 – 06/2007) first-class honours degree
Faculty of Science, Masaryk University Brno, Czech R.
Additional scientific training (12/2012) European course in radiobiology, Paris, France
Soft skills training (09/2010 - 02/2011) Organization, Management and Project Management of Research and Development, Masaryk University Brno, Czech R.

FUNDED PROJECTS:

2013: Marie Curie Intra-European Fellowship „NanoHapy“ FP7-PEOPLE-2013-IEF
2013, 2012: Three Short-Term Scientific Mission (STSM) grants, European Cooperation in Science and Technology (COST), Nano-scale insights in ion beam cancer therapy (Nano-IBCT)
2009: Exchange grant European Science Foundation (ESF) activity Frontiers of Functional Genomics (FFG)

COMMUNICATIONS OF THE RESULTS:

Publications as first author: 4
Štefančíková L et al., Cancer nanotechnology. 2014; 5:6-21.
Stefancikova L et al., Int. J. Oncol. 2011; 39: 1413-1420.
Stefancikova L et al., Int. J. Oncol. 2010; 36: 699-706.
Stefancikova L t al., J. Clin. Pathol. 2009; 62: 948-950.
Publications as co-author: 10 (2012-2014)
Invited talks: 3
2014 XXIII International Materials Research Congress, Cancun, Mexico
2014 “Dynamics of Systems on the Nanoscale” DySoN Conference 2014, Edinbourgh, GB
2013 COST Action: MP1002 Working Group Meeting; Experimental data, biological aspects, Belfast, GB
Oral presentations as first author: 6 (2009-2015; 2009: first prize for young scientists)
Posters: 5 (2008-2012)

EXPERIENCE IN SUPERVISION AND TEACHING:

- co-supervision of a postdoc (OPVK, Czech R.), 2 PhD students (Marie Curie ITN, France and JINR, Rusia) and a master student (Erasmus Mundus, France)
- teaching assistant in 1) European training course in radiobiology, practical training; 2) Radiation physics - lab training 3) Methods of Molecular Biology – lab training; 4) Molecular Biology of Eucaryots – lab training
- regular lectures in molecular biology and radiobiology within Operational Program Education for Competitiveness (IBP, ASCR; Masaryk and Charles Universities, Czech R.)

Carmen Streich

Date of birth: May 9, 1988
University of Duisburg-Essen
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Carmen Streich studied Water Science at the University of Duisburg-Essen, Germany. For her Master thesis she joined the Technical Chemistry group of Professor Stephan Barcikowski in Essen, where she employed laser ablation in liquid to generate ultra-pure, colloidal gold and platinum nanoparticles. With these particles,

Mohammed Syed Ali

Head and Assistant Professor, PG & Research Department of Biotechnology
Division of Nanomedicine and Infectious Disease

I am DR .Syed Ali, I was born in 1983 and now currently working Head of the department and Research Department of Biotechnology, Mohamed Sathak College of Arts and Science (Affiliated to University of Madras), Chennai, India. I have completed Phd in Oceanography – Marine Biotechnology2011 (Title: Screening of various biological resources from Gulf of Mannar for the management of Dengue fever) and also Master of Philosophy and Master of Science in Marine Biotechnolgy, 2006. My field of research work marine pharmacology based upon marine biotechnology. Now my self and my research scholars are working Marine Nanomedicine from Marine Resources. To be developed the nano target drug and formulation based upon the Siddha medicine. In My institution have ethical clearance so we do work to Mice and Rat model. And also my research group working zebra fish model also like cancer, neuroscience and infectous disease like malaria and Dengue using nanoparticles. I got international travel grant was awarded by orgainsing committee to attend the international conference climatic change, Beging, (June 2-21,2014) China and International conference on parasitology at Pakistan (October,27-29,2014). I delivered guest talk about vector borne disease using nanomedicine targets drug. I have published 33 international publication in peer reviewed journals and I have contributed I three book chapter. Recently I have published one book Safety and Bio-efficacy of mosquito control form marine resources using nanoparticle.I have member of five scientific committee to all over the world especially European Society of Clinical Microbiology and Infectious Disease, Europe (Member ID:123822) and Advisory Board Member of all of the world. Finally my research team working nanomedicine and development using marine resource.

Shima Tavakol

Assistant Professor
Razi Drug Research Center, Iran University of Medical Sciences, Tehran, Iran
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ACADEMIC BACKGROUND

Post-Doc researcher of Medical Nanotechnology, Tehran University of Medical Sciences, School of Advanced Technologies in Medicine, Tehran, Iran (September 2014).
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
- Ranked 2nd, in the M.Sc Entrance Examination held by Ministry of Health and Medical Education. 2007
- Ranked 3rd, among Technician degree Graduate. 2003

SOME ARTICLES PUBLISHED TO REFEREED JOURNALS:

- 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.
- n 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) DOI: 10.1007/s12035-014-8812-8.
- Investigating the effects of particle size and chemical structure on cytotoxicity and bacteriostatic potential of nano hydroxyapatite/chitosan/silica and nano hydroxyapatite/chitosan/silver; as antibacterial bone substitutes. Shima Tavakol, Mohammad Reza Nikpour, Elham Hoveizi, Behnaz Tavakol, Seyed Mahdi Rezayat, Mahdi Adabi, Sahebeh Shajari Abokheili, Mohsen Jahanshahi. Journal of Nanoparticle Research (2014) 16:2622.
- Small molecules differentiate definitive endoderm from human induced pluripotent stem cells on PCL scaffold. Elham hoveizi, Siruse Khodadadi, Shima Tavakol, Oveise Karima, Mohaamad Ali Nasiri. Applied Biochemistry and Biotechnology. (2014) 173(7):1727-36.
- Thermogel nanofiber induces neural-like cells from human Endometrial-Derived Stromal Cells; an in-vitro and in-vivo study in Rat. Shima Tavakol, Hadi Aligholi, Ali Gorji, Aresou Eshagh Abadi, Elham Hoveizi, Behnaz Tavakol, Seyed Mahdi Rezayat, Jafar Ai. Journal of Biomedical Materials Research: Part A. (2014) 102(12):4590-7.
- The effect of Noggin supplementation in Matrigel nanofiber-based cell culture system for derivation of neural-like cells from human Endometrial-Derived Stromal Cells. Shima Tavakol, Mohammad Masummi, Seyed Mostafa Modarres Mousavi, Amir Amani, Seyed Mahdi Rezayat, Jafar Ai. Journal of Biomedical Materials Research: Part A. (2015) 27;103(1):1-7.
- The Effect of Laminated Hydroxyapatite/Gelatin Nanocomposite Scaffold Structure on Osteogenesis using Unrestricted Somatic Stem Cells and in Rat. Shima Tavakol, Mahmoud Azami, Ahad Khoshzaban, Iraj Ragerdi Kashani, Behnaz Tavakol, Elham Hoveizi, Seyed Mahdi Rezayat Sorkhabadi. Cell Biology International. (2013) 37: 1181.
- The Effect of Carrier Type on Bone Regeneration of Demineralized Bone Matrix in Rat. Shima Tavakol, Ahad Khoshzaban, Mahmoud Azami, Iraj Ragerdi Kashani, Hani Tavakol, Seyed Mahdi Rezayat. Craniofacial Surgery. (2013) 24(6):2135-40.
- Programming of human endometrial-derived stromal cells (EnSCs) into preoligodendrocyte cells by overexpression of miR-219. Somaye Ebrahimibarough, Hamid Reza Kouchesfehani, Jafar Ai, Mahmoodinia M, Shima Tavakol, mohammad Massumi. Neuroscience Letters. (2013) 537: 65.

PATENT

- Hydrogel based peptide nanofiber containing long motif of laminin for application in neurodegenerative disorders. (Under PCT filling).
- Hydrogel based peptide nanofiber containing long motif of laminin for application in medical studies; International category A61, Patent no 82433.
- A Biodegradable and biocompatible nano composite T- plate implant and method of synthesizing the same. United States Patent Application 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.

Zsutsanna Toth

Zsuzsanna Tóth is working as a researcher in the Medical Informatics Laboratory, in Miskolc since 2015.
After a Master degree in Biology and Environmental studies in 2009 at the University of Pécs (Hungary), she started PhD studies in the program "Molecular analysis of microbial metabolism" at the International Doctoral School for Biology in Pécs (Hungary) under the supervision of dr. Csaba Fekete, and worked in the Microbial Biotechnology Research Group located at the János Szentágothay Research Centre. During her PhD she pursued research in new generation sequencing based transcriptomic characterization of three dimensional in vitro cell systems.
In 2015 she joined the group of Medical Informatics of Bay Zoltán Nonprofit Ltd. For Applied Research (Hungary), located at Knowledge Management Centre in Miskolc. She is currently involved in the development of methods for cancer diagnosis and non-invasive pre-impalntation aneuploidia testing based on high-throughput sequencing technologies.

Martina Tuttolomondo

PhD

Martina Tuttolomonda was born 1985 in Palermo, Italy, and started studying Pharmaceutical Chemistry and Technologies in 2004. The final year of her combined Bachelor's/Master's Degree Course training was spent as student at the department of Molecular and Biomolecular Science and Technology (STEMBIO) - University of Palermo, Italy, where Martina Tuttolomondo was working on a project in the area of drug delivery. She graduated in 2012 with the final mark of 110/110 Cum Laude with the thesis "Lipid Micromatrices for the Topic Treatment of Oral Cavity Mycosis". In the same year she received the license to work as a Pharmacist. Since March 2014 Martina Tuttolomondo is PhD student in Health Sciences at the Lundbeckfonden Center of Excellence NanoCAN at the University of Southern Denmark as Marie Curie ESR fellow within the ITN network Pathchooser, where she started exploring natural pattern recognition mechanisms for drug delivery under the supervision of Prof. Dr. Jan Mollenhauer and Prof. Dr. Kenneth A. Dawson.

Mehmet Hikmet Ucisik

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RESEARCH AREAS

Nanotechnology; Nanomedicine; Drug Delivery Systems (DDS); Surface Functionalization; Emulsomes; Cell Targeting; Pharmaceutical Biotechnology.

EDUCATION

2009-2014	PhD (with distinction) – Department of NanoBiotechnology, University of Natural Resources and Applied Life Sciences (BOKU), Vienna, Austria
2007-2009	PhD (started) – Department of Molecular Biology and Genetics, Istanbul Technical University (ITU), Istanbul, Turkey
2004-2006	MSc – Department of Biotechnology, TU Delft, Delft, The Netherlands
2000-2004	BSc – Department of Chemical Engineering, Bogazici University, Istanbul, Turkey
1992-2000	Gymnasium, "Deutsche Schule Istanbul", Istanbul, Turkey

LANGUAGES

Fluent in both English and German; native language Turkish

Publications

- Ucisik, M. H., Küpcü, S.*, Debreczeny, M., Schuster, B. and Sleytr, U. B.* (2013) S layer Coated Emulsomes as Potential Nanocarriers. Small, 9: 2895 2904.
- Ucisik M.H.*, Küpcü S., Schuster B., Sleytr U.B. (2013) Characterization of CurcuEmulsomes: Nanoformulation for Enhanced Solubility and Delivery of Curcumin, Journal of Nanobiotechnology, 11:37:1-13.
- Ucisik M.H.*, Küpcü S., Breitwieser A., Gelbmann N., Schuster B., Sleytr U.B. (2015) S-layer Fusion Protein as a Tool functionalizing Emulsomes and CurcuEmulsomes for Antibody Binding and Targeting, Colloids and Surfaces B: Biointerfaces, 128, 132-139
- Ucisik M.H.*, Schuster B., Sleyt U.B. (2015) Emulsomes meet S-layer Proteins: An emerging Targeted Drug Delivery System, Current Pharmaceutical Biotechnology, 16:4, 392-405

Hennig, R., Pollinger, K., Veser, A., Breunig, M., Göpferich, A.: Nanoparticle multivalency counterbalances the ligand affinity loss upon PEGylation. Journal of Controlled Release. 2014, 194, 20–27

Anika Veser

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Address: Universitaetstrasse 31
93053 Regensburg, Germany

Anika was born in Ravensburg in 1986. She studied Molecular Biotechnology at the University of Munich until 2012. She finished her master´s thesis with the title: "Studies on peptide-lipid interactions in liposomes". Afterwards she joined the Department of Pharmaceutical Technology in Regensburg as a Ph.D. student. Her scientific work focuses on multivalent binding antagonists for the neurokinin-1 receptor as potential drugs for the therapy of rheumatoid arthritis.

PUBLICATIONS

Langer, M., Sah, R., Veser, A., Gütlich, M., Langosch, D.: Structural Properties of Model Phosphatidylcholine Flippases. Chemistry & Biology. 2013, 20, 63–72

Hennig, R., Pollinger, K., Veser, A., Breunig, M., Göpferich, A.: Nanoparticle multivalency counterbalances the ligand affinity loss upon PEGylation. Journal of Controlled Release. 2014, 194, 20–27

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. Currently he is the Chief Scientific Officer of the Nano-Bioanalytik-Zentrum Münster (NBZ) and manages the Nano-Characterization-Lab Muenster (www.NCL-Muenster.de) of 11 local companies, which develop new and certified methods for characterization of Nanomaterials in consumer products and biological systems.

Jan Zaloga

Mr Zaloga studied Pharmacy at the University of Regensburg. After finishing his second state examination in May 2011, he spent 6 months as a research fellow at the Robert Gordon University in Aberdeen, Scotland. He is currently a PhD student in the Section of Experimental Oncology and Nanomedicine (SEON; Chair: Prof. C. Alexiou) at the University Hospital in Erlangen. His main research topic is the development of an iron-oxide based drug delivery system for magnetic drug targeting and its establishment in a good manufacturing practice (GMP) compliant environment.

ABSTRACTS OF THE INTERVENTIONS

LIGHT-EMITTING ∏-PROBES FOR DIAGNOSTIC APPLICATIONS

DONG JUNE AHN, Department of Chemical & Biological Engineering, College of Engineering KU-KIST Graduate School of Converging Science & Technology Korea University, Seoul 136-701, South Korea, ahn@korea.ac.kr

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.

CARBON NANOTUBES UPTAKE IN BRAIN AFTER INTRAVENOUS INJECTION IN MICE

KHULOUD T AL-JAMAL, Institute of Pharmaceutical Science, King's College London, London, United Kingdom
Khuloud.al-jamal@kcl.ac.uk

ABSTRACT SUMMARY

The ability of amino-functionalized multi-walled carbon nanotubes (MWNTs-NH_3^+) to cross the Blood-Brain Barrier (BBB) in vitro and in vivo was evaluated using the primary porcine brain endothelial cells (PBEC)/rat astrocytes co-culture and C57Bl6 mice, respectively. MWNTs-NH_3^+ were able to cross the in vitro BBB model, reaching a maximum transport of 10.8 ± 0.9 % after 72 hours. [^{111}In]DTPA-MWNTs displayed brain uptake of ~2-5% ID/g, after intravenous administration in mice, which is significantly higher than any other reported values for other nanomaterials.

INTRODUCTION

Brain disorders are on the rise accounting for almost 12% of world mortalities every year. Despite extensive research in drug development, brain disorders are still largely untreated due to the inability to deliver current therapeutics to the brain across the BBB (1).
Chemically functionalized carbon nanotubes (f-CNT) constitute a novel class of nanomaterials with attractive physical, chemical and electronic properties (2). The key advantage of f-CNTs is the extremely high surface area to size ratio allowing a high degree of chemical functionalization making them invaluable tools for designing drug delivery systems to the brain (3). One of the most interesting characteristics of f-CNTs is their ability to translocate across plasma membranes and enter the cells either passively by direct translocation across membranes or actively via endocytosis (4). Herein, we confirmed the ability of chemically functionalized MWNT (f-MWNT) to cross the BBB and reach the brain in in vitro and in vivo model, respectively.

EXPERIMENTAL METHODS

Pristine MWNTs were functionalized with 1, 3-dipolar cycloaddition reaction yielding MWNTs-NH_3^+ which were further modified to generate [^{111}In]DTPA-MWNTs. To set up the BBB model, PBEC and astrocytes were isolated from porcine brain and 1- 2 days old rat brain, respectively. After purifying the primary cultures, PBEC and astrocytes were co-cultured in a Transwell™ system with 3.0 µm pore size. The modified lactate dehydrogenase (LDH) assay was employed to study the toxicological impact of MWNTs-NH_3^+ on PBEC. Trans-endothelial electrical resistance (TEER) was measured to assess the tightness of the BBB model. When TEER was > 200 Ω.cm^2 permeability experiments were carried out using [^{111}In]DTPA-MWNTs. Briefly, [^{111}In]DTPA-MWNTs (20 µg/ml) were added to the apical chamber and the plate was incubated at 37 ◦C up to 72 h. The energy dependency of the transport was studied by reducing the incubation temperature to 4 ◦C and incubating the [111In]DTPA-MWNTs with cells for 4 h. The ^{111}In signal was quantified as counts per minute (CPM). Ultrathin sections of the glutaraldehyde-fixed PBEC were imaged using electron microscopy, to understand the mechanism of uptake. For in vivo studies, [111In]DTPA-MWNTs were injected intravenously in mice. Brain uptake was imaged and quantified by SPECT/CT imaging and gamma counting, respectively.

RESULTS AND DISCUSSION

In this work we demonstrate, for the first time, the ability of MWNTs-NH_3^+ to cross the BBB, using a co-culture model of PBEC and primary rat astrocytes. The lack of potential cytotoxicity of MWNTs-NH_3^+ under the studied conditions was confirmed by lactate dehydrogenase assay (LDH). The percentage transport of [^{111}In]DTPA-MWNTs across PBEC (Figure 1) was 4.2 ± 0.3 % at 4 h. The percentage transported increased considerably after 24 h to 7.5 ± 0.8 %, and reached a maximum of 10.8 ± 0.9 % at 72 h. Incubating the cells at 4 ◦C for the initial 4 h resulted in a slight but significantly lower % transport (2.0 ± 0.5 %) than that obtained at 37 ◦C (P= 0.0005). This difference was abolished upon the re-incubation of Transwells™ at 37 ◦C (10.0 ±1.2 %) at 72 h.

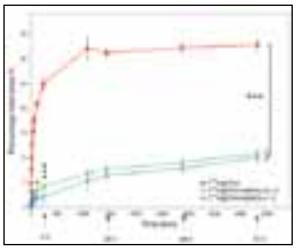

Fig. 1: The percentage of [^{111}In]DTPA-MWNTs transported across the PBEC monolayer over 72 h.

The STEM images (Figure 2, i) show MWNTs-NH_3^+ within endoplasmic vesicles after 4 and 24 h. This vesicular uptake allowed the clusters of MWNTs-NH_3^+ into the PBEC monolayer after interaction with the plasma membrane occurred. The electron microscopy images captured after 48 h of starting the uptake experiment confirmed complete crossing of MWNTs-NH_3^+ across PBEC (Figure 2, iii). The images show a partly open vesicle facing the basal chamber providing the first evidence of the complete crossing of the endothelial cell via transcytosis.

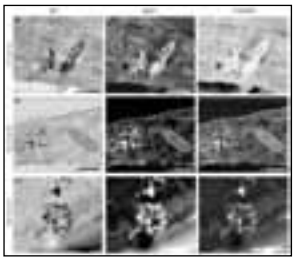

Fig.2: The tanscytosis pattern of MWNTs-NH_3^+ across the PBEC monolayer following the incubation of MWNTs-NH_3^+ with an in vitro BBB model.

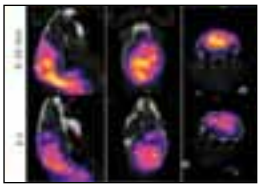

Fig.2: SPECT/CT images confirming uptake of [^{111}In]DTPA-MWNTs in mice brain, after systemic injection.

Whole body live mice imaging (Figure 3), confirmed the ability of f-MWNT to accumulate in brain after i.v. injection. Gamma counting, after whole body saline perfusion, confirmed their superior brain uptake of ~2-5% ID/g, the highest reported so far among nanomaterials.

CONCLUSION

This is the first evidence of MWNT-NH_3+ translocation across the BBB. The significant reduction in the transport of MWNT-NH_3^+ across the BBB at 4 °C confirmed that the uptake was driven by an energy-dependent pathway. Electron micrographs confirmed transcytosis of MWNT-NH_3^+ and its sequence as a function of time.. MWNT-NH_3^+ are able to access mice brain after i.v. injection.

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NEUTRON CAPTURE THERAPY WITH BORON CONTAINING MAGNETIC NANOPARTICLES – TARGETED LOCAL RADIATION ENHANCEMENT

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INTRODUCTION:

Focusing the therapeutic action in the tumor while sparing healthy tissues is always a precondition for a successful tumor therapy concept. Therefore, Boron Neutron Capture Therapy (BNCT) is since a long time under study. Currently a more effective boron delivery agent is highly desirable to perform successful BNCT drafts. A promising strategy to deliver boron into tumor tissues is using magnetically directed nanoparticles. In our previous work on Magnetic Drug Targeting (MDT) we could achieve both: superior enrichment of superparamagnetic iron oxide nanoparticles (SPIONs) in animal tumors of up to 415 ng/mg and a high selective (450-fold) tumor versus blood enrichment of SPIONs. Combining both, the requirement of boron amount in irradiated tissues (20 ppm) and maximum enrichment of SPIONs in tumor tissue after magnetic drug targeting leads to a required boron payload of 4.8%. In our studies, we launched pilot experiments to merge BNCT and MDT concepts investigating the neutron flux behavior and biological outcome by irradiating three-dimensional cell-cultures.

MATERIALS AND METHODS:

Experiments of the neutron beam behavior were performed at the prompt gamma activation analysis (PGAA) facility of the Forschungs-Neutronenquelle Heinz Maier-Leibnitz (FRM II) research reactor in Munich, Germany. The thermal neutron flux equivalent chosen for the measurements was 2.35 x 109 n/cm²s in air. The important feature of this neutron beam was its homogeneity over an area covering the samples completely (ca. 30×30 mm2). This is appropriate to irradiate agarose gel (1.5 %) cubes for evaluating the irradiation of physiological tissues. The neutron flux attenuation was measured in dependence on the depth, concentration of the boron containing layer, co-presence of SPIONs in the boron-layer, co-presence of nitrogen and neutron beam attenuation behind bone material. The results are necessary for neutron flux density determination and dose calculations. Flux density was measured using the gold foil activation method. Au gets activated and enables decay corrected analysis. Magnetic boron containing nanoparticles have been prepared by precipitation and subsequent surface modification using carboranes. A three-dimensional cell-culture system was excogitated and built to determine the biological outcome derived from irradiations. Therefore tumor spheroids have been embedded inside the phantom structure at different positions. They were generated by sowing a suspension of a defined number of cells in an agarose-coated 96-well plate yielding in small tumor spheroids at the deepest point of the agarose-coated area 36-27h later.

RESULTS:

Neutron flux density was hardly affected by boron (100-200 ppm), co-presence of SPIONs (15-300 µg/mg), co-presence of nitrogen in physiological concentrations (18-20 mg/g) or bone material. Derived from the flux attenuation data, dose calculations were done. A 100-fold increased dose of nitrogen containing gels in comparison to pure agarose gels was detected. Boron containment further increases the dose up to 1000-fold. PGAA and ICP-AES (plasma coupled atomic emission spectroscopy) revealed a boron payload of the SPIONs that is considerably above the required valued of 4.8% (m/m). Tumor spheroids mimic tiny metastases and areas of solid tumors, and thus represent a more complex in vivo situation more similar model for a variety of applications in tumor cell research. Flow cytometry analysis of the before irradiated spheroids clearly monitored the biological outcome.

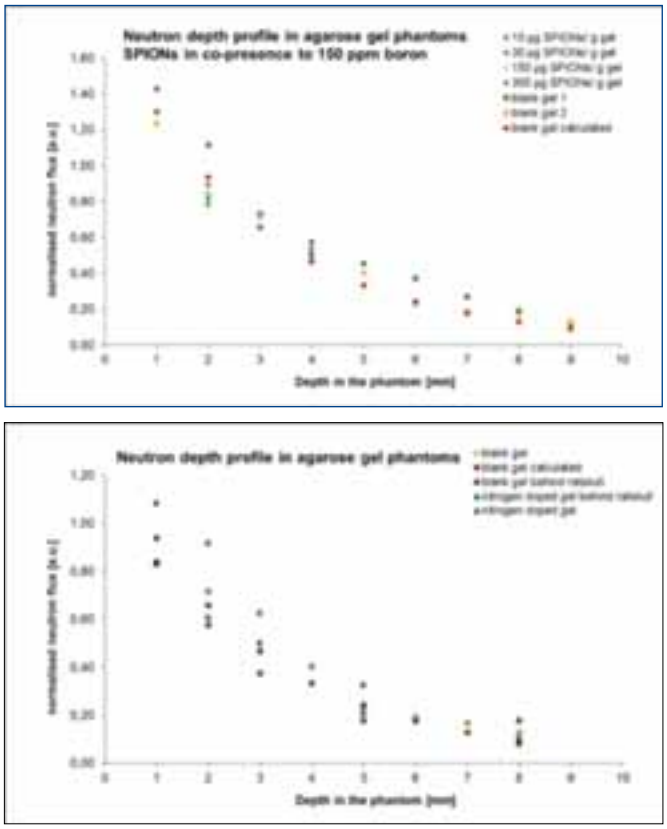

Fig. 1: Neutron depth profile in dependance of various conditions.

CONCLUSION:

Our experimental setting resulted in the attempted concentration of the radiation doses in the targeted area and opens the opportunity for a successful combination of MDT and BNCT. The high linear energy transfer of BNCT in our experiments still prevails which is a precondition for further studies.

MULTI-LAYER NANOCAPSULES AS CARRIERS FOR MULTI-TARGET ONCOLOGICAL NANOTHERAPIES

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CIMUS Research Institute, University of Santiago de Compostela

Most of the work performed so far in the area of nano-oncologicals has been directed to achieve passive targeting of the drug to the tumor tissue. This work has led to the development of a few currently marketed nano-drugs and a greater number under clinical development. However, the benefit of this approach, which has mainly relied on the decrease of the systemic toxicity of the drugs, has been found to be insufficient for the current demands in this field. As a step forward, and within the frame of the LYMPHOTARG-Euronanomed project, we have attempted to target not only the tumoral tissue but also the lymphatics. With this goal in mind we have designed an innovative nano-drug delivery platform consisting of polymeric nanocapsules. These nanocapsules have an oily core, in which we can incorporate hydrophobic drugs, and a shell formed by one or multiple polymer layers, onto which we can associate polynucleotides. The polymers investigated so far for the formation of these layers include polyaminoacids, polysaccharides and polypeptides. In order to assess the value of this technology, we have used docetaxel as a standard model drug. The results obtained with specific formulations administered i.v. to nude mice bearing an orthotopic and metastatic lung cancer model have made clear the possibility to target simultaneously both the tumoral tissue and the lymphatics. In fact, docetaxel concentrations were up to 37 times and 4 times higher than those achieved upon the administration of the same dose of the commercial docetaxel formulation (Taxotere®), in the tumor and the lymphatics respectively. From our knowledge, this is the most significant tumor targeting accumulation reported so far, based on the use of nanocarriers.
The understanding of these very positive results should be put into the context of the knowledge accumulated for this delivery technology. Indeed, these nanocapsules exhibit a number of properties that make them particularly attractive for the targeting of anticancer drugs: (i) they are stable in plasma; (ii) they can control the release of the encapsulated drug; (iii) they are able to reduce the toxicity of the associated drug, docetaxel and (iv) they were shown to increase the efficacy of docetaxel not only in terms of the reduction of the tumor size but also in terms of the elimination of metastatic cells in the lymphatics. Consequently, our work in this field has confirmed the necessity for the anti-cancer delivery nanocarriers to fulfill a number of key properties and to emphasize the potential of lymphatic targeting as a strategy to deal with metastatic cells and, probably, with immune-suppressor cells.
Our work related to the use of polymer nanocapsules for the delivery of oncological drugs has been done in collaboration with a number of laboratories and the authors are very thankful to the following researchers: Raquel Abellan, Ana Cadete, Dolores Torres, Marcos García-Fuentes, Erea Borrajo, Anxo Vidal, Marta Alonso, María de la Fuente, Rafael López, Elena Santidrián, Laura Sánchez, Illaria Marigo and Vicenzo Bronte. The authors are also thankful to the projects LYMPHOTARG (PS09/02670) and NICHE ((ENM-II – JTC20139) of the EuroNanoMed programs, financed by the Carlos III Health Institute, Ministry of Health, Spain.

MACROPHAGE-TARGETED NANO-DELIVERY SYSTEMS FOR ANTI-INFLAMMATORY THERAPY

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Inflammation is a defense mechanism adopted by the body in response to variety of stimuli, including pathogens, injury, and autoimmune responses. Monocytes and macrophages originate from a mononuclear phagocyte system (MPS) in the bone marrow. Tissue-associated macrophages are highly plastic cells that can acquire a distinct functional phenotype (M1 or M2) upon encountering stimuli in their local microenvironment. The classical M1 macrophage phenotype is characterized by upregulation of iNOS, TNFα, IFNγ and other pro-inflammatory cytokines and chemokines, while the alternate M2 anti-inflammatory phenotype is characterized by up-regulation of IL-4, IL-10, TGFβ and arginase expression.
In this presentation, I will describe our approaches to effectively switch the macrophage phenotype from a predominant M1 to M2 polarization state in order to control inflammation in various debilitating disease conditions, such as rheumatoid arthritis (RA) and liver inflammatory diseases. Specifically, we have engineered tuftsin-modified alginate nanoparticles for peritoneal macrophage specific delivery of plasmid DNA expressing murine IL-10 and showed transfection and therapeutic effectiveness in RA model established in Lewis rats. Additionally, we have utilized CD44 targeting hyaluronic acid-poly(ethylene imine) (HA-PEI) nanoparticles encapsulating plasmid DNA expressing IL-10 and IL-4 expressing plasmid for re-polarization of peritoneal macrophages with potential to treat liver inflammatory diseases.
In both of these clinically translatable examples, our focus has been to utilize peritoneal macrophages as Trojan horses with inherent tropism to inflammatory sites in the body for effective repolarization and inflammatory effects. Using alginate and hyaluronic acid – naturally-derived, biocompatible, and biodegradable polymeric materials – we show tremendous potential of this approach to treat debilitating chronic inflammatory diseases.

INTERACTION OF NOVEL ANTIMALARIAL NANO-FORMULATIONS WITH CELLS

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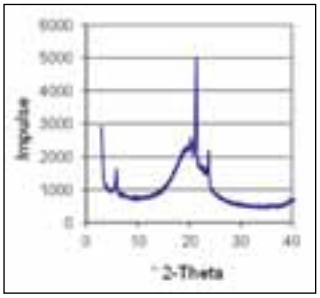
ABSTRACT

Artemisinins, the main stay in the treatment of malaria today are used in combinations with other antimalarials to forestall resistance, as artemisinin-combination therapies. In line with the World Health Organisation's recommendation in that respect, solid lipid nanoparticles (SLN) were formulated to encapsulate two antimalarial drugs- artemether and lumefantrine. The nanoparticles were evaluated for size and solid state properties. The molecular environment of the lipid particles was studied by fluorimetric spectrophotometry using SLN containing a lipophilic fluorescent marker (Coumarin 6). Caco-2 cells were used to investigate the ability of the SLN to interact with the absorptive interface of the GIT. Mice heart endothelial cells were also used as marker cells to assess cellular trafficking with imaging by confocal laser scanning microscopy. Result of this study revealed different crystal properties for artemether and lumefantrine, which affected their solubility in the lipid matrix and thus, loading in the lipid nanoparticles. The particles of the SLN were within the range of 150 nm - 600 nm with varied polydispersity indices. Wide angle X-ray diffraction analysis indicated presence of particles of solid nature (Fig. 1A). Interaction of the nanoparticles with cells indicated Coumarin 6 uptake from the labeled SLN as shown in Fig. 1B.



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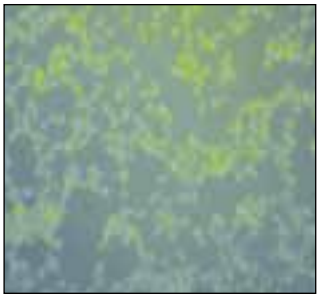
B

Fig. 1. (A): Representative diffractogram of mixture of artemether and lumefantrine (1% of 1:1 ratio) in lipid marix. (B) Fluorescent image of Coumarin 6 uptake from nanoparticles in the presence of cells (MHEC5-T).

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BIODEGRADABLE MAGNETIC NANOCAPSULES OF IXABEPILONE: IN VITRO STUDY

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1. INTRODUCTION

Ixabepilone (IXA) is a microtubule inhibitor belonging to epothilones which has recently been approved for the treatment of metastatic breast cancer. Although it targets microtubules similarly to docetaxel and paclitaxel, ixabepilone has activity in patients that are refractory to taxanes [1]. Ixabepilone pharmacotherapy is associated with serious side effects, which could be alleviated by selective ixabepilone delivery to tumor cells. In this work, novel hybrid (organic-inorganic) magnetic nanocapsules of IXA (MAG-IXA-NCS), based on biodegradable and biocompatible poly(lactide)-poly(ethyleneglycol) (PLA-PEG) copolymers were prepared and evaluated in vitro. Magnetic nanocarriers of anticancer agents represent promising theranostic anticancer formulations as they could combine targetability, imaging, and dual anticancer activity (killing of tumor cells through drug action and magnetic hyperthermia).[2].

2. METHODS

2.1 Synthesis of Polymers: PLA-PEG block copolymers having different PLA/PEG ratios were synthesized as described previously [3]. The copolymers were designated as PLA(X)-PEG(Y), where X, Y stand for the molecular weight (x10-3) of the respective block.

2.2 Preparation and characterization of nanocapsules: PLA-PEG-IXA magnetic nanocapsules were prepared by an interfacial polymer deposition technique. The prepared nanocapsules were characterized with regard to drug loading and encapsulation efficiency by HPLC, size by dynamic light scattering (DLS), ζ-potential by microelectrophoresis, morphology by Transmission Electron Microscopy (TEM) and colloidal stability by monitoring the size and ζ-potential characteristics of the nanocapsules in the presence of increasing NaCl concentrations. The in vitro drug release properties of nanocapsules in phosphate buffered saline (pH 7.4) at 37°C were also investigated with or without the application of an external AC magnetic field.

2.3 Cell studies:The cytotoxicity of blank and ixabepilone-loaded magnetic nanocapsules against A549 human lung cancer cell line was assessed by flow cytometric measurement of cellular fluorescence after staining with propidium iodide (PI). The cellular uptake of the nanocapsules was quantified, as well. For this purpose, the magnetic nanocapsuless were surface loaded with PI.

3. RESULTS

The basic physicochemical characteristics of the prepared PLA-PEG magnetic nanocapsules are shown in Table 1. The average size of nanocapsules depended on the composition of PLA-PEG copolymer and was found to increase from 200 to 440 nm as the molecular weight of the PLA -PEG increased. The ζ-potential of the nanocapsules assumed low negative values. Furthermore, nanocapsules' yield was satisfactory, ranging between 65% and 80%. Drug loading ranged between 0.2%-0.9% for the different nanocapsuules compositions for a theoretical loading of 1.1 %.

TABLE 1.
Physicochemical characteristics of the prepared PLA-PEG magnetic nanocapsules (NCs) of ixabepilone.

Sample	Average size (nm)	PDI	Z-potential (mV)	NCs yield (%)	IXA loading (%)
PLA(10)-PEG(5)	270	0.180	-1.8	69.2	0.2
PLA(20)-PEG(5)	280	0.160	-2.0	64.8	0.9
PLA(40)-PEG(5)	410	0.350	-4.8	77.0	0.6

Morphological examination of nanocapsules was performed with Transmission Electron Microscopy, following negative staining with phosphotungstic acid solution (1%). TEM images indicated that nanocapsules had spherical shape and a rather low size variability (Fig. 1).

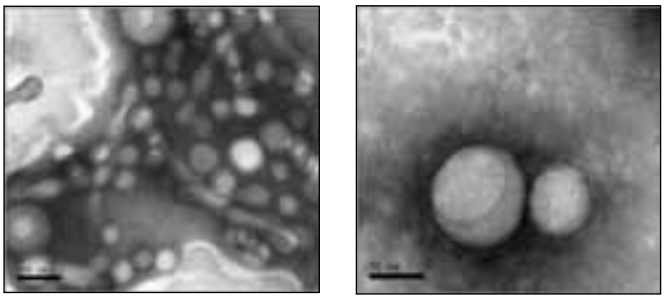

*Fig.1: TEM image for PLA(20)-PEG(5)-IXA nanocapsu*les.

The nanocapsules exhibited satisfactory colloidal stability, as indicated by monitoring size and ζ-potential changes in the presence of increasing NaCl concentrations (Figure 2).

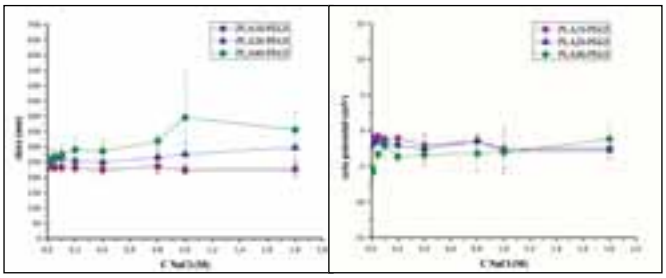

Fig. 2: Hydrodynamic diameter and ζ-potential of nanocapsules in the presence of NaCl (at 25°C).

MAG-IXA-NCs exhibited sustained IXA release in vitro. The more hydrophilic PLA(10)PEG(5) nanocapsules, having the highest PEG proportion in their composition, exhibited faster and higher release compared to the less pegylated nanocapsule compositions. Drug release was accelerated by the application of an AC magnetic field of 19,9 kA/m, 110 KHz (pulses of 10 minutes every 30 min), indicating the magnetic responsiveness of the nanocapsules. The blank nanocapsules did not exhibit cytotoxicity whereas the ixabepilone-loaded nanocapsules exhibited comparable to free drug cytotoxicity against A549 cancer cells.

4. CONCLUSIONS

The PLA-PEG/ixabepilone magnetic nanocapsules exhibit satisfactory physicochemical, colloidal and release characteristics, as well as in vitro anticancer activity, justifying further consideration with regard to their potential application as targetable ixabepilone nanocarriers

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PEER REVIEW, OR NOT PEER REVIEW?

LAJOS P. BALOGH, PhD, Editor-in-Chief, Nanomedicine NBM (Elsevier), Section: Scientific Publishing in Nanomedicine

Acquisition and distribution of information has radically changed in the past decade. Access to scientific information is abundant and publishing is undergoing dynamic changes. Value of a publication may be different for authors and publishers. However, the essential question for science publishers is how to determine the present and future scientific and economic value of research publications and how to monetize this value. Does science include patents and some potential economic value? At what extent should scientific research be practical? Are published results reliable and reproducible? This discussion is now growing and several articles have recently appeared in well-known journals (Science, Nature, The Economist, etc.) questioning the ways of funding research proposals, the use of basic science practices, the validity of scientific results, and the reliability of publications, especially with respect to reproducibility and translation into new products. Getting published is crucial for academicians and researchers. Should the ultimate goal of research be commercial success and products that solve disease specific problems and cure patients? In this talk the speaker will summarize major changes in publishing including the latest business models and methods of manuscript/article evaluations, and demonstrate scientific methods used to determine the value of scientific journals. Examples will be provided to explain how these tools may be used correctly to evaluate publication activities of individual authors, groups, institutions, and countries. In addition to comparing leading nanomedicine journals, a Q&A opportunity will also be provided.

LIPOSOMES' BASED STEROIDAL NANO-DRUG PREVENTS CEREBRAL MALARIA

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Cerebral malaria (CM) is the most severe complication of Plasmodium falciparum infection, and a leading cause of death and long term cognitive damage in children under the age of five in malaria-endemic areas. We report high therapeutic efficacy of a novel formulation of liposome-encapsulated water-soluble glucocorticoid prodrugs, and in particular the novel pro-drug β-methasone hemisuccinate (BMS), for treatment of experimental cerebral malaria (ECM), using the murine Plasmodium berghei ANKA model. BMS is a novel derivative of the potent steroid β-methasone which is an amphiphatic weak acid, and was specially synthesized to enable remote loading into nano-sterically stabilized liposomes (nSSL), to form nSSL-BMS. This novel BMS-nano-drug, composed of nSSL remotely loaded with BMS by trans membrane calcium acetate gradient (Avnir et al 2011), dramatically improves drug efficacy and abolishes the high toxicity seen upon administration of free BMS (Waknine-Grinberg et al 2013). nSSL-BMS reduces ECM rates in a dose-dependent manner and creates a survival time-window, enabling administration of an anti-plasmodial drug, such as artemisone. Administration of artemisone after treatment with the nSSL-BMS results in complete cure of the malaria. Treatment with BMS leads to lower levels of cerebral inflammation, demonstrated by changes in: cell markers cytokines and chemokines levels (in both mRNA and the proteins), as well as diminished hemorrhage and edema, correlating with reduced clinical score. Administration of the liposomal formulation results in accumulation of BMS in the brains of sick mice but not of healthy mice. This steroidal nano-drug effectively eliminates the adverse effects of the cerebral syndrome even when the treatment is started at late stages of disease, in which disruption of the blood-brain barrier has occurred and mice show clear signs of neurological impairment. Overall, combined treatment with nSSL-BMS and artemisone may be an efficacious and well-tolerated therapy for prevention of CM, elimination of parasites, and prevention of long-term cognitive damage.

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UNSOLVED PROBLEMS IN VIRAL INFECTIONS

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Very few medical fields exist where progress has been as impressive as for viral diseases. To name a few examples: Vaccines for Hepatitis B or the human papillomavirus show high-est efficacy and prevent these viral infections associated with the risk of malignancies. AIDS and death in millions of HIV-infected patients can be prevented by highly potent antiretroviral treatment whereas decades ago this was a deadly disease in close to all affected persons. If treatment is started early enough, HIV is now a treatable chronic condition with close to nor-mal life expectancy. Even when treatment is started late HIV-infected patients encounter a good prognosis in 70-90% of cases. Another example is the Hepatitis C virus where newer directly acting antiretroviral agents coming to use in the last couple of years have brought a revolution in the treatment field with cure rates of 90-100%.

Nevertheless, important, partly dramatic, unsolved problems remain for viral infections and ensuing diseases. For acute infections caused by viruses such as Influenza or Ebola, dis-ease severity is high in populations at risk, as in the elderly for influenza, or excessively high, independent of risk factors, for Ebola. For these acute infections, vaccine development is paramount to prevent immediate morbidity and mortality. However, the diversity caused by different viral strains and by mutations impairs vaccine development and efficacy. A further unresolved problem is the fact that vaccine efficacy where most needed, i.e. in very young children and the elderly, is impaired by lower or decreasing immune responses. Chronicity of certain viral infections such as HIV or the Hepatitis B and C virus is another challenge. For HIV, a cure or vaccine, though research has been relaunched, is still far away or even out of reach. For HCV there is no protective immunity, even after clearance of the virus, against a new infection with the identical virus. Hence, the perspective to develop an HCV subunit vac-cine is rather pessimistic. For HIV, reaching protective immunity by vaccines is a major unre-solved problem. Several randomized trials with subunit vaccines taking advantage of the gly-coproteins of HIV have shown no significant efficacy so far. The reasons are not well under-stood but one may speculate that the diversity of the virus, the very high mutation rate, as well as the lack of antibodies are responsible for the lack of efficacy.

An unresolved problem in general for infectious diseases is the difficulty to transfer the partly dramatic advances into action. E.g., still many HIV infected individuals do not know their HIV status and may transmit the virus, attend clinics too late for care, are treated

insufficiently or are lost to follow-up after starting antiretroviral treatments. Breakdowns of health-care sys-tems are associated with outbreaks of infectious diseases as recently observed with the largest ever seen Ebola epidemic leading to vicious cycles. A further unresolved problem lies within key populations at risk and/or transmitting viral diseases not reached. Finally, a lack of political will to build up structured health care systems may be observed in different regions of the world.
In a world with a growing population density, in particular in larger towns and megacities, viral infections may cause paramount problems to individuals but also the society at large. To tackle these unresolved problems basic, clinical, epidemiological and public health science as well as intensive political support will be needed to not only react to uprising outbreaks but also to prevent such epi- and pandemics.

INNOVATIVE TECHNOLOGIES FOR THERANOSTIC BRAIN INTERFACES

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Deciphering and modulating brain function in neurological diseases is a mandatory objective in battle against neurodegenerative, pscychiatric and cancer neuropathologies.
Finding biomarkers and relevant targets for an innovative theranostic management of incurable brain diseases is a major biomedical need. In oncology, direct access to fresh tissues has paved the way for targeted therapies. Neurodegenerative brain remains inaccessible, only post-mortem brain being available with inherent biases due to postmortem processes. Neurostimulation provides a unique non-lesional access to the neurodegenerative brain. Exploiting this, we developed a fingerprinting strategy to capture molecular informations from brain cells and local microenvironment in contact with the DBS stylet. A specific kit was developed that, as well as the connected clinical validation. A second-generation device was developed involving a high innovative strategy using a micro-chemo-silicon chip. . This was recently validated in a pilot phase trial in clinatec translational technology center. Moreover, the last third generation strategy was developed using nanoporous silicon. Local mechanistic deciphering involves classical molecular profiling from genomic to proteomic but also new “physical” annotations of the living also recently possible thanks to nanotechnologies such as Atomic force microscopic investigations. Implementing local therapies is a strong connected objective benefiting from the molecular and physical annotations integrating local tissue pathology heterogeneity.
Again micro-nano-technologies provide unique miniaturized devices from electrophysiological recording for brain-computer-interfaces (BCI) for example for tetraplegia or aphasic patients. The chronicle stability of these devices is a major bottleneck; nanocoating using carbon nanotubes or nano-diamond is a validated alternative to provide safe long-term implant for BCI. Functional validation was done in big animal model. Similarly, we developed innovative strategies to inject locally nano-devices modulating brain microenvironment for brain tumors or neurodegenerative diseases.

SMALL AND BIOCOMPATIBLE COATINGS FOR IRON OXIDE-BASED NANOPARTICLES

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Superparamagnetic iron oxide nanoparticles (SPIONs) have been widely studied for medical applications, especially as contrast agents for magnetic resonance imaging (MRI) and as a source of local heating (hyperthermia) for tumor treatment. As their in vivo performance strongly depends on their stability in aqueous solutions, we developed biocompatible coatings to prevent their aggregation in unbuffered water. For this purpose, SPIONs were until now typically coated with high molecular weight molecules, like sugars (e.g. dextran) or polymers, but these coatings have been shown to make SPIONs accumulate in the liver and the spleen. Moreover, the use of these large coating molecules prevents the loading of large numbers of SPIONs into small volumes, which is important to promote efficient hyperthermia treatment of small metastases in, for instance, lymph nodes. Therefore, the coatings that we present are based on very small molecules, aiming at loading as many SPIONs as possible in small metastases (< 5 mm), in order to specifically detect them and treat them efficiently by hyperthermia. 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 capability of SPIONs to be specifically and exclusively located in the targeted tumor tissue is challenging but highly needed for both the detection and treatment of tumors. Unfortunately, the lack of specific targeting towards the diseased tissue has often been the cause of false negatives in MR images (tumors which appear as healthy spots). Furthermore, as the essence of hyperthermia therapy is to destroy the tissue by the heat generated around SPIONs, the latter must be located only in the targeted tumor. As a consequence, the need for specific targeting is absolutely essential for hyperthermia applications to avoid serious secondary damage of healthy body tissues.
In order to fulfil the prerequisite for clinical applications, we propose 11 different coating strategies in aqueous solution without the use of organic solvents and toxic molecules as aid to the coating processing. The biocompatible coating molecules were chosen according to the presence of chemical groups with high affinity towards the iron oxide surface. The affinity of the coating molecules for iron oxide was determined by isothermal titration calorimetry (ITC), which showed very different kinetic behaviours between coating molecules. The presence of the coating molecules was proved by Fourier transform infrared spectroscopy (FTIR) and quantified by ultraviolet-visible spectroscopy (UV-vis). The coated-SPIONs were also examined with Cs-corrected, monochromated high-resolution transmission electron microscopy (TEM) using a FEI Titan Themis at 80 kV high tension, which revealed that the coating molecules were laying in 2 to 3 layers longitudinally to the SPIONs, contradicting most of the schematics found in literature (fig. 1). To estimate the size of the coated-SPIONs, their primary particle sizes and hydrodynamic diameters were determined by various methods: TEM, dynamic light scattering (DLS) and differential centrifugal sedimentation. As the biological environment responds very differently in contact with SPIONs of different surface charges1, the zeta potential of the coated-SPIONs was determined by DLS to predict their in vivo behaviour. In addition, qualitative and quantitative analysis of the chemical groups available for further functionalization of the SPIONs with targeting ligands was done by X-ray photoelectron spectroscopy (XPS). Besides the physico-chemical characterization of the coated-SPIONs, we investigated their in vitro behaviour as a function of coating and cell line. We especially focused on the toxicity and cellular up-take of coated-SPIONs by different cell types (primary versus metastatic cells).

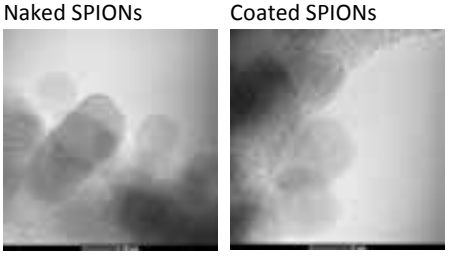

Fig. 1: High-resolution transmission electron microscopy micrographs showing the iron oxide surface of naked SPIONs versus

SPIONs coated with one of the coating molecules. The coating molecules arrange longitudinally around the surface of SPIONs in several layers.

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LESS IS MORE? NANOSCALE SYSTEMS FOR THE DELIVERY OF A TUBERCULOSIS DNA VACCINE

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Plasmid DNA (pDNA), encoding the Mtb antigen 85A, was adsorbed on the outer surface of cationic nanoparticles. These nanoparticles were made of trimethyl chitosan (TMC), a modified biopolymer having proinflammatory features, to deliver pDNA and to induce predominantly Th1 responses. To further enhance these responses, muramyl dipeptide (MDP), an innate immune receptor ligand, was encapsulated into the pDNA-nanoparticle formulation. The formulations were administered in C57BL/6 mice by intramuscular administration. IFN-γ release from spleen cells was detected via ELISPOT and serum IgG isotypes via endpoint ELISA.
Mice immunized with the pDNA-nanoparticle formulations induced a 1.7-fold higher secretion of antigen-specific IFN-γ from lymphocytes then mice having received the naked pDNA only. The incorporation of MDP provoked an additional 1.5-fold IFN-γ increase compared to pDNA nanoparticles without MDP, being significantly more effective than non-adjuvanted pDNA. The clear polarization towards a Th1 response by pDNA-nanoparticle formulations was indicated by detection of serum IgG2c/IgG1 titers above 1, whereas the naked pDNA induced Th2 biased IgG responses.
These results demonstrate that application of TMC nanoparticles in a DNA vaccine formulation are able to influence the nature of immune responses, and can substantially be increased by incorporation of MDP. This may provide an effective strategy to improve the prophylactic and therapeutic efficacy of DNA vaccines and may render TMC nanoparticles a strong candidate for the formulation of genetic vaccines against tuberculosis.

PEPTIDE-FUNCTIONALIZED NANOPARTICLES AS DUAL-TARGETING DRUG DELIVERY SYSTEM FOR BRAIN TUMORS THERAPY

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INTRODUCTION

Nanoparticles (NPs) have several advantages as delivery vehicles that make them useful for cancer therapy. Their small size, allows them to overcome some biological barriers, access tumor tissue through porous vasculature [1,2], and achieve cellular uptake [3]. However, in the case of brain tumors such as glioblastoma multiforme (GBM), a careful engineering of polymeric nanoparticles including functionalization with targeting ligands is needed to provide the NPs with the ability to cross the blood brain barrier (BBB), the most restrictive physiological barrier in the body, and also to promote receptor mediated uptake into cancer cells.
In addition to NPs' surface engineering to increase blood circulation and influence biodistribution [4], in recent years different investigations about targeting ligands attached to the surface and their effect on the uptake by target tissue have been carried out [5]. Encapsulation of chemotherapeutic drugs inside targeted NPs can further increase the therapeutic index by delivering an elevated dose directly to a tumor while limiting systemic toxicity [6].
The aim of our project is to develop a dual-targeting drug delivery system for the chemotherapeutic drug paclitaxel (PTX) by functionalizing novel polyester-based NPs with peptides possessing special affinity for the low-density lipoprotein receptor-related protein 1 (LRP-1). This receptor is overexpressed both in glioma cells and in the endothelial cells of the BBB, allowing the decorated NPs to overcome the limitations of current chemotherapy strategies to shuttle drugs from blood to brain, and then target glioma cells.
The present work focuses on the preparation of these NPs and the study of their therapeutic efficacy both in vitro and in an in vivo glioblastoma model.

RESULTS

Peptide-functionalized PTX-loaded NPs were obtained by a nanoprecipitation process. In particular, the organic phase containing two preformed block co-polymers, P and 2P, was co-precipitated with PTX into the aqueous phase to form NPs. The suspension was purified and concentrated by tangential flow filtration (TFF) to obtain the working samples. Nanoparticles were characterized by dynamic light scattering (DLS) and particles sizes around 100-120 nm (PDI < 0.1) and negative surface charge were obtained. The amount of the BBB penetrating peptide (BPP) in the nanoparticles and their drug content were determined by amino acids analysis and PTX quantification using a UPLC, respectively.
Previous results obtained in our group, and already presented at the 2014 CLINAM meeting, demonstrated that the brain penetrating peptide (BPP) used to functionalize our NPs did interact with the LRP-1 receptor and that the BPP-decorated NPs were able to cross the BBB in an in vitro model.
In order to assess in vivo the brain penetration capacity of our BPP-decorated PTX-loaded nanoparticles in comparison to free paclitaxel, we performed an in situ brain perfusion study.
After the administration of PTX (in solution or encapsulated) through the carotid artery, rats were decapitated and capillary depletion took place in order to separate brain parenchyma from brain capillaries. PTX levels were detected by UPLC analysis and two important pharmacokinetic parameters from both free PTX and BPP-decorated PTX-loaded NPs were determined: brain uptake and the influx rate constant (Kin).
Interestingly, statistically significant differences were observed when these two parameters were compared reaching 20 and 19-fold increase values for the brain uptake and the Kin, respectively when PTX was encapsulated (figure 1).

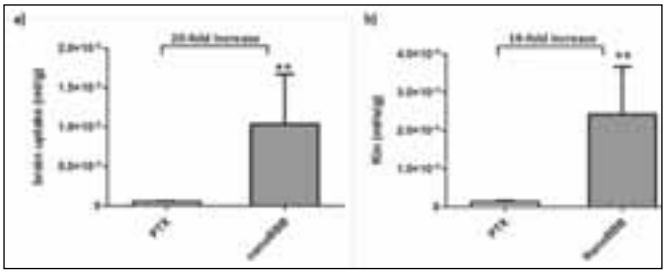

Fig. 1: Brain Uptake and Kin obtained after the perfusion of PTX in solution or encapsulated in BPP-decorated NPs at 1mg/animal (n=4)

Next, in order to determine the cytotoxic effect of the BPP-decorated PTX-loaded NPs, and to compare it with the anti-tumor efficacy of the non-encapsulated drug, we performed a kinetic study where U87MG cells were incubated with the same dose of PTX (20nM) for different time periods: 1, 4, 6 and 8 days. In particular, cells were also incubated with PTX-loaded non-decorated NPs and empty decorated-NPs as controls. Then, a MTS colorimetric assay was performed in order to assess cell viability obtained in each condition.
BPP-decorated PTX-loaded nanoparticles had a cytotoxic effect similar to free PTX. Furthermore, non-decorated NPs and empty decorated NPs did not induce significant levels of cell death, indicating that peptide decoration enhances NPs' therapeutic effect, and that NPs were not toxic per se.

Finally, in order to assess the therapeutic effect of BPP-decorated PTX-loaded NPs, we performed an efficacy in vivo study in a human glioblastoma murine model. In particular, 6-week old SCID mice were stereotactically inoculated with 1x105 Pluc-G-U87 cells. One week post implantation, Bioluminiscence images (BLI) were taken to monitor tumor development and animals were randomly divided into two groups: control and NPs-treated (n= 10 each group). Next day, treatments were initiated (t= 0) and weekly BLI was performed to follow tumor growth during the experiment.
As shown in figure 2, the administration of BPP-decorated PTX-loaded NPs resulted in a significant delay of the tumor growth compared to the control group, treated with the NPs vehicle.

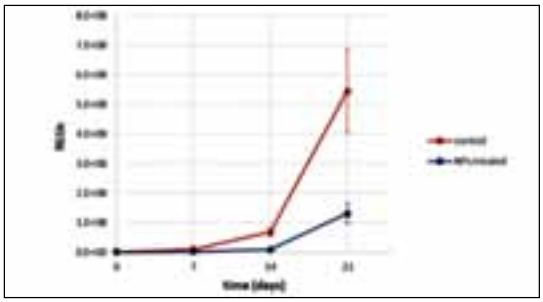

Fig. 2: In vivo changes in light production by U87 tumors expressing Pluc, resulting from the intravenous administration of PTX-loaded nanoparticles (NPs treated group n=10) or the vehicle (control group n=10).

In addition to the delayed tumor growth observed in the treated mice group, BPP-decorated PTX-loaded NPs also resulted in a significant increase in animal survival.
As shown in figure 3, the increase in life span (ILS) obtained with the therapeutic treatment was 25%, with a median time survival (MTS) of 30 days for the treated animals and 24 days for the control mice.

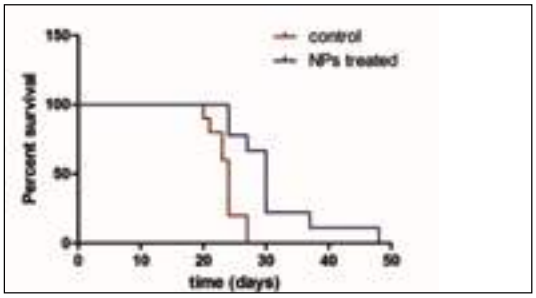

Fig. 3: Kaplan-Meyer graph summarizing mice survival. Median survival, treated mice, 30 d; control mice, 24 d (p= 0.002).

CONCLUSIONS

Our results demonstrate the efficacy of the therapeutic strategy based on the use of BPP-decorated PTX-loaded nanoparticles in a human glioblastoma murine model. We observed that, when compared to the control group, this treatment resulted not only in a significant delay of the tumor growth, but also in a significant increase in animal survival.

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PLAYING GAMES TO HELP PEOPLE UNDERSTAND NANOMEDICINE

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The leading cause of mortality in the Western world is atherosclerotic plaque disruption with superimposed thrombosis, leading to heart attacks and strokes. The EC FP7 project NanoAthero aims to demonstrate the preliminary clinical feasibility of the use of nanosystems to achieve a). targeted imaging to show key processes and early indications of adverse conditions in a patient, and b). targeted delivery and improved efficacy of drugs for plaque and stroke treatments. The potential for treating patients is clear but the use of unfamiliar nanotechnologies might also prove a barrier for some. Past experience suggests that to engage with patients can help in designing trials and in the successful of novel treatments. How then do we get people thinking constructively and creatively about this emerging area?
A Democs card game is being created in the NanoAthero project to explore a range of nanomedical issues, and we offer you the opportunity to play a shortened pilot version of the game during the Clinam conference. Democs is a group discussion, for 6-8 people, using cards as the source of information and as the stimulus for reflection and debate. The game takes people through basic factual information, and some of the ethical and social issues, posing questions, giving different viewpoints, using case studies to illustrate dilemmas. It invites people to come to their own conclusions, and also vote on potential policies or applications. It's free and suitable for 16 years and upwards, it assumes no prior knowledge of the subject, and can be played anywhere where you have a table, some chairs, some friends, and some coffee. For 10 years the Democs card game, originally devised by Perry Walker of the New Economics Foundation, has offered a unique and effective way of enabling general publics to explore novel issues like these for themselves, in their own social settings. It is now in use in many countries and languages on topics as diverse as cloning and climate change.
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NEGLECTED DISEASES, CURRENT STATUS AND FUTURE NEEDS

RETO BRUN

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), the Medicines for Malaria Venture (MMV) or the Drugs for Neglected Diseases initiative (DNDi) 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. Hundreds of patients were treated and cured so far. The second one is the oxaborole SCYX-7158, another oral drug with excellent pharmacokinetic properties. It passed safety in humans and will be tested in patients soon.
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. Elimination of sleeping sickness seems realistic with the number of patients at a very low level (<8000/year) and two new oral drugs in the development pipeline. 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.

COMPUTER-AIDED DESIGN OF LIPOSOMAL DRUGS: IN SILICO PREDICTION AND EXPERIMENTAL VALIDATION OF DRUG CANDIDATES

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Liposomes are the most extensively clinically used - drug delivery system. Since the FDA approval of the first nano-drug Doxil® [1] more than 12 other liposomal drugs were approved by the FDA and more liposomal drugs are under development. Doxil is based on the combination of high and stable drug loading which is also responsible for the controlled drug release as well as on the use of nano-pegylated liposomes. Pegylated nano-liposomes are important for treating cancers, neurodegenerative and inflammatory disorders, as they take advantage of the enhanced permeability and retention (EPR) effect and deliver drugs to the site of disease. Development of liposomal formulations is a time consuming process which requires major efforts. A more rational and less labor intense process is taking advantage of computational modeling approaches capable of predicting whether an active pharmaceutical ingredient (API) could be loaded to and delivered by liposomes.
Towards that end, Quantitative Structure– Property Relationship (QSPR) models were developed with Iterative Stochastic Elimination[2] and k-Nearest Neighbors[3] approaches to predict drug loading efficiency (high vs. low) in liposomes. Chemical as well as formulation descriptors were employed and the resulting statistically validated models[4] were used to screen a few thousand biologically active molecules from the Comprehensive Medicinal Chemistry database. Three drugs were selected for experimental testing of their loading into nano-liposomes, also taking into account challenges of nano-liposomal development. Two of the selected drugs were high- and one was low-loading, confirming the predictions. Ten other negative molecules from literature were also confirmed, to a total prediction accuracy of 92%[5]. Figure 1 presents the screening results of CMC database by the two computational approaches (ISE and kNN)
One of the tested drugs- mupirocin, was remotely loaded into pegylated nano-liposomes, and stabilized by intraliposomal hydroxypropyl-β-cyclodextrin to form Nano-mupirocin[6] which was evaluated in vivo for its therapeutic efficacy. Mupirocin, an antibiotic with a unique mode of action is currently restricted to topical administration due to its rapid degradation in the blood. Intravenous administration of Nano-mupiricin to mice in necrotizing fasciitis model showed significant superiority of Nano-mupirocin over Mupirocin. Our approach demonstrates the utility of QSPR models in screening API libraries for identifying candidates that should benefit from being administered as nano-drugs.

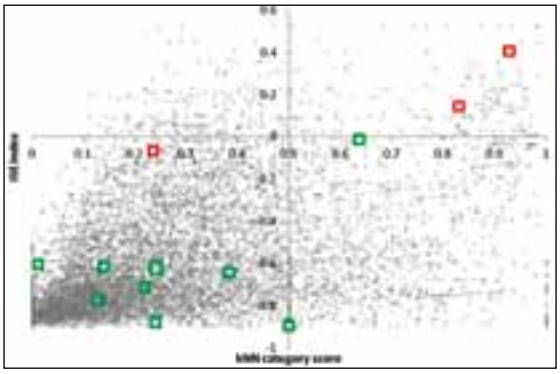

Fig. 1: The relationship between ISE index and kNN category score. Hits identified by both models as positives are found in the upper right quadrant. Negative hits are found in the lower left quadrant. Red squares are molecules tested in this study and green squares are molecules found in the literature; all these molecules comprised an additional external validation set to test our models.

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BROAD-SPECTRUM VIROCIDAL PEPTIDES: A NOVEL APPROACH TO HEPATITIS C VIRUS THERAPY

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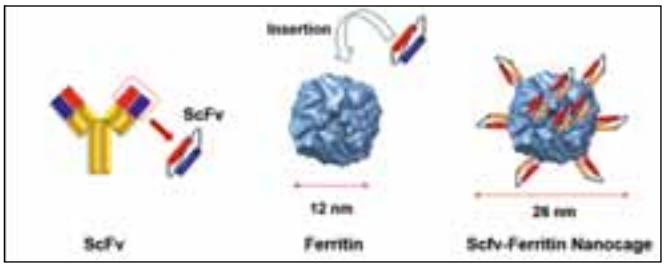
Over 160 million people worldwide are infected with hepatitis C virus (HCV) and it is a leading cause of liver transplantation worldwide. Current therapies are largely ineffective for many patients, and there is no available vaccine. These issues emphasize the importance of developing new classes of antiviral drugs. To address this challenge, we have discovered an amphipathic, α-helical (AH) peptide that represents a breakthrough, broad-spectrum antiviral drug candidate. It ruptures the lipid membrane surrounding enveloped virus particles in a membrane curvature-dependent manner. The size range of virus particles susceptible to treatment with AH peptide encompasses a wide range of deadly viral pathogens including dengue, HCV and HIV. Unlike antibacterial peptides which exhibit high selectivity based on membrane surface charge, this antiviral peptide demonstrates membrane curvature sensing, with preferential targeting of highly curved membranes. Importantly, the mechanism of action of AH peptide confers a high barrier to resistance developing among enveloped virus populations. Recent evidence supports that AH peptide interacts with lipid membranes to induce pore formation and membrane destabilization. The preference to selectively rupture lipid vesicles and virus particles of small size appears to be related to membrane strain-dependent pore formation. Various factors including the cholesterol fraction and envelope proteins stabilize curved membranes, in turn requiring higher peptide concentrations for membrane-activity. We have further demonstrated that AH peptide exerts potent antiviral activity against a wide range of enveloped viruses. In vivo experiments are ongoing in order to determine if AH peptide can reduce viral titer in the bloodstream. Taken together, these results lay the groundwork to explore membrane-active peptides as a new class of direct-acting antivirals for HCV therapy.

FUNCTIONALIZED PROTEIN NANOCAGES AS A PLATFORM OF TARGETED THERAPY AND IMMUNODETECTION

SANG J. CHUNG, Hyo Jin Kang, Geon Go

nm-sized PEG-coated AuNPs (AuNPPEG) as a core NP and carboxyl or amine groups were conjugated to AuNPPEG to generate negative (AuNPCOOH) or positive AuNP (AuNPNH2), respectively. Each type of AuNP was intravenously injected into mice at a dose of 1 mg/kg body weight and the concentration of Au was measured in different organs by ICP-MS. The levels of AuNPCOOH and AuNPNH2 in the blood were minimal even at 30 min post-injection whilst AuNPPEG showed high levels at 30 min and 4 h post-injection. The organ distribution also showed the higher deposition rate depending on their functional groups: AuNPPEG for mesenteric lymph node, kidney, brain, and testis; AuNPCOOH for liver; AuNPNH2 for spleen, lung, and heart. All AuNPs had a low elimination rate but the major route of elimination was different depending on their functional groups: AuNPCOOH for bile; AuNPPEG and AuNPNH2 for urine. In conclusion, both size and surface charge of AuNPs produce differences in blood kinetics, organ distribution, and elimination pattern which can be important information for directing NPs to specific organs or improving the kinetic properties.



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LONGITUDINAL BIODISTRIBUTION STUDIES OF INHALED NANOMEDICINES USING SPECT/CT IMAGING

LEA ANN DAILEY

The development of clinically acceptable nanoparticle formulations for use in pulmonary drug delivery has been hindered by concerns about the toxicity of nanomaterials in the lung combined with a lack of information on nanoparticle clearance kinetics and biodistribution. In this study, the biodistribution and clearance profiles of 111In labelled albumin nanoparticles (~200 nm) and pegylated lipid nanocapsules (~50 nm) were assessed over 48 h following a single administration to the mouse lung via oral aspiration. Biodistribution was assessed both by single photon emission computed tomography and X-ray computed tomography imaging and terminal organ harvest studies. 111In labelled albumin nanoparticles were cleared in a two-phase process with 64.1 ± 8.5% of the original dose remaining in the lung at t= 48 h. Over the 48 h period, the majority of the lung dose partitioned from the lung lining fluid into the lung tissue, with ~6% found in alveolar macrophages (cellular clearance), ~6% in the liver (indicative of systemic translocation) and ~6% in the intestines (indicative of mucociliary clearance) at 48 h. In contrast, pegylated lipid nanocapsules, were cleared rapidly from the lungs fitting a first order kinetic model with only 7.1 ± 5.7% of the original dose remaining in the lung at t= 48 h. Over the first 24 h period, the majority of the lung dose partitioned from the lung lining fluid into the out of the lung, with very low fractions (<1%) found in alveolar macrophages (cellular clearance), ~ 10% in the liver (indicative of systemic translocation) and no signal in the intestines (indicative of a lack of mucociliary clearance at later time points). This study provides important longitudinal information on the general behaviour of two different nanoparticle vehicles in the lung, which may be used to direct future formulation design of inhaled nanomedicines.

USING A QUANTITATIVE METRIC FOR HYDROPHOBICITY AS A SELECTION CRITERIA FOR NANOMEDICINE BIOCOMPATIBILITY

It is often postulated that nanomaterial surface hydrophobicity plays an important role nanomedicine biocompatibility and bio-nano interactions, yet quantitative studies relating nanoparticle hydrophobicity to particle behaviour in biological systems have been lacking. In this study, hydrophobic interaction chromatography (HIC) was used to assess a panel of nanoparticles spanning several biomaterial types and a purpose-developed hydrophobicity scale, the HIC index, ranging from 0.00 (hydrophilic) to 1.00 (hydrophobic), was used to generate a quantitative metric for surface hydrophobicity. This enabled the relationship between the nanomaterial HIC index value and acute lung inflammation after pulmonary administration to mice to be investigated. The nanomaterials with low HIC index values (between 0.50–0.64) elicited little or no lung inflammation, whereas a dose-dependent acute inflammatory response was observed for nanoparticles with high HIC index values (0.88–0.96). In a separate study, the HIC method was also used to characterize nanoformulation stability and protein corona formation. Polymeric nanoparticles with highly hydrophobic cores were formulated with a coating of different pegylated surfactants and exposed to serum-supplemented biomimetic fluids. HIC index values were determined before and after incubation with serum. Surfactant coatings with a low affinity to the nanoparticle core exhibited high HIC index values (~0.96), while high affinity coatings exhibited low HIC index values (~0.30). Protein corona formation altered the HIC index of different systems dependent on the stability of the surfactant coating and was useful in characterising changes to the nanoparticle surface in the presence of biomolecules. In summary, the HIC index provides a versatile, discriminatory, and widely available quantitative measure of nanoparticle surface hydrophobicity.

HOW ARE ANISAMIDE-DECORATED COLLOIDS TAKEN UP BY TUMORAL CELLS?

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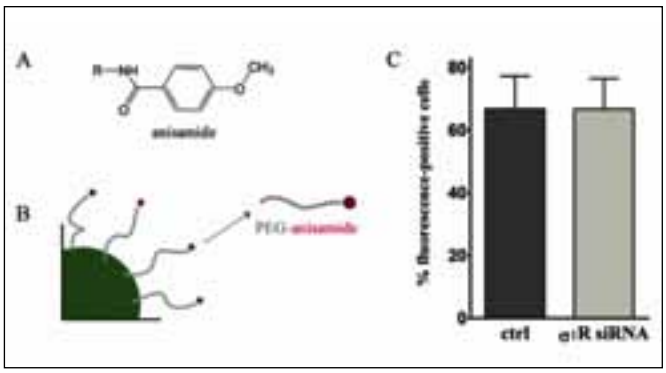
Strategies aimed at increasing the efficacy of tumor-directed nanocarriers for therapy or imaging include the discovery of targeting ligands with high specificity and affinity to tumor tissues. Among the existing tumor-targeting moieties, proteins and peptides have a leading role, fulfilling to a high extent these criteria. Small organic molecules, however, such as folic acid, present several advantages, including lower risk of immunogenicity and ease of incorporation on the nanoparticle surface by simple chemistry. A small organic molecule with promising tumor-targeting properties when grafted to nanocarriers is anisamide (Figure 1, A). Its use as a tumor-selective moiety was inspired by the larger iodinated benzamides[1], comprising the p-methoxy benzamide in their structure, previously studied as tumor imaging agents via the recognition of the Sigma-1 receptor (σ_1R). Following an initial publication reporting the grafting of anisamide onto the surface of liposomes and subsequent enhanced uptake by tumor cells[2], this ligand has been used for the decoration of numerous types of colloidal systems and has shown tumor-targeting potential in vitro as well as in vivo. In all these reports, the suggested cell component to interact with anisamide is the σ_1R, based on the binding of the larger iodobenzamides. However, to date no thorough characterization of the interaction between the receptor and the molecule has been performed. The

goal of this project is to study if and how anisamide interacts with its putative cell target, the σ_1R.
Fluorescent non-biodegradable particles of 1 µm-diameter were used as the main tool of the study. The particles were PEGylated and decorated with anisamide (Figure 1, B). The ligand-functionalized particles showed enhanced uptake by B16F10 murine melanoma cells compared to control cells (macrophages). σ_1R silencing using siRNA did not influence the targeted-particle uptake (Figure 1, C), questioning the involvement of the receptor in the uptake process. Competition experiments with a σ_1R ligand also did not affect the particle uptake (data not shown), confirming the lack of involvement of σ_1R in the uptake of anisamide-coated colloids. Investigation of the targeted-particle uptake process will follow to explore the mechanism of endocytosis triggered by the presence of anisamide.
This project is financially supported by Krebsliga Schweiz (KFS-2821-08-2011).



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GOLD NANO PARTICLES IN DIABETES IMMUNOTHERAPY

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Type 1 diabetes (T1D) is an antigen specific autoimmune disease in which the insulin producing beta cells of the pancreas are destroyed resulting in a life-long absolute dependence on insulin injections for survival. In 50% of cases the onset is in childhood, and the incidence of cases under the age of 5 is increasing worldwide. Despite modern insulin regimes, less than 20% of those affected can achieve target glucose control and hence patients are exposed to the long-term risks of retinopathy, amputation, neuropathy and renal failure as well as premature coronary artery disease and episodes of disabling hypoglycaemia. Over 60,000 people in the UK are affected with more than 5000 new cases annually. Currently no long-term immunotherapeutic options exist that have an acceptable safety profile. The optimal approach would be antigen specific immunotherapy (ASI), in which tolerance is specifically restored to the antigens targeted in the autoimmune process. T1D is ideally placed for this as many of the target antigens and epitopes are known (proinsulin, GAD, IA-2, IGRP, ZnT8, Chromogranin) and individuals at risk of disease can be reliably identified many years prior to clinical presentation by the use of islet specific antibody testing. ASI has been developed in animal models of T1D, but not in a form that can be effectively translated into humans. Here we present data on complexing peptide antigen relevant to T1D to small gold nanoparticles. We show that the particles deliver antigen efficiently to the immune system without inducing inflammation and hence have multiple properties that are relevant to inducing tolerance to self-antigens.

DIABETES IMMUNOTHERAPY – IN VIVO DATA

Colin M Dayan, Cardiff University – on behalf of the EE-ASI Consortium (www.ee-asi.eu)

The EE-ASI consortium is seeking to develop novel ways to reverse the autoimmine process in type 1 diabetes (T1D). We have developed a system that couples an antigenic peptide relevant to T1D to small gold nanoparticles and delivers these intradermally via microneedles to promote immune tolerance. In vitro these particles deliver antigen to the immune system without inducing inflammation. We have used ex-vivo human skin organ culture as well as preclinical models to explore the effects and fate of these particles in vivo. We have observed that the particles spread widely in the dermal compartment and appear to be transported to the draining lymph nodes predominantly via migratory antigen presenting cells. The antigen and other components attached to the particle can also be detected in distant lymph nodes and the spleen, suggesting efficient delivery to the immune system. Early evidence suggests that this may be a favourable mechanism of antigen delivery to promote immune tolerance in T1D.

HOW PUBLIC PRIVATE PARTNERSHIPS DRIVE INNOVATION IN DRUG RESEARCH AND DEVELOPMENT

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The declining productivity of drug research and development is of major concern for private and public stakeholders in the pharmaceutical industry and in health care more broadly. One strategy to tackle this challenge that has gained momentum in recent years is the establishment of precompetitive public–private partnerships (PPPs) to focus on issues that are too large for single organizations to effectively address alone[1)]. Examples of such partnerships include the Innovative Medicines Initiative in the European Union, the Biomarkers Consortium in the United States and Top Institute Pharma in the Netherlands.
Less than a decade ago, partnership between academic organisations, major industry players and SMEs was rare, and when they did occur, they were usually within isolated silos of activity. The FES-funded (Dutch natural gas reserve fund) research portfolio triggered the creation of the Top Institute Pharma (TI Pharma). Following an invitation from large and small industry players and leading academic organisations, the Dutch government stepped in to encourage partnerships that could improve pharmaceutical research. This followed up on a report on priority medicines, commissioned by the government of the Netherlands during its EU presidency in 2004 and written by the WHO. No effective structure had previously existed to bring public and private organisations together, and the WHO report gave a clear strategic definition of public health priorities for medicine research – in particular for some high-impact topics that were not being addressed.
Since the first FES project started in 2007, scientific, financial and educational outcomes have exceeded all expectations[2)]. They include direct impacts on patient health and on the economy. There have been notable scientific and even clinical successes. Positive results have emerged for many topics defined in the seminal 2004 WHO report 'Priority Medicines for Europe and the World', and awareness of those topics has grown dramatically. A highly skilled research cohort has been nurtured and given new, broader skills, and there is a strong and unprecedented atmosphere of collaboration.
This presentation provides background information on public private partnerships, discusses the key learnings from running a unique collaboration of 96 partners, including 26 knowledge institutes, 21 large and 43 smaller industrial players, as well as organizations such as the Dutch Medicines Evaluation Board and patient organizations. It considers the impact of such a programme on scientific know-how; on progress with priority medicines; on human capital development; and on the growth of collaboration within new networks. It also looks at what the future holds for collaborative research.

[1)]Denee TR, Sneekes A, Stolk P, Juliens A, Raaijmakers JA, Goldman M, Crommelin DJ, Janssen JW.

Nat Rev Drug Discov. 2012 Mar 30;11(5):419. doi: 10.1038/nrd3078-c1 “Measuring the value of public-private partnerships in the pharmaceutical sciences.”
[2)]TI Pharma Report; New Track to Medicines, 2014 https://www.tipharma.com/fileadmin/user_upload/Afbeeldingen/News/TI_Pharma_Report_DIGITAL.pdf

ABRAXANE IN NEODJUVANT TREATMENT OF EARLY STAGE BREAST CANCER

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nab-Paclitaxel (ABRAXANE®) is an albumin-bound nanoparticle form of paclitaxel. Albumin is a key plasmic carrier of hydrophobic molecules, such as paclitaxel, and is highly accumulated in tumors, which may play a key role in the more efficient accumulation of nab-paclitaxel in tumor tissues versus organs. To date, there have been 4 large completed global phase 3 trials with nab-paclitaxel in patients with metastatic disease and all 4 trials successfully met their endpoints. As a result, nab-paclitaxel was approved for the treatment of metastatic breast cancer (MBC) in over 40 other countries globally, and more recently, it was approved in the US for the first-line treatment of locally advanced or metastatic non small-cell lung cancer (NSCLC), in combination with carboplatin and in US and Europe for the first-line treatment of metastatic pancreatic ductal adenocarcinoma (mPDA), in combination with gemcitabine. The recently completed phase 3 trial in chemotherapy-naïve patients with metastatic melanoma has also met its primary endpoint of progression-free survival (PFS), produced an improved trend in overall survival (OS), and an improved safety profile versus standard dacarbazine therapy (Hersh, SMR 2012).
Recently, a new large randomized phase 3 study conducted by Untch et al. (SABCS 2014) in early stage breast cancer with neoadjuvant treatment was completed in 1204 patients. The goal of this study was to compare the pathologic complete response (pCR) following combination chemotherapy (epirubicin+cyclophosphamide) with either conventional paclitaxel or nab-paclitaxel. In addition, patients that were Her 2+ were given trastuzumab and pertuzumab. The primary endpoint of pCR was achieved for 38% of patients receiving nab-paclitaxel and 29% of patients receiving conventional paclitaxel (n=1204, odds ratio 1.53, p=0.001). Strikingly, in the subgroup of patients with triple negative breast cancer, pCR was achieved for 48.2% or patient receiving nab-paclitaxel and 25.7% of patients receiving conventional paclitaxel (n=275, odds ratio 2.69, p<0.001). These results are particularly encouraging for the patients in the triple negative breast cancer subgroup where there are limited therapies currently available. Additional results will be discussed during the presentation.

UNSOLVED PROBLEMS IN DIABETES

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Current treatments for type 2 diabetes include insulin, metformin, sulphonylureas, thiazolidinediones, α glucosidase inhibitors, incretin hormone-based therapy and sodium-dependent glucose co transporter inhibitors. Each of these treatments can improve glycaemia and some may even delay the onset of diabetes. Unfortunately, none of them has diseasemodifying characteristics, which would require slowing the progressive decline in insulin secretion. Furthermore, type 2 diabetes is associated with dyslipidaemia and hypertension, and is a major risk factor for cardiovascular disease, nephropathy, neuropathy and retinopathy.
To prevent and treat these related conditions, several drugs are prescribed to most patients with type 2 diabetes in addition to glucose lowering medications. This multi-drug approach is often associated with decreased patient compliance, as the number of pills prescribed is inversely proportional to adherence to treatment. Moreover, several antidiabetic drugs are associated with adverse effects. The most problematic are gastrointestinal symptoms in patients treated with metformin; hypoglycaemia and an increase in body weight in patients treated with sulphonylureas or insulin; an increase in body weight and bone fractures in patients treated with thiazolidinediones; and genital infections in patients taking sodium-dependent glucose cotransporter inhibitors.
Therefore, an ideal treatment for patients with diabetes should not only palliate glycaemia but also prevent the progression of the disease, target its comorbidities and have long-lasting effects with minimal adverse effects. In our presentation, we will speculate on the extent to which this ambitious but urgently needed profile could be achieved by anti-inflammatory drugs

VIRTUAL NANOMEDICINE TO REAL NANOMEDICINE

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Nanomedicine is an applied science to help patients and it is essential that projects whilst remaining cutting edge should be capable of development in what is a heavily regulated sector. Whilst this may seem daunting at first, the assessment of commercial potential can be done and is being done successfully in Europe. The outcome is much more diverse projects and a new patient focused culture.This does require change and some guidance is provided in this talk and in the documents referred to. Following the advice will enable researchers to see precisely where they are currently and measure their progress against industrial and investor standards and of course define what they should be doing.
Paramount in moving the community from virtual nanomedicine to the market is a paradigm change on the part of funders. Without funders leading the way no real progress can be made.

FOR 2 SESSIONS: FOLLOW-ON NANOMEDICINES (NANOSIMILARS) AND INTERNATIONAL REGULATORY FORUM

FALK EHMANN MD, PhD, MSc

Over the last three decades many first-generation nanomedicines have successfully entered routine clinical use and it is now important for medicines regulatory agencies to consider the mechanisms needed to ensure safe introduction of ‘follow-on’ nanomedicine products, ‘nanosimilars’. Moreover, drug regulators need to ensure that ‘next’-generation nanomedicines enter clinical development and consequently the market in a safe and timely way for the benefit of public health.
Recent European Medicines Agency initiatives to facilitate the development of nanomedicines include the publication of 4 Reflection Papers on:

- block copolymer micelles,
- liposomal products,
- nanosized colloidal iron-based preparations and
- coating of nanomedicines

The EMA has been chairing the International Working Group on Nanomedicines established in 2009. In November 2014 it has been agreed to establish a Working Group on Nanomedicines under the International Pharmaceutical Regulators’ Forum (IPRF) promoting discussions among a wider group of regulators, facilitating and enabling global harmonisation in the area.

AC5 SURGICAL HEMOSTATTM IS AN EFFECTIVE HEMOSTATIC AGENT IN ANTICOAGULATED ANIMALS USING A NON-COMPRESSIBLE, PENETRATING LIVER WOUND MODEL

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Intra-operative and postoperative bleeding is a major concern in surgical procedures for patients taking anticoagulant medications, or where anticoagulants are used to prevent potential life-threatening embolic complications. Heparin and other anticoagulants are used frequently and have an immediate effect on blood clotting, lasting 4 to 6 hours. Although synthetic self-assembling peptides have been shown to achieve rapid hemostasis in small animals, none have adequately addressed the potential for hemostasis in the presence of anticoagulant therapy in-vivo. Our goal was to investigate the hemostatic activity of a known synthetic self-assembling peptide in animals treated and untreated with anticoagulation therapy. Using a rat liver puncture model, animals were treated with known synthetic peptide AC5 Surgical Hemostatic Device™, or saline controls. Time-to-hemostasis and coagulation times were recorded in both anticoagulated and normal animals. Here we show that AC5™ was able to achieve rapid hemostasis equivalently in both anticoagulated and normal animals.

TARGETED NANOTECHNOLOGIES FOR CANCER THERAPY: FROM DISCOVERY TO CLINICAL TRIALS AND LESSONS LEARNED

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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 [1, 2]. 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. The annual worldwide market of controlled release polymer systems which extends beyond drug delivery is now estimated at $100 billion and these systems are used by over 100 million people each year. 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 [3]. The surface engineering of these nanoparticles may yield them "stealth" to prolong their residence in blood [4] and the functionalization of these particles with targeting ligands can differentially target their delivery or uptake by a subset of cells [5], further increasing their specificity and efficacy [6]. 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. More recently 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 [7]. 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 the first-in-human targeted and controlled-release nanoparticles, BIND-014 and SEL-068 [8, 9].

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INHALABLE TARGETED NANOMEDICINE: SAFETY AND EFFICACY

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INTRODUCTION

Nanoparticles (NPs) offer great potentialities for pulmonary drug delivery since it is today possible to combine the good aerodynamic properties of porous particles, their avoidance of muco-cillary clearance and the excellent biovailability of NPs using the so-called trojan microparticles (1). Moreover, targeting specific receptors on macrophages or dendritic cells can be achieved through specific fixation of ligands to NPs surface. However, the very same properties that make NPs exciting drug carriers might induce harmful effects as they interact with specific cells hampering their use as lung drug

delivery systems (2). Our group is covering the different aspects approaching particle and cell engineering to study the efficacy and toxicity of biodegradable NPs.

TARGETING APPROACHES TO LUNGS MACROPHAGES

Lung macrophages are the site of many intracellular bacterial infections such as Legionella pneumophila or Mycobacterium tuberculosis, this one being responsible of tuberculosis (TB). With about 1.8 millions deaths per year, TB is the infectious disease that kills most people worldwide. The pathogen resides mainly in alveolar macrophages and is hardly accessible for solubilized anti-TB drugs. Therefore, one of the main challenges in the treatment of TB is to achieve high concentrations of anti-TB drugs at its intracellular localization.

To be able to be taken up by lung macrophages, the delivery system must be at best in the colloidal range. However, although very small particles are able to reach the alveoli through Brownian diffusion, most of them are exhaled from the lungs owing to their low inertia and a very little number is taken up by macrophages. To overcome this drawback, we have combined the therapeutic potential of Poly(lactide-co-glycolide) (PLGA) nanoparticles systems with the ease of manipulation of microparticles by developing a hybrid vector named Trojan particles (1). We have developed and characterize this new delivery vehicle. In vitro release kinetics were performed for both nanoparticles and Trojan particles. Although a burst effect can be observed with both systems, the extent of the burst is lower for Trojan particles but as shown for rifampicin, the release profile is not completely adapted for sustained release (1).

In any case, although nanoparticles can improve, through the use of Trojan microparticles, the aerodynamic properties of the native nanoparticles, addressing nanoparticles to macrophages in order to enhance the uptake should increase drug retention in the lungs and the affinity to the target cells. This is the reason why we have developed targeted PLGA NPs through their surface-modification, the concept being to develop bacteria-like NPs in order to have them co-localize with the infectious agent. It is known that in the lungs, M. tuberculosis exploits a surfactant protein A (SP-A) receptor to enter the macrophages. Furthermore, it is known that SP-A has a high affinity for mannose, a common sugar in biological patterns present on bacterial membranes. Therefore, a mannosylated poly(lactide)-block–poly(ethylene glycol) (PLA-PEG) co-polymer was synthesized in order to prepare NPs that are capable to selectively adsorb SP-A, and mimic hereby the pathway by which M. tuberculosis invades macrophages. The mannosylated PLA-PEG copolymers, we have synthesized, allow formulation of NPs with increased cellular interaction in dependence on the presence of mannose residues on the NP surface. We investigate the effect of SP-A on the uptake for these formulations by macrophages and demonstrate that SP-A-coated NPs could mediate the NP internalization by macrophages in vitro but also in vivo in mice.

NANOPARTICLE SAFETY TO THE LUNGS

The very same properties that make NPs exciting drug carriers might induce harmful effects as they interact with specific cells hampering their use as lung drug delivery systems. Using in vitro and in vivo models, the aim of this work was to correlate the potential toxicity of biodegradable NPs to their cellular bioavailability or biodistribution and to their physicochemical properties. For this purpose, three types of surface-modified NPs were designed: positively and negatively charged as well as neutral. NPs were prepared using PLGA. Positively and negatively charged as well as neutral PLGA NPs were obtained by coating their surface with chitosan (CS), poloxamer (PF68) or poly(vinyl alcohol) (PVA), respectively.

We have first used an in vitro model of Calu-3 cell to mimic the bronchial epithelial barrier. The role of NP surface chemistry and charge on the epithelial resistance and mucus turnover was investigated. MUC5AC was used as a marker of mucus production (3). It was shown that the interaction with mucin reduced the penetration of CS- and PVA-coated NPs while the hydrophilic PF68-coated NPs were able to diffuse across the mucus barrier leading to a higher intracellular accumulation. NPs did not interfere with the formation and maintenance of tight junctions, with the exception of CS-coated NPs which caused a transient but reversible decrease of the trans-epithelial electrical resistance. NPs did not increase the MUC5AC mRNA expression or the protein levels regardless of their surface properties. Moreover, non inflammation was observed as evaluated by measurement of proinflammatory cytokines (4).

A co-culture model of THP-1/A549 cells was then used to evaluate the toxicity of biodegradable NPs. This model was shown to be relevant for in vitro pulmonary nanotoxicology studies. It was possible to detect a mild inflammatory response to PLGA NPs stabilized by three different hydrophilic polymers PVA, CS and PF68, but very limited compared to well-known inflammatory compounds (5). In vivo in mice the administration of biodegradable NPs did not induce an inflammation process as opposed to non biodegradable NPs for which all parameters measured clearly evidenced a toxicity after acute administration (6).

CONCLUSION

In conclusion, we have shown that it was possible to deliver PLGA NPs into microparticulate forms and moreover to functionalize these NPs for better targeting of macrophages. In addition, whatever is the surface modification, we found out little adverse effects of these particles in vitro and in vivo.

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IMPROVEMENT OF PATIENT'S CARE AND THERAPEUTIC EFFICIENCY TOWARDS MELANOMA WITH DENDRITIC NANOOBJECTS

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A proper surface coating of nanoprobes (NPs) can be central to in vivo targeting efficiency. It can stabilize particles, avoiding agglomeration, and increasing the sensitivity of NP-based sensors or ligands. 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, accumulation, circulation and clearance from the body. In addition, 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. 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 high 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.
Metastatic melanoma is a very aggressive disease for which no long-term (curative) treatment is currently available. Since 2011, six therapies targeting deregulated kinases or immune system used alone or in combination (i.e. Vemurafenib, Dabrafenib, Trametinib, Ipilimumab, Peginterferon alfa-2b, and Pembrolizumab) were approved by the U.S. Food and Drug Administration (FDA) for late-stage melanoma. Despite promising early results, these treatments are limited by their high relapse rates and undesired side-effects. Due to the high mortality rates still observed in the advanced stages of the disease, extensive researches for both new diagnosis modalities and effective therapies are still of interest. Among the panel of therapeutic options, targeted radionuclide therapy (TRT) emerged as a potential tool to selectively treat disseminated forms of melanoma. In this context, radiolabeled ligands directed towards Melanocortin-1 (MC1) receptor or melanin-producing cells have been the most extensively studied. A recent first human study underscored the great potential of melanin-targeting radiopharmaceuticals to significantly increase the survival rate of
patients with disseminated melanoma. Nevertheless, the inter-individual heterogeneity in metastasis melanin expression, pointed out the necessity to identify melanin-positive subpopulation before
performing TRT. Because the disseminated metastatic lesions of melanoma could occur throughout the body, patient stratification should be based on molecular imaging. In vitro confocal microscopy studies on human samples but also in vivo scintigraphic or magnetic resonance imaging in animal models showed that our versatile dendritic nanoobjects could have impacts on: (i) Sentinel lymph node (SNL) biopsy, by limiting the number and the associated morbidity of regional lymphadenectomy; (ii) In situ lentigo malignant melanoma delineation of tumor margins for limited exeresis; (iii) therapy outcome and prognosis of high grade melanoma; (iv) therapy of cutaneous metastatic lesions in elderly persons.

Fig 1: SPECT acquired 2 hours after bilateral intra-dermic injection at the extremity of lower legs of 99mTc radiolabeled dendritic NP

Fig 2: TEM image of a calcinated melanoma tumor after IV injection of dendronized NPs bearing melanocyte targeting ligands.

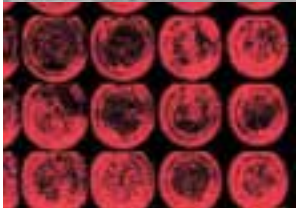

Fig 3: Confocal images of a melanoma tumor taken at 658 nm + reflectance, showing the co-localization of the melanocytes and functional dendronized NPs, IV injected.

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Mater. Chem. B 2015, DOI: 10.1039/C4TB01954G

POLYAMIDOAMINE NANOPARTICLES AS CARRIERS FOR THE DELIVERY OF DRUGS TO MALARIA PARASITE STAGES IN THE MOSQUITO VECTOR

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Malaria is arguably one of the main medical concerns worldwide because of the numbers of people affected, the severity of the disease and the complexity of the life cycle of its causative agent, the protist Plasmodium spp. With malaria elimination now firmly on the global research agenda, but resistance to the currently available drugs on the rise, there is an urgent need to invest in the research and development of new therapeutic strategies. Drugs can potentially target a suite of pathogen life stages inside two different hosts: humans and the insect vector. Infection starts when a parasitized female Anopheles mosquito inoculates sporozoites of the malaria parasite into a person while taking a blood meal. Within a few minutes, sporozoites have migrated through the skin and bloodstream to the liver, where they invade hepatocytes. Sporozoites develop into merozoites, which enter the circulation, invade red blood cells (RBCs), and replicate asexually to produce daughter cells that invade new RBCs to perpetuate the blood-stage cycle. Some parasites eventually differentiate into sexual stages, female or male gametocytes that are ingested by a mosquito from peripheral blood. When an infected bloodmeal reaches the insect's midgut, micro- and macrogametocytes develop into male and female gametes. Following fertilization, the zygote differentiates into an ookinete that moves through the midgut epithelium and forms an oocyst, which releases sporozoites. The malaria transmission cycle is restarted when sporozoites migrate to the salivary glands and are injected into a human with the mosquito's next bite. We have developed polyamidoamine (PAA)-derived nanovectors that combine into a single chemical structure drug encapsulating capacity, antimalarial activity, low unspecific toxicity, specific targeting to parasitized RBCs (pRBCs), optimal in vivo activity, and affordable synthesis cost. Our recent data suggest that the antiparasitic mechanism of PAAs can be based on blocking the erythrocyte invasion of egressed parasites (Figure 1).

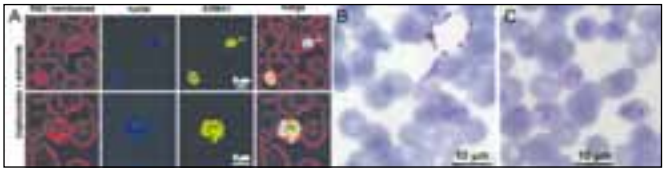

Figure 1: (A) Fluorescence microscopy targeting study of the PAA AGMA1 to pRBCs. FITC-labeled AGMA1was added to living P. falciparum cultures and, after 90 min of incubation, the samples were processed for confocal fluorescence microscopy analysis. (B,C) Microscopy study of the effect of AGMA1 on the invasion of RBCs. (B) Image from a Giemsa-stained P. falciparum culture at ring stage

after a complete cycle following treatment with AGMA1. (C) Control sample without AGMA1. From (1).

The ensuing prolonged exposure of the pathogens to the immunitary system might be applied to the design of new malaria vaccination approaches where PAAs could play a dual role as carriers of antimalarial drugs and as vaccination adjuvants. In addition to binding egressed merozoites, PAA-based nanoparticles are capable of penetrating late-stage pRBCs and of adsorbing on intracellular merozoites of both human and murine malarias. The mechanism of pRBC recognition by PAAs has not been elucidated yet but it seems to be related with the known increased permeability to small nanostructures via the tubulovesicular network. This unexpected synergistic effect combining therapeutics and prophylaxis represents a radically new approach to the treatment of malaria for which we propose the new term theralaxis.

A largely unexplored avenue in antimalarial drug development is targeting Plasmodium stages in the vector itself. While the innate immune system of mosquitoes is capable of completely clearing a malaria infection, it is far from the sophisticated arsenal providing long-term protection in mammalian adaptive immunity. This might result in parasite stages with reduced defenses because they only need to survive for a few weeks inside the insect, facing an immune surveillance not as demanding as in the human host. Drugs targeting early Anopheles stages must kill only ca. 5 × 103 parasites to free a mosquito from Plasmodium infection (2), and the absolute low corresponds to oocysts, of which there are only 2 to 5 in a single insect (3), being around for over a week. However, the advantage of few cells to be reached per individual is counterbalanced by a large population. Although at first sight delivering drugs to mosquitoes seems impractical as a potential method of control in the field, we believe that it can provide important advantages if one can avoid the obvious drawbacks (4). Strategies that control malaria using direct action against Anopheles are not new, but most of them aim at eliminating the vector, either by killing it with pesticides (5) or through the release of sterile males (6). Since eradicating an insect species might have as a consequence unpredictable disruptions of ecosystems with potential undesirable side effects, mosquito-friendly antimalarial strategies should be favored whenever possible. Such an ecologically-minded and fascinating alternative is the recent proposal of using Wolbachia, a maternally transmitted symbiotic bacterium of arthropods, to induce in Anopheles refractoriness to Plasmodium infection (7). Thus, administration of drugs to mosquitoes to free them of malaria with the objective of blocking transmission of the disease might not be so far-fetched.

It is known that natural plant sugars are an essential pool of energy for female mosquitoes. This need could be exploited to develop a sugar meal trap that attracts and drugs them while they feed, although this would only be effective as a control measure if the insects rely on other nutrient origins in between bloodmeals. The pathophysiology of the malaria parasite might facilitate attraction of Plasmodium-infected mosquitoes to alternative feeding places. To keep blood fluid and prevent quick coagulation, Anopheles synthesizes an anti-hemostatic armamentarium containing, among others, the enzyme apyrase. Plasmodium inhibits apyrase, and in this way entices its carrier to bite more because blood coagulates faster and Anopheles has to probe longer to get its full dinner, thereby increasing potentially infective host contacts. It can be expected, then, that infected insects will have a larger probability of searching for non-human sources than those which are not parasitized. A trap emitting human volatiles (8) and consisting of a blood-like substance could contain highly concentrated drugs against Plasmodium gametes, ookinetes, oocysts or sporozoites without fear of overdoses or toxic side effects for people. These “fake human” traps, hung on the walls of dwellings in malaria endemic areas, could likely be made at an affordable cost. Such a strategy, because it is not designed for administering antimalarials to humans, will bypass clinical trials that often delay for years the development and deployment of new medicines. An additional advantage of direct delivery from a fixed-volume container is that the drug does not become diluted with time.

The three elements that constitute a targeted therapeutic nanovector (nanocapsule, targeting molecule and the drug itself) can be exchanged, as if they were LEGO parts, to obtain new structures better suited to each particular situation. Heparin and heparan sulfate are targets for the circumsporozoite protein in the sporozoite attachment to hepatocytes during the primary stage of malaria infection in the liver(9), and chondroitin sulfate proteoglycans in the mosquito midgut have been described to bind P. falciparum ookinetes as an essential step of host epithelial cell invasion (10). This body of accumulated evidence suggests that glycosaminoglycans might be adequate to target antimalarial-loaded nanovectors to Plasmodium mosquito stages. Future antimalarial strategies relying on drugs working through radically new mechanisms might demand direct delivery to Plasmodium stages in the mosquito of targeted nanovectors loaded with these new medicines. The specifications to which the nanocarriers will likely have to fit are (i) a long half-life of months without losing integrity before being ingested by the mosquito while preserving drug activity, (ii) an adequate degradation rate once inside female Anopheles to allow the drug entering Plasmodium, (iii) a slower degradation rate once inside male Anopheles to allow the nanocarriers being horizontally transferred to females upon mating, (iv) a high solubility in mosquito artificial diets to allow for the maximum affordable concentrations, and (v) a targeting as specific as possible to Plasmodium stages inside Anopheles (gametocytes, ookinetes, oocysts and sporozoites).

A final bonus of delivering the nanocarriers directly to mosquitoes is that the range of sizes becomes greatly expanded between a few nm and up to several microns for the direct delivery to females. Delivery to insects will allow also for a not so strict vigilance on other nanocarrier characteristics such as, among others: zeta potential; toxicity of the chemical units constituting the nanovector; nature, type and number of targeting units; nature, number, and amount of drug(s) loaded. Our preliminary data offer promising perspectives for the use of nanocarriers to shuttle drugs into mosquito stages of Plasmodium (Figure 2).

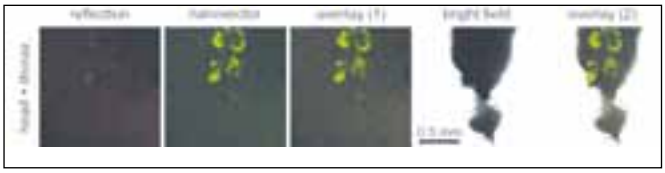

Figure 2. Preliminary data on the delivery of polymeric nanovectors to Anopheles. Newly hatched Anopheles atroparvus were fed the fluorescein-labeled PAA ISA1 incorporated in their sugar meal. After three days, fluorescence microscopy was used to observe the polymer distribution in living mosquitoes (fluorescein, green). Reflection is shown in red as a control to indicate external parts of the mosquito. The center image is an overlay of fluorescein and reflection images to confirm the presence of ISA1 in a region of the thorax, which indicates the distribution of nanoparticles throughout the insect’s organism.

ACKNOWLEDGEMENTS

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MULTISTAGE VECTORS FOR THERAPEUTIC DELIVERY

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The principal biological barriers that govern the distribution of systemically injected therapeutic agents, including molecularly targeted biomolecules, conventional chemotherapy/small molecules, and nanotherapeutic agents, comprise those of epithelial/endothelial nature, those that are associated with transport through the microenvironment, and those of cellular/subcellular nature. Biological recognition is active only after these barriers are crossed, and in many cases biological recognition agents add to specificity of recognition, but render more difficult and unlikely the passage across biological barriers. The MSV system is designed exactly to enhance the ability of their therapeutic payloads to cross biological barriers, and concentrate preferentially at the target sites. In the MSV system, nanoporous silicon-based first-stage particles concentrate preferentially at the vascular endothelium of the therapeutic target, such as within the cancer microenvironment.

The preferential concentration is the result of the choice of the most suitable size, shape, surface charge, and (if needed) molecular targeting agents. We have designed and demonstrated first stage vectors with superior ability to concentrate preferentially in organ targets (most notably lungs, liver, bone marrow) and at tumor microenvironment vasculature walls. The second-stage nanoparticles are released by harmless degradation of the first-stage vector, with tunable kinetics of degradation and release. The second-stage nanoparticles penetrate across the tumor microenvironment and, depending on the nanoparticle type, are up-taken by target cells through receptor-mediated endocytosis, or fuse with the target cell membrane.

We have demonstrated the release of many different second-stage particles, and their superior efficacy in the therapy of metastatic disease in animals models. Among the nanoparticles we have released are liposomes, as well as nanoparticles made of biodegradable polymers, albumin (abraxane), iron oxide, silica, quantum dots, carbon nanotubes, gold nanoshells, and their combinations. Through these nanoparticles we have successfully delivered in animal models a broad spectrum of therapeutic agents, which are the "third-stages" in a preferred MSV strategy. These include conventional chemotherapeutics (such as doxorubicin, taxanes, rapamycin), biological molecules (such as siRNAs, miRNAs, proteins), thermal ablation agents, contrast agents, and their combinations.

The second-stage can itself by further targeted by molecular recognition moieties. The different fates of the third-stage, therapeutic payloads, may be controlled to be such as active vesicular transport to perinuclear locations following receptor-mediated endocytosis, or direct release of the payload in the cytoplasm. Different choices of second-stage particles can be used to ensure these different fates, as desired, including lysosomal escape. The full MSV system comprising all three stages yields therapeutic results in animal models of metastatic cancer that are much superior to those obtained with the third-stage (i.e. naked drug) only, or second- and third-stage (i.e., nanoparticle-encapsulated drugs) only.

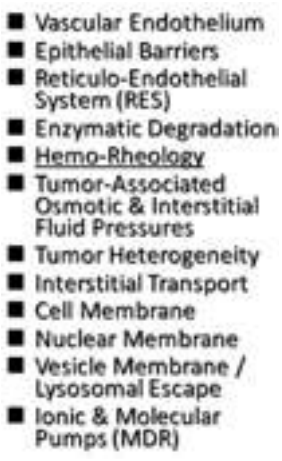


Fig 1: The principal biological barriers that govern the distribution of systemically injected therapeutic agents, including molecularly targeted biomolecules, conventional chemotherapy/small molecules, and nanotherapeutic agent

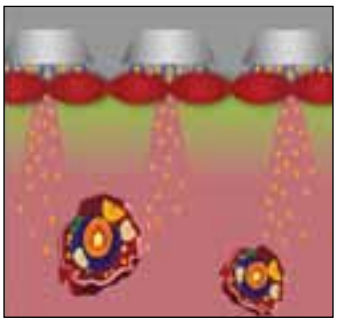


Fig 2: First-stage particles concentrate preferentially at the vascular endothelium of the therapeutic target, such as within the cancer microenvironment. The preferential concentration is the result of the choice of the most suitable size, shape, surface charge, and (if needed) molecular targeting agents. targeting agents.

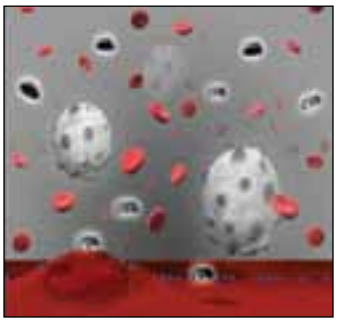


Fig 3: The second-stage nanoparticles are released by harmless degradation of the first-stage vector, with tunable kinetics of degradation and release. The second-stage nanoparticles penetrate across the tumor microenvironment and, depending on the nanoparticle type, are up-taken by target cells through receptor-mediated endocytosis, or fuse with the target cell membrane.

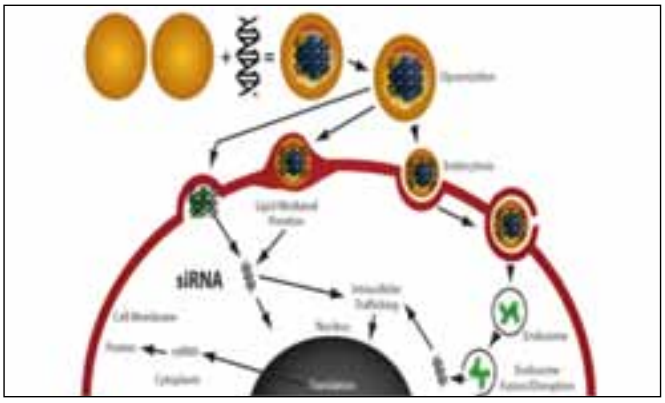


Fig 4: The different fates of the third-stage, therapeutic payloads, such as active vesicular transport to perinuclear locations following receptor-mediated endocytosis, or direct release of the payload in the cytoplasm. Different choices of second-stage particles can be used to ensure these different fates, as desired, including lysosomal escape.

TEN THINGS YOU SHOULD BE AWARE OF WHEN WORKING WITH NANOPARTICLES & CELLS

ALKE FINK AND BARBARA ROTHEN-RUTISHAUSER, Adolphe Merkle Institute, University of Fribourg, Ch. de Verdiers 4, CH-1700 Fribourg, Switzerland.

Engineered nanoparticles meet the biological world at the nano-bio interface. This encounter holds many promises e.g. for application in medicine ("nanomedicine"), but also raises concerns for the safe use of nanomaterials ("nanotoxicology") and has led to novel science investigating the interaction of nanoparticles with cells.

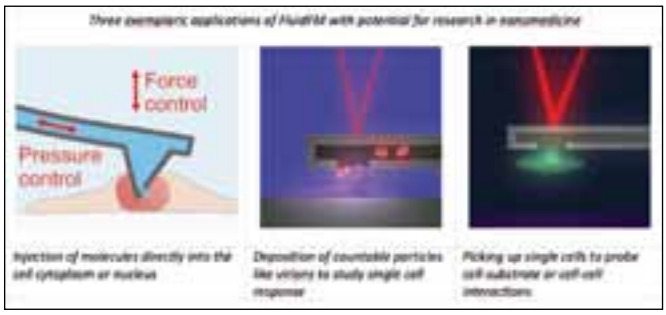
Despite significant progress in the past years and due to the complex ever-changing biological environment, it will still be a long time until we understand or even can control the nano-bio interface, in particular since crucial pieces of the puzzle are being badly neglected at present. Although the community has gained considerable knowledge on nanomaterial handling and cell experiments, it is rarely known what really enters into the study. One clearly missing link between lab-synthesized nanoparticles and measured cellular endpoints is the aggregation behaviour of nanoparticles in the biological environment as well as a thorough cell characterisation. For example, are the originally monodisperse particles still monodisperse or do we measure aggregates? What are the con-

sequences with regards to dose, uptake mechanism, intracellular fate, or cell reactions? In addition, the proper characterization of the cellular systems is often missing leading to misinterpretations of the results. We should much more carefully investing time to answer the following questions: Do the cell systems present the structural-function properties of the tissue/organ we would like to investigate? Which endpoints should be measured?
An overview will be given regarding the above mentioned pitfalls in this research field including a thorough discussion about limitations and pitfalls one can stumble when designing experiments.

NOVEL SINGLE CELL MANIPULATION APPROACHES FOR NANOMEDICINE BY FLUIDFM

PATRICK FREDERIX, Head of Applications and service, Nanosurf AG, Liestal, Switzerland

Nanosurf is an AFM (Atomic Force Microscopy) manufacturer producing a full range of solutions, from compact systems for the industrial and teaching market to comprehensive solutions for research and material characterization. In this presentation I will address the potential of a new manipulation solution, called FluidFM, for nanomedicine.
FluidFM which is developed together with the company Cytosurge is an AFM based nano-manipulation tool using microfluidic cantilevers. The cantilevers are connected to a pressure controlling system that can either apply an overpressure for local liquid delivery or underpressure for local aspiration. With the integration in an optical microscope, the FluidFM system comprises position, force, pressure and optical control. This opens a wide avenue of experiments [1]: With a syringe-like tip molecules can be injected in the cytoplasm or in the cell nucleus to study the cell response to the injected molecules [2,3]. These can be plasmids, but is also applicable for other molecules. Rather than injection through the membrane, molecules can also be administered onto a cell membrane at gentle contact. This soft deposition can be extended to larger particles compared to injection. This has been demonstrated to study the cooperativity of vaccinia virions at the single cell level [4]. Cell-cell and cell-substrate interactions are important for cell functionality, and body-implant interactions. I will show examples in mammalian, yeast and bacterial cells that are examined with respect to their substrate adhesion characteristics [5-7]. Finally by introduction of an electrode in the microfluidic channel mechano-electrophysiological response of e.g. beating heart cells can be studied [8].
In summary, the manipulation possibilities of FluidFM are versatile allowing a large variety of nanomedicine experiments at the single cell level.



Three exemplaric applications of FluidFM with potential for research in nanomedicine

Injection of molecules directly into the cell cytoplasm or nucleus
Deposition of countable particles like virions to study single cell response Picking up single cells to probe cell-substrate or cell-cell interactions

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THE PATHWAY OF INDUSTRY TO HORIZON 2020

HEICO FRIMA, European Commission, Directorate-General for Research & Innovation

The first Call for Proposals of the European Union Horizon 2020 Framework Programme for Research & Innovation (2014–2020) was published in December 2013. Horizon 2020 has a budget of € 80 Billion. It has three main pillars: ‘Excellent Science’, ‘Industrial Leadership’ and ‘Societal Challenges’, each with several specific objectives. Together, the funding opportunities in Horizon 2020 span the entire innovation cycle, from frontier research to rather close to market activities.
Horizon 2020 offers many opportunities for funding research and innovation in the medical and pharmaceutical field, notably for nanomedicine. The presentation will give an overview of Horizon 2020 with an emphasis on pathways of industry in the pillars ‘Industrial Leadership’ and ‘Societal Challenges’. It will highlight the important role of the ‘Key Enabling Technologies’ (nanotechnology, advanced materials, industrial biotechnology, nano-electronics, nano-photonics and advanced manufacturing), the HEALTH Programme, the Innovative Medicines Initiative, the Infrastructure Programme, and the opportunities for innovation with the SME Instrument and the ‘Fast Track to Innovation’.
The nanomedicine community with the European Technology Platform for Nanomedicine has been very active and successful in the preceding Framework Programme 7. The FP7 NMP (Nanotechnology, Materials and Production Technologies) Programme funded about 85 nanomedicine projects with more than € 400 million EU funding, and involving 240 SME’s. These projects develop novel biomaterials for implants and regenerative medicine, novel nano-enabled diagnostics and therapy of diseases, and smart implants using bio-nano-info convergence. A strong pre-clinical nanotechnology competence for applications in medicine has meanwhile been established in European R&D laboratories.
The presentation will give a summary overview of funding for nanomedicine projects from the first Calls for Proposals in Horizon 2020. Compared to FP7 there is more emphasis on the translation of preclinical results into therapies and products that can be used by the clinicians, for the benefit of the patients. This includes pilot manufacturing projects for scaling-up the production of nano-pharmaceuticals from the laboratory scale to the quantities that are needed for clinical testing.
The physical, chemical, structural and biological characterisation of

nanomedicines is a challenging issue. The Infrastructure programme supports a new distributed EU Nano-Characterization Laboratory (EU-NCL), a consortium that includes the US-NCL.
The Horizon 2020 Health Programme provides funding for translation and clinical testing of new nano-enabled diagnostics and therapies.
There is also a new Support Action 'ENATRANS' to provide networking and advice on translation for SME's in the nano-biomedical sector.
Finally, the complementarity will be highlighted between Horizon 2020 and the EU Structural and Investment Funds (ESIF) that have € 80 Billion available for funding research and innovation in Europe, through regional programmes using smart specialisation strategies.
[1]Disclaimer: All views expressed are entirely of the author, do not reflect the position of the European Institutions or bodies and do not in any way engage any of them.

FROM ANTIMICROBIAL COATINGS FOR IMPLANTS TO SMART BACTERIAL DETECTION AND DRUG DELIVERY

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More and more implants and different implant materials get inserted into the human body each year. With increasing implant operation numbers and at the same time more resistant bacteria, the absolute number of implant infections is steadily augmenting. [1] We therefore aimed at developing coatings in order to prevent such device infections.
Our first approach consisted in the development of silver-based, nanostructured coatings, which turned out to possess excellent antimicrobial properties while at the same time being biocompatible. Such coatings are ideal for the healing phase after an operation, as these coatings are active for approximately three months after insertion and can hence prevent infections stemming from contacts with e.g. S. epidermidis.[2, 3]
In order to make our coatings last longer, we developed the synthesis of encapsulated silver nanoparticles. Using two techniques, the microemulsion technique and the template approach, we can tune the sizes of these nanocontainers between 20 nm and 200 nm, depending on the desired application. Multiple coatings allow to renders these nanocapsules biocompatible, while they release antimicrobial silver ions over a period of up to three years. [4, 5]
All of the currently developed coatings have in common that they release active compounds independently whether bacteria are present or not. We therefore developed a bacterial sensor, which is able to detect (by fluorescence or colorimetry) at room temperature in a fast (5 min) and specific way (based on genetic code) the presence of bacteria at very low concentrations. Alternatively, the "fitting" antibiotic can be set free with respect to the detected bacteria. Finally, the sensor can be coupled to a nanoencapsulated drug reservoir for a triggered drug release.

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PEGYLATED LIPOSOMAL MITOMYCIN-C PRODRUG ENHANCES TOLERANCE OF MITOMYCIN C: A PHASE 1 STUDY IN ADVANCED SOLID TUMOR PATIENTS

ALBERTO A. GABIZON, Talia Golan, Tal Grenader, Patricia Ohana, Yasmine Amitay, Hilary Shmeeda, Ninh M. La-Beck, Esther Tahover, Raanan Berger

Purpose: Mitomycin C (MMC) has potent cytotoxicity but a problematic toxicity profile limits widespread systemic use. In animals, pegylated liposomal mitomycin C lipid-based prodrug (PL-MLP) was well tolerated and more effective than free MMC. We evaluated PL-MLP in patients with advanced cancer.
Patients and methods: 27 patients were treated in escalating dose cohorts of 0.5-3.5 mg/kg (equivalent to 0.15-1.03 mg/kg MMC) every 4 weeks for up to 12 cycles, unless disease progression or unacceptable toxicity occurred. Pharmacokinetics were assessed during cycles 1 and 3.
Post-infusion activation of the complement systemwas also evaluated in cycle 1.
Results: Per protocol maximum tolerated dose was not reached at 3.5 mg/kg. However, prolonged thrombocytopenia developed after repeated doses of 3mg/kg or cumulative doses of 10-12 mg/kg. Dose-related grade 3 or higher adverse events included fatigue, anemia, and decreased platelets. C_{max} and $AUC0_{-\infty}$ increased linearly over the dose range 0.5-2.0 mg/kg, and greater than linearly from 2.5-3.5 mg/kg; there were no significant differences in clearance of MLP between cycles 1 and 3. Median $t_{1/2}$ was 23 hours among dose cohorts, with no trend by dose or cycle. One patient had a partial response. Stable disease was observed in 10 patients across all dose levels.
Conclusions: PL-MLP has a long circulation time, was well tolerated and can be administered to heavily pretreated patients at a single dose of 3.0 mg/kg and cumulative dose of 10-12 mg/kg before development of prolonged thrombocytopenia; this is nearly three-fold the equivalent dose of MMC tolerated historically. This formulation may be active in a variety of tumor types and is better tolerated than free MMC.

APIDSOL NANOCARRIERS FOR LOCAL DRUG DELIVERY

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Apidel is a pharmaceutical startup company developing innovative medicines based on its proprietary technologies ApidSOL and ApidCOR. Apidel's mission is to improve the therapeutic performance of existing and new drugs in various disease areas.
ApidSOL technology is based on co-polymers of methoxy polyethyleneglycol (mPEG) and hexyl substituted poly-lactic acid (hexPLA). In a self-assembly process, amphiphilic mPEGhexPLA polymers form nanosized micelles in aqueous environments; Preclinical toxicity studies showed good biocompatibility after topical administration [1].
Three distinct mechanisms enable ApidSOL nanocarriers to achieve efficient loco-regional delivery of poorly soluble drugs: i) incorporation of the drug into the hydrophobic micelle core increases solubility and actual bioavailability ii) particle size in the lower nano-range (20 -50 nm) 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 [2,3]. Local tissue targeting is achieved by topical application of the drug loaded ApidSOL formulations. Above mentioned mechanisms help to obtain high local drug concentrations, to increase therapeutic efficacy and to reduce systemic side effects.
Apidel will present data from its preclinical trials evaluating ApidSOL in dermatology, gastroenterology and ophthalmology.

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SAFETY OF NANOLIPIDS IN DIFFERENT RESEARCH LINES AND POTENTIAL OF LIPIDIC THERAPY AFTER CLINICAL TRIAL IN ONCOLOGY

EUSEBIO GAINZA, BioPraxis AIE, Miñano, Spain.

Nanotechnology for new safety medicines has been widely investigated of the scientific community being a strategic research area of Praxis Pharmaceutical S.A. In the last decades the elaboration of different nanometric particles such as nanostructured lipid nanocarriers (NLC) become an important challenge to overcome different drawbacks appears with biomolecules, peptides and chemotherapeutics. The nanoencapsulation of different compounds and molecules achieve a higher efficiency to the treatments; permit the administration of lower doses of drug with the same therapeutic effect; decrease the toxicity of the drug; diminish the number of doses per treatment and enhanced patient compliance and quality of life. Nevertheless the registration of these nanoencapsulated molecules it is not easy and according with National Products Safety Agencies, EMA and EFSA additional safety confirmations are needed. That is why, in this year's Praxis Pharmaceutical has been working actively in NLC particle nanosafety demonstration in the framework of different European and National projects with promising results in the nanosafety context.
On the other hand, the company is also involved in lipidic therapy to treat different cancer obtaining promising results in different animal models and cell lines. Moreover, currently there is an ongoing clinical trial coordinated by Praxis, using Hidroxyoleic molecule for the treatment of glyoma.

UNDERSTAND TUMOR PROGRESSION AND IMMUNE REACTION AGAINST CANCER BY USING INTEGRATIVE BIOLOGY AND BIOINFORMATICS

JÉRÔME GALON
An overview will be given regarding the above mentioned pitfalls in INSERM, UMRS1138, Cordeliers Research Center, Paris, France
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To date the anatomic extent of tumor (TNM classifications) has been by far the most important factors to predict the prognosis of cancer patients. However, this classification provides limited prognostic information in estimating the outcome in cancer and does not predict response to therapy.
Using large-scale technologies, quantitative measurements, and integrative biology approaches we evaluated the importance of the host-immune response within human tumors.
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 showed that the combination of immune parameters associating the nature, the density, the functional 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", and factors modulating it will be discussed. 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. Analysis of chromosomal instability revealed mechanisms associated with intratumoral lymphocyte proliferation. Based on the immune contexture, a standardized, simple and powerful immune stratification system, termed "Immunoscore", was delineated that may bear a prognostic power superior to that of the currently used cancer staging system. Tumor invasion parameters were statistically dependent on the natural host-immune reaction. Thus, these results provide a novel paradigm for cancer. A worldwide Immunoscore consortium is testing the prognostic value of Immunoscore, using a standardized assay to routinely measure the immune status of a cancer patient.
The functional orientation of the immune contexture is characterized by immune signatures qualitatively similar to those predicting response to immunotherapy, Thus, the continuum of immune response existing, spanning a balance between tumor cell growth and elimination, will be discussed.

NEW FUNCTIONAL NANO-MATERIALS FOR MOLECULAR THERANOSTICS: IMPLICATIONS FOR DEGENERATIVE DISORDERS, METABOLIC DISEASES AND CANCER

EHUD GAZIT, FRSC

Nanoparticles serve as preferred vehicles for numerous theranostics applications. The particles are being frequently decorated with functional biological entities to provide specificity. An alternative direction in which the targeting moieties also act as building blocks for the formation of functional nano-assemblies. Diphenylalanine, a minimal highly potent self-assembling module was incorporated with targeting moieties which preferentially binds to cancer cell. The co-assembly of the engineered hormone together with structurally diverse diphenylalanine derivative allowed the formation of bioactive homogenous spherical nanostructures. The binding capacity of the loaded co-assembled nanospheres to cancer cells and the enhanced tumor selectivity were demonstrated. These findings present a new strategy for the molecular engineering of hormones and other bioactive peptide molecules by minor modification with remarkably potent association motif.
The described use of the diphenylalanine-polypeptide hybrid represent a new front in organic nanotechnology which is clearly a new front in the field of molecular self-assembly of new structures and composite families at the nano-scale. Additional aromatic homodipeptides (including those with non-coded amino acids as DOPA) could self-assemble in nano-spheres, nano-plates, nano-fibrils and hydrogels with nano-scale order. The modification of peptide building blocks with the Fmoc protecting group allows the formation of hydrogels with nano-scale order that are also useful for various theranostics applications. We demonstrated that the peptide nanostructures have unique chemical, physical and mechanical properties including ultra-rigidity as aramides, semi-conductive, piezoelectric and non-linear optic properties. We also demonstrated the ability to use these peptide nanostructures as casting mould for the fabrication of metallic nano-wires and coaxial nano-cables.
The application of the nanostructures was demonstrated in various fields including electrochemical biosensors, tissue engineering, and molecular imaging. We had developed ways for depositing of the peptide nanostructures and their organization. We had use inkjet technology as well as vapour deposition methods to coat surface and from the peptide "nano-forests". We recently demonstrated that even a single phenylalanine amino-acid can form well-ordered fibrilar assemblies of distinct electron diffraction pattern and toxic properties. The combination of DNA properties and peptide backbone in the form of Peptide Nucleic Acid (PNA) resulted in light emitting assemblies that exhibit both stacking and Watson-Crick base-pairing.
Finally, the aggregation of the aromatic moieties into ordered nano-structures could be related to metabolic disorders. The formation of ordered amyloid fibrils is associated with several notable

human disorders. These nano-scale assemblies are predominantly rich in β-sheet secondary structure and specifically bind dyes, such as ThT and Congo-red. The formation of the amyloid fibrils or earlier pre-fibrillar forms correlates with an apoptotic effect in various tissues. While the formation of these cytotoxic supramolecular entities has previously been linked to proteins and peptides, we had later demonstrated that the single phenylalanine amino acid can also form amyloid-like fibrils possessing typical ultrastructural, biophysical and biochemical properties. Moreover, these fibrillar assemblies are cytotoxic by the induction of apoptotic programmed cell death. Physiological phenylalanine accumulation is detected in the plasma, cerebrospinal fluid and brain tissues of phenylketonuria (PKU) patients. The generation of antibodies in a PKU mice model and identification of aggregate deposits post mortem in patient brains suggested a pathological role for these assemblies. Thus, the formation of tangled amyloid-like fibrils by phenylalanine may propose a new amyloidal etiology for PKU. Furthermore, these findings may offer that materials known to inhibit amyloid structure formation may also inhibit other amyloid-like structures and thus, may lead to new innovative direction of treatments for PKU patients, which suffer from profound and permanent mental retardation.

NANOTECHNOLOGY IN MEDICAL DEVICES: HORIZON SCAN, RISK ASSESSMENT AND REGULATORY DEVELOPMENTS

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Innovative nanotechnologies are increasingly used in medical applications and are expected to have a major impact on healthcare in the future. These nanotechnology enabled medical innovations relate to both medicinal products and medical devices. In this study we have focused on medical devices.
Nanotechnology applications in the field of medical devices, also referred to as "nanomedical devices", span a wide range of very diverse products, technologies and application areas. Their intended use can be in the context of therapy, diagnosis, monitoring and/or prevention of disease. All medical disciplines are benefiting from nanomedical devices, especially orthopaedics, dentistry, oncology, and cardiology. Also a number of innovations in clinical chemistry laboratories are enabled by nanotechnologies. Nanomedical devices can involve the use of nanomaterials, however, nanotechnologies also enable innovative devices without using nanomaterials, for example by applying nano-electronics or lab-on-a-chip technologies. The use of nanomaterials in medical devices poses a particular challenge for the safety evaluation and risk assessment of these medical devices as the specific character of the nanomaterial used should be taken into consideration. Nanomedical devices can be non-invasive or invasive, resulting in potential contact with any kind of tissue. It is important to have clear insights into the state of affairs with regard to the availability of nanomedical devices and their specific benefits and risks, not only for regulators and industry, but also for physicians and pharmacists.
In the present study, we have carried out a horizon scan to identify nanomedical devices that are currently available on the market or under evaluation in clinical studies. In addition, we have analyzed the most promising research developments, potentially leading to products in the future. A variety of data sources was explored, including a search of scientific literature and clinical trial registries, analysis of databases from FDA and Health Canada, patent analysis and commercial market reports.
Furthermore, the various aspects of safety evaluation and risk assessment of medical devices containing nanomaterials were addressed, based on the Scientific Opinion "Guidance on the Determination of Potential Health Effects of Nanomaterials Used in Medical Devices", published in January 2015 by the European Commission's Scientific Committee on Newly Identified Health Risks (SCENIHR). SCENIHR recommends a phased approach for evaluating the risk of the use of nanomaterials in medical devices based on potential release and characteristics of the nanomaterials, in order to avoid unnecessary testing. The phases cover particle release (phase 1), particle distribution and persistence (phase 2), hazard assessment (toxicological evaluations) (phase 3), risk characterisation/risk assessment (phase 4). See Figure 1 for a graphical representation of this phased approach.

Finally, an overview of current developments in regulation and standards for medical devices with relevance for the application of nanotechnologies is provided. This includes activities of the European Commission's Working Group on New and Emerging Technologies in medical Devices (NET WG) related to the ongoing revision of the regulatory framework for medical devices. Also, a guidance document is currently under development within the International Organization for Standardization (ISO), entitled "ISO/NP TR 10993-22 Biological evaluation of medical devices – Part 22: Guidance on nanomaterials".

ETHICAL ISSUES IN PATIENTS' INVOLVEMENT IN DECISIONS MAKING ON PERSONALIZED TREATMENTS WITH NANOMEDICINE/S: PROTECTION VERSUS THE FRONTIERS OF HOPE

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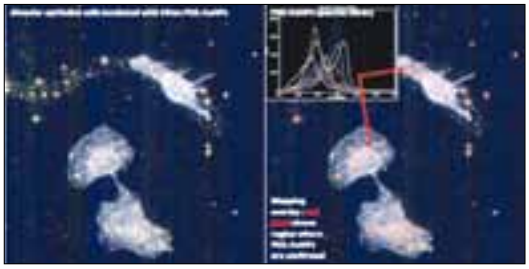
Nanomedicine, in an ever increasing manner and speed, becomes a constitutive part of the new medicine of the 21st century. It permeates all its disciplines with new, revolutionary materials, devices, nano-medicines, and never-before-encountered technological possibilities. Distinguishing between treatment 'hypes' and real hopes for cure of the most dreadful human diseases and ailments does become difficult even for a well-educated health professional. Needless to say anything about a vulnerable patient, navigating anxiously, with his or her semi-blind confidence, the vast, sometimes rather ugly and dangerous 'waters' of nowadays' Internet (dis)information. 'Pirates' of the 'profit business for all costs' are prepared, hiding well in the 'information fog', to prey with insatiable greed on his or her infinite hopes, earthly despair, and immitigable suffering; making profits without borders and leaving the exploited individual alone, when he or she has failed the expectations of alleged 'miraculous cures'. Media and politics are not providing much help either.
In contrast to this dreadful imaginary, there is a colossal effort of the real science and research, pushing the 'frontiers of possible' towards ever new horizons, while taking into account the patient, present and future, with his or her still unmet (and growing) needs; coloured with real human fears and sorrows. Seeking the informed participation of the patient as a partner and subject of their honourable, very expensive, and sometimes even risky efforts.
These complex and dynamically evolving realities provide for as yet rather poorly explored universe of the moral/ethical inquiry. It

might help, subject to fulfilment of yet another hope, to lead those involved in pursuing the real, 'substantive' good of the individual, his or her family and other close persons, and also of the broader society, present and future. The contemporary man, he or she, is quite ill-prepared for such reflection. Moreover, it often seems to him or her as being superfluous, 'purely philosophical', 'un-practical', 'non-pragmatic', almost deleterious. In the end of the day, however, it is the only option left to him or her, if seeking for a rational guidance in decision making concerned with issues of life, health and needy help. Such as when considering participation in a clinical trial, research project, or subduing to a novel, possibly health-saving or even life-saving treatment. The human nature has it that these decisions are by default deeply personal, individual and subjective in nature, but, paradoxically enough, they are best made in connection, communication and closeness with our fellow human beings that share parts of our way in life, our hopes and our fears. In present day biomedical research, or clinical medicine settings, the 'procedural' aspects of these unique human encounters are deemed very important, such as the work of ethics committees or other similar bodies; also the 'transparency' in the complex decision-making procedures – that are, at the same time, required, paradoxically enough, to respect the privacy, the intimacy of the individual, his dignity, his 'human mystery'. Patients and their carers, their physicians in particular, but their relatives and the society enlarge, all we are in the unmet need of education and some guidance here and there. However, even with all this in proper place and quality, we are still very much keen to approach our friends, relatives, and 'persons of trust' to get help, insight and humane support, when facing those important decisions. They probably would still, for hopefully (?) a long time to go, be not replaced by an 'empathetic cyborg'. Or would they?

OPTICAL OBSERVATION AND HYPERSPECTRAL CHARACTERIZATION OF LABEL FREE NANOMATERIALS IN BIOLOGICAL MATRICES

NICOLAS GONZALES

Critical research is being conducted to quantify the potential benefits of nanomaterials for use as anti-microbials, therapeutics, drug delivery vectors and biomarkers. Important efforts are also ongoing to better understand the effects these materials on the environment and population from a toxicology perspective. This work requires an ability to observe and characterize these nanomaterials in their natural form (without fluorescent markers) as they interact with non-labeled biological matrixes, including cells, tissue and whole animal organisms. A novel hyperspectral microscope technology has been specifically developed to support these research needs. This technology utilizes patented darkfield-based illumination optics, creating high signal-to-noise images of non-fluorescent nanomaterials interacting with both biological and materials samples. The integration of hyperspectral imaging with this high signal-to-noise microscopy technology allows the creation of high resolution spectral images. This enables the characterization of nano-materials based on their chemical composition and added functional groups. It also provides the ability to spectrally confirm and map the presence and location of nanomaterials as they are integrated into many different biological environments. Examples illustrating the use of this technology to detect and characterize multiple types of nanomaterials in a wide range of biological matrixes will be presented.



Schaefer-Tec group supply a wide range of instruments for research and process controls in nano-micro technology. We recently partnered with CytoViva (Auburn, USA) to promote their hyperspectral microscopy technology in Europe.

INDUSTRY CASE STUDY #2 GLATIRAMOIDS

IRIS GROSSMAN, PH.D. (CONFIRMED), Vice President, Global Head of Personalized Medicine & Pharmacogenomics, Teva Pharmaceutical Industries.

While methods for evaluating the "sameness" of a small-molecule drug and a corresponding generic are well established, determining a set of methods that is sufficient to establish "sameness" of biosimilars or non-biological complex drugs (NBCDs) remains an area of active research.

Glatiramoids are non-biologic complex drugs (NBCDs) comprising four naturally occurring amino acids in a complex copolymeric mixture. The first and most thoroughly studied glatiramoid, glatiramer acetate (Copaxone®, Teva Pharmaceutical Industries, Ltd.) is approved for treatment of relapsing-remitting forms of multiple sclerosis, an autoimmune disorder characterized by neuro-inflammation and progressive neurodegeneration. Glatiramoid mixtures comprise a potentially incalculable number of structurally closely related active peptide moieties that, at present, cannot be isolated, quantified, or identified using even the most sophisticated available multidimensional separation techniques. Numerous studies have demonstrated that the glatiramer acetate in Copaxone® modulates innate and adaptive immune cell responses to promote anti-inflammatory and neuroprotective activities; however, the active epitopes in Copaxone® are unknown and the precise mechanisms of immunomodulatory activity responsible for its therapeutic efficacy are not entirely elucidated. The identity, quality, and consistency of a glatiramoid are inexorably linked to its own manufacturing process.

Several manufacturers now market glatiramoids in various countries that are purported to be generic or follow-on versions of Copaxone®; however, a full set of long term safety and efficacy data for these products is not yet available in the peer-reviewed medical literature. Sophisticated analysis techniques, though unable to completely characterize glatiramoid mixtures, can differentiate among them based on orthogonal physicochemical features and biological activities in multiple relevant test systems.

Traditional bioequivalence cannot be established for Copaxone® - neither pharmacodynamic nor pharmacokinetic validated markers could be established for Copaxone®, and its precise mechanism remains to be fully characterized. To this end, Teva applied microarray technology to characterize Copaxone® and compare it with differently manufactured glatiramoids in immunologically-relevant model-systems, including human monocytes and PBMCs, as well as and mouse splenocytes. Such studies identified significant differences in expression of genes and pathways associated with safety and efficacy, capturing cell-specific immunological signatures as well as pan-system shared patterns. The microarray findings in model systems were validated by three lines of evidence: (1) qRT-PCR; (2) human primary monocytes; (3) proteomic analysis. The presentation will include discussion on Probioglat, a purported generic glatiramer acetate that caused a tripling of serious adverse events and a seven-fold increase in relapses when introduced in Mexico. A second example will be presented in which Synthon's purported generic glatiramer acetate (Polimunol) was found to induce a different pattern of gene expression across hundreds of genes, enriching for dozens of relevant pathways, including inflammation and others associated with safety concerns, in multiple model systems. These genomic profiles may help explain the reported clinical inconsistencies in the GATE study.

These data demonstrate that applying microarray technologies to measure gene expression is an emerging approach to compare the biological impact of two medicines, particularly relevant for NBCDs. Such an approach is powerful because microarrays quantify the expression of 40,000+ genes in a single assay, enabling investigators to evaluate the impact of each medicine on a broad range

of relevant biological pathways and processes. Yet careful experimental design, including selection of appropriate model systems, and careful data analysis methods, are critical to ensure that these studies yield robust, relevant findings. Additional published studies using gene expression techniques to compare medicines will be reviewed and discussed, demonstrating how and when such technologies should (and should not) be utilized.
Following this critical review of the evidence base supporting standards for equivalence of glatiramoids, a proposed experimental framework for establishing "sameness" of two complex medicines will be presented.

ICG-LOADED PEGYLATED LIPOSOMAL DOXORUBICIN AS A THERANOSTIC NANODRUG DRUG DELIVERY

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INTRODUCTION

Cancer mortality is progression-dependent thus its treatment relies on effective therapy and monitoring of responses. Theranostic systems provide both imaging and therapeutic capabilities to address this problem. The photoacoustic contrast agent ICG was successfully incorporated into the PEGylated doxorubicin-encapsulating liposome, DOXIL® marrying two FDA approved entities. This was achieved through different protocols by varying the chronology of ICG incorporation: film insertion, freeze fracture and post-insertion. The method of liposome nanoscale sizing was also compared between extrusion and sonication.

METHODS

The lipids HSPC, cholesterol and DSPE-PEG_{2000} (56:38:6) were dissolved in an organic solvent prior to lipid film generation, its hydration and subsequent liposome downsizing. Doxorubicin is then loaded via active loading following establishment of a transmembrane pH/salt gradient. In the film insertion protocol, ICG is solubilised with the lipids in the organic solvent. In the freeze fracture protocol, the lipid film is hydrated with ICG solution and incorporated via freeze fracture. In the post insertion protocol, ICG is added to the preformed liposomes in the final stages of doxorubicin loading.
ICG incorporation efficiency was measured via absorbance in DMSO. Lipid concentration was calculated via Stewart assay. Doxorubicin encapsulation efficiency was measured via fluorescence. Optical properties were analysed via UV/Vis spectroscopy. Size and zeta-potential were measured via dynamic light scattering and electrophoretic mobility. In-vivo imaging was carried out through multispectral optoacoustic tomography (MSOT) imaging of 4T1 tumour bearing mice and validated through fluorescence cryosection following IV administration.

RESULTS

Using extrusion for the vesicle preparation led to less than half the intensity in optical density compared to the much more effective sonication. The optical property of all liposomes was stable over 18 days. Doxorubicin loading of the extruded systems was 82% for the film insertion and freeze fracture systems, and 90% for the post insertion. It was possible to load 94-98% for the sonicated systems. There was no leakage during storage of these systems at 4°C. At 37°C in 50% serum doxorubicin release was 20% or below for each system. All liposomes were 100-150nm in diameter carrying a surface charge between -15mV and -35mV. MSOT data showed peak tumour accumulation of sonicated, post inserted liposomes after 24 hours. Validation of liposomal drug delivery was proven through co-localisation of ICG and doxorubicin via fluorescence cryo-sectioning.

DISCUSSION

This work represents three different protocols for the construction of ICG-incorporated DOXIL® vesicles containing therapeutic concentrations of doxorubicin. Sonication rather than extrusion allowed a higher ICG incorporation efficiency. The post insertion protocol proved the most efficient in terms of ICG incorporation. It generated the highest resultant optical density, and successfully accumulated in the tumour as observed by MSOT imaging in vivo.

ACKNOWLEDGMENTS

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DESIGN, PREPARATION AND IN VITRO AND IN VIVO ANTITUMOR EFFECTS OF INTRACELLULARLY DEGRADABLE AMPHIPHILIC BLOCK COPOLYCURCUMIN NANOPARTICLES

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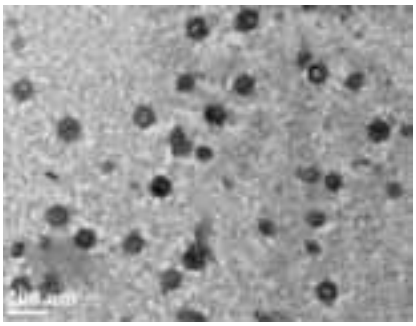
Nanoscale drug delivery systems (NDDSs) have been widely used for cancer treatment. For most of the current NDDSs, drugs are physically encapsulated or entrapped into the NDDSs, where drug release is mainly achieved via passive diffusion, making it difficult to achieve controlled release, a vital property for high therapeutic efficacy. As a result, most of the drug payloads may have been released before reaching the target sites, leading to reduced therapeutic efficacy and causing adverse side-effects. Furthermore, most NDDSs also suffer from drawbacks such as low drug loading (e.g. antibody-drug conjugates) and/or burst release (e.g. micelles/liposomes). To date, none of the current NDDSs can satisfy all of the requirements of an "idea NDDS" outlined by Langer et al.[1]
To make best use of the advantages of current NDDSs (passive and active targeting delivery to tumour) and overcome their drawbacks (unnecessary and even harmful on-way drug release before entering tumour cells and/or only a small quantity of drugs together with nano-carriers entering tumour cells) due to the physical incorporation or encapsulation of drugs in NDDSs, which often leads to uncontrolled drug release via diffusion, we take a new approach to prepare NDDSs which is based on so-called poly(active pharmaceutical ingredient) (PAPI) strategy where the APIs are incorporated into an intracellular cleavable polymer backbone (not physically incorporated in drug carriers) in combination with self-assembly characteristics of amphiphilic block copolymers. Here PAPI is defined as a polymer prepared by polycondensation of an API or its derivative having the same or similar bioactivity as a co- or sole-monomer. Considerable advantages here are that the physical-chemical properties of the PAPIs can be readily tailored by changing co-monomers or via chemical modifications. The PAPIs can be further made into various NDDSs where the API release can be controlled via stimuli triggered polymer degradation, overcoming the drawback of un-controlled, diffusion based drug release character commonly experienced in physically incorporated systems. Curcumin (Cur) is extracted from turmeric as food additive or traditional medicine for centuries in China and India, which can be used as chemopreventive agent and chemo-sensitizer for tumor cells, as we reviewed.[2] However, Cur's clinical trials or in vivo applications are limited due to its poor water solubility, poor stability at physiological pH. We encapsulated Cur in poly(anhydride-ester)-b-poly(ethylene glycol) copolymer micelles which were completely water-dispersible, overcoming the problem of poor water solubility that limited its efficacy and bioavailability and thus enhancing Cur anticancer efficacy.[3-5] In this paper, Cur containing 2 hydroxyl groups each molecule is used as a model example to demonstrate our PAPI strategy. We envisaged that an amphiphilic biotin-PEG-b-poly(curcumin-dithiodipropionic acid) (Biotin-PEG-PCDA), consisting of a high molecular weight (MW) hydrophobic PCDA block and a long PEG block, would assemble into stable core-shell nanoparticles (NPs), resulting in greatly increased water-solubility and

offering effective protection against recognition and uptake by the reticuloendothelial system, allowing for prolonged circulation and effective tumour targeted accumulation via the EPR effect. Moreover, the hydrophobic core made of PCDA should lead to high drug loading and providing effective protection for Cur against hydrolysis. The Biotin-PEG-PCDA NP should be stable during the blood transport but can readily release its API (Cur) once enters the target cancer cells/tissues triggered by the high intracellular glutathione (GSH, 1-10 mMv.s.~10 μM in blood)[6]and esterase[7]contents. The over-expressed biotin receptors found on cancer cells can be exploited for effective, active cancer targeting.[8]Importantly, the Biotin-PEG-PCDA NP can be loaded with a second anticancer drug (e.g.doxorubicin, Dox) to exploit the synergy of combinational dual-drug therapy to further enhance in vivo anticancer efficacy.

Cur was first copolymerized with dithiodi-propionic acid (DTDPA) to form a hydrophobic PCDA block, which was then coupled to a biotin-PEG to produce the amphiphilic biotin-PEG-PCDA di-block copolymer. The obtained copolymers were characterized by IR, NMR, GPC and had expected structures.

The Biotin-PEG-PCDA NP and doxorubicin (DOX) loaded Biotin-PEG-PCDA NP were prepared by a simple O/W emulsion followed by solvent evaporation method without using emulsifiers or surfactants. The Biotin-PEG-PCDA or DOX/Biotin-PEG-PCDA NP was spherical with a diameter of 76.5 or 82.1 nm, as shown in Figure 1.

A



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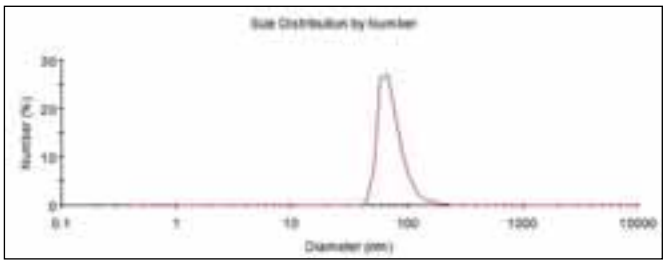

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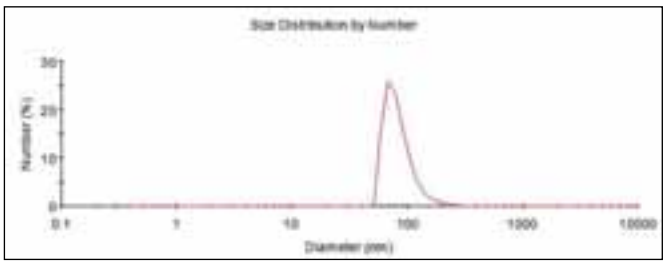

Fig1: Figure 1 The TEM graph and dynamic lasar scattering profiles of Biotin-PEG-PCDA NPs (A and B) and DOX/Biotin-PEG-PCDA NPs (C).

We did comprehensive research on Biotin-PEG-PCDA or DOX/Biotin-PEG-PCDA NP in terms of stability, drug release, antitumor effects and harvested lots of very interesting results. These results are summarized below.

The Biotin-PEG-PCDA has a much higher water-solubility, being 470 μg/ml (corresponding to 127 μg/ml equivalent of free Cur), respectively, representing an enhanced solubility of >500 fold over free Cur; its UV-vis spectra in phosphate buffered saline (pH 7.4) over a course of 24 h are almost identical, indicating that the Biotin-PEG-PCDA is stable under such conditions. In contrast, a dramatic decrease (>50%) of absorbance was observed for free Cur in PBS over 30 min, illustrating a high instability.

The Biotin-PEG-PCDA NP is stable at a low GSH concenration (e.g. 10 μM, similar to that in blood transportation), but is readily cleaved to release the Cur based APIs at 5 mM GSH (similar to the intracellular GSH level) and esterase.

Compared to the PEG-PCDA NP, the Biotin-PEG-PCDA NP exerts significantly higher cytotoxicities against both the human cervical HeLa and breast EMT6 cells.

The DOX/Biotin-PEG-PCDA NPs are significantly accumulated in the tumor, much higher in amount than those in other organs such as heart, spleen, lung or kidney after intravenous injection in the EMT6 xenograft mouse model, yielding the high inhibition rate of tumour growth (IRT, 79%), higher than that of free Cur (with IRT of 32%). The histological and immunohistochemical analyses results also reveal that the Biotin-PEG-PCDA NP are highly effective in inducing tumour cell apoptosis and inhibiting cell proliferation by providing effective anti-angeogenisis properties.

The DOX/Biotin-PEG-PCDA NP presents a GSH triggered DOX release profile, that is, DOX is slowly released at a low GSH concentration (10 μM) but much faster released at a high concentration of GSH (5 mM).

The DOX/Biotin-PEG-PCDA NP has effective in vitro and in vivo anti multidrug resistant (MDR) cancer effects. It can greatly increase the uptake of DOX by human MDR breast cancer cell line MCF-7/ADR and decrease its efflux, down-regulate P-gp expression and inhibit the activity of ATP in MCF-7/ADR, thus presenting the DOX/Biotin-PEG-PCDA NP's excellent reversal effects on MCF-7/ADR cells. The in vivo results with MCF-7/ADR bearing multidrug tumor nude mice model also show its significant in vivo anti MDR tumor efficacy.

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RNA NANOMEDICINES FOR INDIVIDUALIZED TUMOR IMMUNOTHERAPY: TRANSLATION FROM BASIC RESEARCH INTO CLINICAL PHASE I TRIALS

HEINRICH HAAS

Messenger RNA (mRNA)-based nanomedicines constitute a new class of pharmaceutical products with potential applications in tumor therapy, vaccination, or protein substitution therapy. In tumor immunotherapy, tumor antigen-encoding mRNA is delivered into antigen presenting cells (APCs) in order to induce T-cell mediated antitumoral responses. Manufacturing of tailored RNA pharmaceuticals for individual patients enables truly personalized cancer immunotherapies. For translation of this concept into clinical practice, various technological challenges regarding GMP-compliant manufacturing need to be solved. Pharmaceutical formulations for regular administration of the mRNA to the patients and delivery to the target site are required.

Here, a technology platform of injectable RNA nanomedicines that dispose high biopharmaceutical availability as well as efficient and organ-selective expression of the RNA is presented. Based on this technology, novel RNA pharmaceuticals for tumor immunotherapy could be translated into clinical development. The dedicated manufacturing processes offer a fully GMP-compliant, cost-effective and highly scalable production. Clinical trials are underway or in preparation.

LIPOSOMAL DRUG TARGETING USING GLYCANS AS LIGANDS FOR LECTINS – THE APPLICATION OF DOXORUBICIN IN CANCER TREATMENT

STEFAN HALBHERR

The research on nanoparticle-based drug delivery systems has been at the forefront of scientific inquiry for many years. So called nanomedicines provide new possibilities for reducing undesired adverse effects and improving clinical activity of anti-cancer drugs by using targeted delivery systems. Up to now, a number of liposomal drug formulations have been approved for clinical application for many different types of cancer such as Caelyx/Doxil, DaunoXome and Myocet. A major limitation of liposomes is the lack of organ and cell specificity that causes undesired side effects and premature, uncontrolled drug release.

In contrast to predominate targeting approaches that rely on protein-protein interactions (e.g. antibody or peptide mediated targeting) for drug delivery, the innovative technology of Dr. Noboru Yamazaki and InnoMedica is based on the active targeting function of specific glycans on the surface of liposomes. The modification of the liposomal surface with pre-selected glycans enables the site-directed binding to lectins that are up-regulated and associated with cancer related inflammation and the tumor microenvironment. Various lectins have already been described to play a pivotal role in tumor differentiation and metastasis in humans, rendering them ideal targets for specific drug delivery. InnoMedica's delivery system represents a step forward towards the refinement of current state of the art chemotherapy, allowing for the reduction of administered doxorubicin and associated side effects on the basis of its cancer targeting properties.

TRANSLATION OF BIOPHARMACEUTICALS FROM BENCH TO CLINICS – EXAMPLES FOR A NEW RECOMBINANT TUBERCULOSIS VACCINE AND DENSE BODIES AS CYTOMEGALOVIRUS VACCINE

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Translational research, i.e. the transfer from bench to bedside is more and more becoming the focus of funding programs and commitment of academic institutions. However, the step from excellent academic research into clinical testing is often hampered by missing expertise, regulatory experience, financial resources and lack of time on behalf of the researchers. Thus we risk missing high potential candidates which remain confined to the laboratories and never get to prove their value in patients.

It is crucial for successfully performing translational research that already early on the course is set for later gaining the acceptance of regulators and possible business partners. This includes securing of intellectual property, careful planning of a non-clinical and clinical roadmap and early involvement of regulators. In a reverse planning approach the developmental chain is designed stepwise backwards from the final goal, for example the target product profile for a clearly defined indication and patient population. A critical chain is defined how to achieve this final goal and tollgates are implemented at critical points to assess whether development is still on track or else adjustments are required. Such strategic planning significantly reduces the risk and developmental time so that a faster translation from bench to bedside is achieved. Furthermore, successful translational research can only succeed if the right partners from academia, contract manufacturers, GLP laboratories, clinical institutions, regulators, finance, and industry are joined together (Fig. 1).

Fig. 1: Translation of Biopharmaceuticals from bench to clinics requires competent partners from different fields of expertise coordinated by a straightforward and goal-oriented project management process.

We present two best practice stories where vaccine candidates from academia have been moved forward to proof of concept at different stages.

VPM2001 is a vaccine candidate against human cytomegalovirus (HCMV) infection originating from the Institute for Virology at the university medical Center Mainz. Infection with HCMV may cause serious and life-threatening disease manifestations in children and in immunosuppressed individuals. HCMV is the most frequent viral cause of birth defects in the developed world and no prophylactic treatment is available until today. VPM2001 is based on Dense Bodies (DB), subviral particles produced naturally in cell culture. They contain all major viral proteins in a natural confirmation while lacking the Nucleocapsid and the viral DNA. The particles of about 300 nm diameter are non-infectious and induce strong and lasting antiviral humoral and cellular immune responses in animals. The preclinical proof-of-principle has been shown in different animals and the next step will be a safety assessment in animals and humans.

VPM1002 is a tuberculosis (TB) vaccine candidate originating from the Department of Immunology at the Max Planck Institute for Infection Biology, Berlin. Conventional live Mycobacterium bovis Bacille Calmette Guérin (BCG) is still being used to vaccinate neonates in TB endemic regions of the world and protects against severe childhood complications of TB infection, mainly TB induced meningitis. It does not protect against the most common form of lung TB in adults and poses safety risks such as disseminated BCGosis, especially in neonates with compromised immune status, caused for example by HIV infection of the mother or child. VPM1002 is a recombinant BCG ΔureC::hly where the urease C gene (ureC) of BCG was functionally deleted by integration of the listeriolysin gene (hly). The genetic modification enables the antigens to escape from the lysosom, improves MHCI presentation of antigens and cross-priming and induces an improved immune response, characterized by multi-functional (IFN-gamma, IL-2, TNF-alpha) CD4 and CD8 T cells, central memory cells, and production of Th1 and Th17 type cytokines. Furthermore, activation of apoptosis acts as a safety feature by self limiting dissemination of VPM1002 within 40 days. The vaccine has successfully passed phase I in Germany in healthy male adults and phase Ib in South Africa, i.e. in the TB endemic environment, as well as phase IIa in healthy neonates in South Africa, always in comparison to conventional BCG. Currently a phase II in newborn infants, partially born by HIV infected mothers, is about to start in South Africa, with regulatory and ethical approval already available. In the previous trials VPM1002 was found to be safe and well tolerated, with significantly lower rate of abscesses at the injections site compared to BCG in neonates. Immunogenicity was at least equivalent to BCG with a tendency to superiority with regards to generating multi-functional CD4 and CD8 responses.

In conclusion, by carefully planning a lean non-clinical program in close cooperation between academics, regulators, and contract research organizations a fast translation from the lab to the first clinical trials (VPM1002: 5 years) is feasible.

DECODING THE EPR EFFECT: INVESTIGATING THE INFLUENCE OF TUMOUR FEATURES ON NANOMEDICINE ACCUMULATION, DISTRIBUTION, AND RETENTION

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INTRODUCTION

The poor pre-clinical to clinical translatability of anti-cancer nanomedicines is a significant challenge in the drug delivery field1. Despite the abundance of promising pre-clinical outcomes, the clinical efficacy of nanomedicinal therapeutics has been largely disappointing. There is an obvious disconnect between the pre-clinical testing of nanomedicines and the reality of treating patient tumours. The limited characterisation and understanding of the EPR effect2 and its heterogeneity across patient tumours makes it difficult to design clinically beneficial nanomedicines3.

Nanomedicine accumulation, distribution, and retention are expected to be influenced significantly by tumour features such as tumour vasculature, stroma, immune infiltrate, and morphology. Since these features vary between and within human tumour types, the EPR effect cannot be treated as a universal property of all solid tumours. The clinical implications of this suggest that only some patients would be expected to benefit from nanomedicine-based therapy.

The aims of this pre-clinical work were to assess the tumoural accumulation, distribution, and retention of a liposomal nanomedicine in two diverse tumour phenotypes. Clear insight into how tumour features and morphology influence nanomedicine behaviour is required to enable disease-driven rational design of new formulations; validation of clinically relevant animal models; and implementation of patient pre-selection efforts.

EXPERIMENTAL METHODS

Features of formalin-fixed, paraffin-embedded pre-clinical tumour material were characterised via standard immunohistochemical staining for CD31, αSMA, and F4/80.

Mice bearing subcutaneous flank Calu-3 or Calu-6 tumours were dosed intravenously with PEGylated liposomes (~90 nm diameter) loaded with doxorubicin (ammonium sulphate gradient liposomes purchased from Nplex Laboratories, United States). To assess liposome accumulation and retention, at various time points post-dose, tumours were excised, homogenised, and doxorubicin concentration was determined via mass spectrometry.

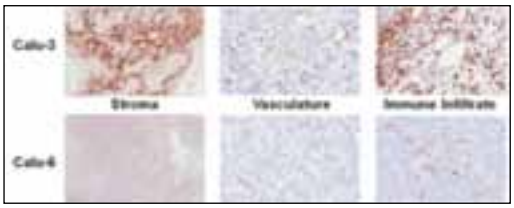
Mice bearing Calu-3 or Calu-6 tumours were dosed intravenously with PEGylated liposomes encapsulating computed tomography (CT) contrast agent (NanoVista, Canada). To determine the 3D intratumoural distribution of the liposomes, high resolution microCT scans were completed on excised tumours. A spatial resolution of ~17 µm was achieved using a Siemens Inveon Pre-clinical MicroPET/CT scanner at 80 kV/500 µA.

The efficacy of liposomal doxorubicin treatment (1.5 mg DXR/kg q7d x2) was evaluated in subcutaneous Calu-3 and Calu-6 tumour models in SCID and nude mice, respectively.

RESULTS AND DISCUSSION

Using two mouse subcutaneous xenograft models of human lung cancer, we investigated the role of tumour phenotype in liposome accumulation and intra-tumoural distribution, using two pre-clinical models known to present with different architectures. The levels of tumour stroma, vasculature, and macrophages were characterised in Calu-3 and Calu-6 tumours, and significant differences were observed (Figure 1). Calu-3 tumours presented with substantially higher levels of all features, relative to Calu-6 tumours.

Figure 1. Immunohistochemical staining for tumour stroma (αSMA; left), vasculature (CD31; middle), and macrophages (F4/80; right) in subcutaneous Calu-3 (top) and Calu-6 (bottom) tumour xenografts



Stark contrasts in nanomedicine behaviour were observed between the models. At 24 h post-dose, more than a 10-fold greater doxorubicin concentration was observed in the Calu-3 tumours, which present with a higher stromal density, vascular area, and level of immune infiltrate than do the Calu-6 tumours. Further, at 5 days post-dose, doxorubicin was still present in Calu-3 tumours, but was not detectable in Calu-6 tumours.

A difference in intra-tumoural liposome distribution between the phenotypes was shown in 3D via high resolution CT imaging (Figure 2). Liposomes localised preferentially in the tumour periphery in Calu-6 tumours, whereas a greater proportion of liposomes were located towards the core of the Calu-3 tumours, consistent with the greater density of blood vessels in the core of Calu-3 tumours. The accumulation of liposomes in Calu-3 tumours was also greater than in Calu-6 tumours. This is postulated to be the result of stromal support for the blood vessels in Calu-3 tumours, but not in Calu-6 tumours, where the vessels are not located within the stromal tissue.

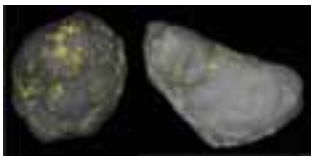


Figure 2. 3D intra-tumoural liposome distribution in Calu-3 (left) and Calu-6 (right) tumours at 72 h post-dose.

The increased drug accumulation and deeper penetration of the liposomes in the Calu-3 tumours correlated with a greater anti-tumour response to liposomal doxorubicin, relative to that observed in the Calu-6 model. This translated to a 73% tumour growth inhibition for liposomal doxorubicin treatment in the Calu-3 tumours, relative to 17% in Calu-6 tumours.

CONCLUSION

We have demonstrated that liposomal drug accumulation and liposome distribution within tumours vary significantly between these two pre-clinical tumour models possessing different phenotypes. In this research, increased accumulation and penetration were correlated with superior anti-tumour efficacy of liposomal doxorubicin in Calu-3 tumours versus Calu-6 tumours. Additional studies evaluating these parameters in other pre-clinical models with complex architectures are required to build an understanding of the relationship between tumour features, nanomedicine behaviour, and therapeutic outcome. This insight could be translated to the pre-selection of the clinical patients who may be more likely to benefit from nanomedicine-based cancer therapy.

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strains. These two compounds could be the next generation antimalarials and will be combined with other agents before general medical use.

The scientific processes for discovering medicines for neglected diseases of the poorer populations is identical to that for diseases of more affluent populations but there is often much less basic science data available for the former diseases.

THE DEVELOPMENT AND CLINICAL EXPERIENCE OF CTL019

JENS HASSKARL

Using the body's own immune system by adoptive transfer of T cells is a promising treatment approach to fight cancer. Human T cells can be modified using viral and non-viral vectors to express engineered T-cell receptors (TCRs) or chimeric antigen receptors (CARs) to specifically target tumor cells. CARs combine a single chain variable fragment (scFv) of an antibody with intracellular signaling domains. First-generation CAR technology used antigen-specific scFV and a signaling transduction domain such as CD3 ζ without co-stimulatory signals. While resulting cells could bind to the tumor cells, efficacy was low. Second generation CAR constructs include a costimulatory domain of either CD28, 4-1BB (CD137) or OX-40, while third generation CARs use more than one costimulatory domain (e.g. CD28 and 4-1BB). Several groups have developed their own CAR constructs using this approach and various CAR therapies are currently tested in clinical trials.
The most frequently tumor antigen targeted is CD19. CD19 is expressed in B-cells and in most B-cell malignancies. Thus, current clinical trials are targeting CD19+ acute lymphoblastic leukemia, chronic lymphocytic leukemia and various CD19+ non-Hodgkin's lymphomas. We will present recent updates from ongoing trials with a second generation CD19-directed CAR using the 4-1BB costimulatory domain that was developed by the University of Pennsylvania group.

NANOMEDICINE: SCIENCE, BUSINESS, AND IMPACT

MICHAEL HEHENBERGER, HM NanoMed

The goal of Translational Nanomedicine is achieved when patients can benefit from the nanomedical breakthroughs conceived by biomedical scientists and engineers.
The talk will examine the steps needed to take a nanomedical idea from concept to established clinical practice. It will cover the respective roles of all stakeholders of the healthcare ecosystem around the patient. Those stakeholders include public and private sponsors of research; diagnostics, device and drug companies; government regulators; health insurance "payers"; and healthcare "providers".
Impact can only occur when the stakeholders are aligned, when a chain of trust has been created among them, and when there is agreement across the ecosystem to incorporate a given novel nanomedical diagnostics and/or therapeutics procedure into medical practice.
When there is real Impact there will also be renewed willingness to provide more funding for basic research, thereby starting a new cycle.
The impact of the Human Genome Project from 1988 to 2010 has been the subject of a recent study by the Battelle Memorial Institute. It led to the conclusion that US Government investments of $5.6B (in 2010 inflation-adjusted US dollars) helped generate a 141-fold financial return based on applications in a number of important areas of human and animal health, agriculture, environmental science, and even forensics, justice and security.
Other potential areas of high nanomedical Impact are cancer diagnostics and therapy, regenerative medicine, neuroscience, and immunotherapy.
Finally, there will be a discussion of the Pros and Cons of recent attempts to accelerate the translation of nanomedical research results by means of targeted public and private investments.

BENEFITS AND LIMITS OF DETECTION OF THE ZETAVIEW NANOPARTICLE TRACKING ANALYSIS (NTA)

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In Nanoparticle Tracking Analysis (NTA) small particles such as extracellular vesicles (EVs) are visualized in a laser scattering video microscope. The tracked translational Brownian motion of the particles in the liquid is evaluated for size distribution and concentration. With an electric field applied, the electrophoresis zeta potential distribution can be determined. With this technique, the size of single particles can be measured between 20 nm and 2 µm. The concentration range is between $4x10^5$ and 10^9 particles / mL. Fluorescence evaluation and multi-variate statistics as a tool for discrimination of sub-populations have been added recently.
The NTA is made fast and reliable for daily clinical research work. Methods to guarantee statistical relevance, trueness and repeatability are discussed on selected examples (Fig. 1).

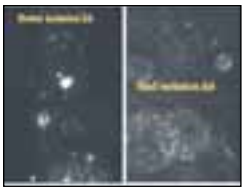


Fig 1: Example of two isolation kits

Yield of production and cleanliness of these EVs are important factors. Subpopulation evaluation concurs the multitude and quality of information (Fig 2).

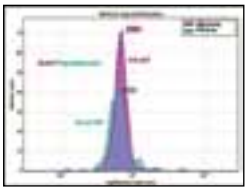
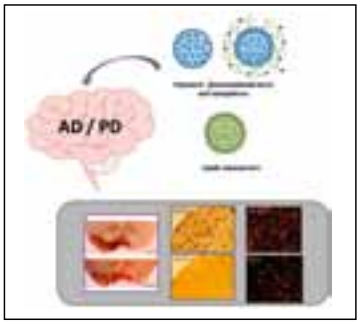


Fig. 2: High resolution discrimination of subpopulations of different particle types of same size

IMPROVING THE TREATMENT OF ALZHEIMER AND PARKINSON BY NOVEL DESIGN OF MICRO- AND NANOPARTICLES

ENARA HERRAN, Department of Pharmacy and Pharmaceutical Technology. University of the Basque Country. Vitoria-Gasteiz. Spain.



Over the past years there has been a remarkable increase in the prevalence of neurodegenerative diseases (NDs) such as Alzheimer's and Parkinson's diseases. Current therapies for these conditions are only able to treat their clinical symptoms, with a temporary effect and without halting the neurodegenerative process. Due to the low effectiveness of these treatments, promising and interesting therapies, such as growth factors (GFs), have been investigated. Nevertheless, the success of these new treatment options not only depends on the application of the specific neurotrophin, but also on a suitable approach for delivering these proteins to the brain. The development of appropriate drug delivery systems (DDS) may allow an enhancement of the GFs concentration in the brain, reaching therapeutic levels. In this sense, micro and nanotechnologies could offer novel opportunities to formulate GFs using a wide variety of biodegradable nanocarriers, including polymeric nanospheres and lipidic nanocarriers, as possible treatments for Alzheimer's and Parkinson's disease. This short communication will introduce results obtained from the in vivo studies that are carried out in our laboratory after the administration of these DDS loaded with neurotrophic factors, in Alzheimer's disease and Parkinson's disease animal models.

DRUG DISCOVERY FOR NEW ANTIMALARIALS

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Currently, the artemisinin combined therapies (ACTs) are the standard of care for the treatment of malaria caused by Plasmodium falciparum. However, about 8 years ago a P. falciparum parasite emerged on the Western border of Cambodia (Phyo AP et al. Lancet 379: 1960–1966, 2012) that is described to be resistant or at least takes distinctly longer treatment times to elimination in patients, a precursor sign for resistance. Because of this observation The Wellcome Trust contacted NITD to explore drug discovery for new antimalarials. A consortium led by NITD was established in 2007 including the Genomics Institute of the Novartis Foundation in La Jolla, California, The Swiss Tropical and Public Health Institute in Basel and the Biomedical Primate Research Center in the Netherlands with the mission to find new antimalarials both for Plasmodium falciparum and vivax. The consortium was jointly financed by the Wellcome Trust, the Economic Development Board of Singapore, Novartis and the Medicines for Malaria Initiatives.
This presentation relates the discovery and development of two compounds resulting from the work of the consortium: NITD 609, a spiroindolone directed at the blood stages of P. falciparum and P. vivax, with a new mechanism of action inhibiting a parasite Na+ pump (PfATP4) and with the potential of a treatment regime shorter than standard of care. NITD609 is in clinical PhIIb and has been shown to rapidly eliminate the parasite in patients. The second compound is a imidazolopiperazine, NITD156, affecting P. falciparum and vivax blood and dividing liver stages, but not P. vivax hypnozoites. NITD156 is in clinical PH IIa with a still unknown mechanism of action but active on all known resistant P. falciparum strains. These two compounds could be the next generation antimalarials and will be combined with other agents before general medical use.
The scientific processes for discovering medicines for neglected diseases of the poorer populations is identical to that for diseases of more affluent populations but there is often much less basic science data available for the former diseases.

NEW APPROACHES FOR SEPSIS DIAGNOSIS AND THERAPY: THE ERA OF MICROVESICLES AND NANOPARTICLES

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Sepsis remains a major cause of mortality in intensive care units and its incidence is increasing along with the antibiotic resistance of the causing microorganisms. Sepsis the presence of systemic inflammatory response syndrome (SIRS) due to infection, and represents a potentially life-threatening condition through resultant multi-organ failure.1 The management of sepsis is technically demanding and costly with only few therapeutic options available. Prompt diagnosis and hence early treatment has a major impact on patient survival. However, diagnosis can be challenging, especially in critically ill patients, most of whom have signs of systemic inflammation, which can be hard to differentiate from sepsis. Diagnostic uncertainty exists; if antibiotic treatment is delayed outcomes are worse, if antibiotics are prescribed too readily then bacterial resistance rates rise, as well as costs and other complications.
In the first part of this contribution, we will discuss the potential of novel microvesicle-based activity assays for sepsis diagnosis. Leukocytes have been shown to release trigger-dependent microvesicle populations in response to bacteria, hence may be utilized to confidently diagnose sepsis. We will show advanced high resolution electron microscopy and Raman spectroscopy data visualizing the process of microvesicle shedding from immune cells in response to Staphylococcus aureus and other bacterial and non-bacterial triggers. Furthermore, we report first pilot study data on the diagnostic performance of a microvesicle-based assays for sepsis diagnosis in a relevant intensive care unit patient cohort, including sepsis patients and patients suffering from non-infectious SIRS.
In a second part, we will briefly discuss novel therapeutic approaches for the rapid removal of pathogenic compounds (e.g., inflammatory mediators, bacterial toxins and bacteria) from whole blood based on magnetic separation. We will show how nano-sized magnetic beads bind to disease-causing factors hence allowing rapid blood cleansing both in vitro and in vivo 2,3 and discuss potential hurdles encountered when translating promising nanomaterial-based approaches into clinical settings.

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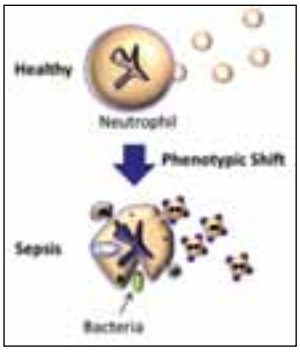

Fig. 1: A disease-specific phenotypic shift in microvesicle plasma populations could be used to confidently diagnose infectious processes.

TRANSLATION OF DRUG EXPOSURE BETWEEN VIRTUAL POPULATIONS TO SUPPORT DRUG DEVELOPMENT

MARTIN HOBE

Due to the massive expenses for research-orientated pharmaceutical companies to be spent during drug discovery and development, there is an increased need to streamline the R&D programs involved and to identify the most promising drug candidates at the earliest time point possible. Physiologically-based pharmacokinetic (PBPK) modelling has proven to be a very useful tool for supporting this process by providing valuable information on drug absorption, distribution, metabolization and elimination.
One area of increasing interest is the prediction of drug pharmacokinetics in a variety of special populations by translating from virtual study populations validated with data from real-life studies to other virtual populations relevant during the development or registration process. By integrating proprietary physiological knowledge and data available in the public domain, we have created a software platform integrating physiologically-based whole-body models for a wide range of such special populations. These include children, hepatically/renally impaired patients, elderly and diseased persons as well as several ethnicities.
Here, we demonstrate the creation, validation and application of such a physiological database using the example of a population of elderly individuals and the consequences of physiological changes on pharmacokinetics associated with increasing age.

IRON OXIDE NANOPARTICLES: CAN WE REACH THE HOSPITAL YET?

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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 transfections1-6. Iron oxide, γ-Fe2O3, 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. During the last 10 years several products based on iron oxide were on the market, especially used as contrast agent for MRI investigation of the liver. A second group of commercial iron oxide nanoparticles are on the market targeting the treatment of iron anaemia. In contrast of the first product group, the latter named the active iron oxide substance as iron complex, even the published TEM investigation shows clearly iron oxide nanoparticles in a similar size range of 3 to 8 nm8. The iron oxide nanoparticles used as MRI contrast agent are recently disappeared from the market. This is in contrast to the research activities resulting in more than 600 papers per year, still within creasing tendency.

THE REASONS FOR THIS DIFFERENCE IN MARKET AND RESEARCH ARE:

The MRI contrast was only for liver approved . The injected nanoparticles are taken up relatively fast in the healthy liver. Therefore this passive targeting was very efficient for this type of investigation. Other target of interest, like nymph nodes showed a much less specific targeting and therefore no new applications for this type of nanoparticles could be established.

New market for iron oxide nanoparticles, which are absolutely necessary to reach a volume which makes this type of nanoparticles economical interesting have to be developed. Key is the more specific targeting of the particles to organs and cells. This is only possible by a more complex coating of the particles with targeting moieties.

Studies conducted in the last few decades on the interaction of engineered nanomaterials with biologically relevant molecules have improved our understanding of the behaviour of these materials in human and animal bodies and have helped to identify in vitro assays that are predictive of in vivo biodistribution/toxicities. However, there are still valid concerns regarding in vitro methods for determining the biocompatibility of NPs or toxicity tests for engineered NPs9. After NPs have been in contact with biological media, their surfaces are covered by various biomolecules (e.g., proteins), which is known as a “protein corona” 9-14. Here, using the unique magnetic properties of the SPIONs, the NPs were extracted from rat sera after in vivo interaction with the rat’s physiological system; thus, one of the very few studies on in vivo protein-NP complexes was performed. The SPION core of PVA coated NPs does not (or minimally) have an influence particle biodistribution and, most likely, the core particles are protected by PVA molecules against direct interaction with plasma proteins and cells. The information from ex vivo protein adsorption and biodistribution bring us more accuracy and understanding of the overall picture of NPs fate in vivo initially from the NP-protein interaction to physiological aspect10.

This presentation will give some insight in the further development of iron oxide nanoparticles, focusing on the challenges to enter new field of medical applications including scientific and regulatory aspects.

ACKNOWLEDGEMENTS

We would like to acknowledge the Nanodiara FP7 Project of the EU Framework 7 Programme, Contract No. NMP4-LA-2009-228929, and the Swiss National Science Foundation (SNSF), Fund No. 205321-120161/1, for their financial support. The work was carried out in close cooperation with Usawadee Sakulkhu1, Lionel Maurizi1, Marie-Gabrielle Ollivier Beuzelin (Laboratory of Powder Technology, Ecole Polytechnique Fédérale de Lausanne, CH-1015 Lausanne, Switzerland.) Morteza Mahmoudi (Department of Nanotechnology and Nanotechnology Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran; and Division of Pediatric Cardiology, Department of Pediatrics, Stanford University School of Medicine, Stanford, California 94305-5101, United States*, Mahdi Motazacker (Departmentof Experimental Vascular Medicine, Academic Medical Center, Amsterdam, the Netherlands) , Marcel Vries and Farhad Rezaee (Department of Cell Biology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands); Azza Gramoun and Jean-Paul Vallée (Department of Radiology, University of Geneva and Geneva University Hospital 1211 Geneva 14, Switzerland.)

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USING NANOPARTICLE TRACKING ANALYSIS (NTA) FOR ACCURATE AND ROBUST NANOSUSPENSION CHARACTERISATION

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The sizing and characterization technique, Nanoparticle Tracking and Analysis (NTA) is described which allows nanoparticles in a suspension to be individually but simultaneously detected and analysed in real time using a laser-based microscope system. NTA has been commercially developed over the past ten years and now, with over 900 systems installed, is a key characterisation technique in many fields of nanoparticle characterisation particularly in the application of nanomedicines, nanopharmaceuticals and exosomes research and development. Here we discuss the technique, its application to these fields and how recent developments are further enabling advancements in these fields. In this method a laser beam passes through a suspension at a low angle. The particles scatter light which is collected onto a digital camera by a microscope-type configuration (Fig 1a). Particles between 10-2000nm are tracked individually (Fig 1b) and their diffusion coefficient, and therefore size, calculated directly from their speed (Fig 1c).

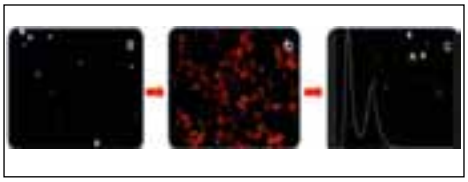

Fig. 1: a) Scattered light from particles collected on CCD, b) particles tracked and c) size distribution calculated

This technique provides significant advantages for sizing over traditional light scatter techniques (e.g. DLS) as the individual tracking of particles results in a greater ability to cope with polydispersed suspensions providing higher resolution.
The technique is also capable of measuring particles in a range of media (not requiring a particular media, or being restricted to pure water). This is relevant across all biological applications, as it is well known that colloidal properties are strongly influenced by their environment.
In addition to its simplistic but powerful sizing approach the technique generates directly a measure of absolute particle concentration, it can be integrated with fluorescence filters to allow fluorescently labelled/loaded particles to be selectively analysed.
In the field of pharmaceuticals, a crucial question under inspection is that of protein aggregation and its measurement thereof [1]. In this field NTA has been identified as a technique for characterizing this potentially over previously available techniques [2].
This technique has variously been applied to drug delivery particles such as liposomes, viral vaccines, bacteriophage, VLPs and controlled release drugs. In viral vaccines, for example, where the standard technique for titering a sample (to measure the concentration of virus) takes several days and could not take into account aggregation, the NTA technique is identified as a rapid and accurate alternative [3]. As the technique can also be integrated with fluorescence filters this gives the potential for fluorescently labelled/loaded particles to be selectively analysed. This can be of particular import where a suspension is not purified.

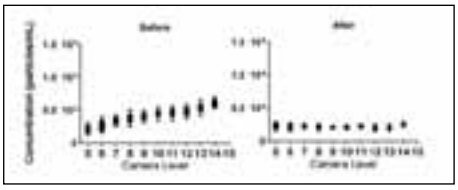

Fig. 2: Concentration data showing the influence of camera level before and after concentration measurement improvements.

Recently NTA has been significantly improved for its robustness and ease of use with automated camera setup settings and an autofocus function. In addition developments have been made to the concentration measurement improving the reproducibility, which has been verified by application of an inter-laboratory comparison (ILC). This has improved both repeatability and susceptibility to analysis settings (see Figure 2).
The technique, these novel developments and their application to the above fields will be described, explaining the importance of obtaining a complete particle characterisation.

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REPROGRAMMING T-CELLS: MANUFACTURING CHALLENGES OF CAR THERAPIES

ALEXANDER HUBER

Adoptive cell therapy (ACT) is a term to describe the transfer of lymphocytes to mediate treatment effect. ACT is a “living” treatment because the administered cells can proliferate in vivo and maintain their antitumor activity. A major factor limiting the successful use of ACT in humans is the identification of cells that can target antigens selectively expressed on the cancer and not on normal tissues. Presently several types of therapies are advancing on a path toward regulatory approval. Chimeric antigen receptor therapies (CART) may pave the way for this truly personalized therapy: CTL019 is used to equip patients’ T-cells with a chimeric antigen receptor that recognizes CD19. Thus, CTL019 reprogrammed T-cells recognize CD19+ B-cell malignancies and they can be used to treat cancer indications such as acute lymphoblastic leukemia and non-Hodgkin’s lymphoma (NHL).
CART therapy needs a new business model since one batch produced is only for one patient. A decentralized production is required with significant investments in facilities that are able to deal with cell processing in large scale under GMP. Overall, a novel supply chain needs to be established to supply worldwide demand with the same raw materials and consumables. Many materials and the whole manufacturing process needed to be adapted from an academic setting into a fully approved GMP process that is able to supply the global demand for the indications mentioned. The fact that regulatory requirements are still being formulated by the agencies adds layer of complexity to manufacturing and supply for cellular therapies.
Challenges for supply and for large scale manufacturing of ACT will be explained using CTL019 as an example case.

THE PRINCIPLES OF KNOWLEDGE BASED MEDICINE AND THE ROLE OF NANOMEDICINE

PATRICK HUNZIKER

Knowledge-based medicine goes beyond current medical and pharmaceutical paradigms by integrating the broad spectrum of medical technology with the individualized view of a patient and his disease in a comprehensive manner, and is fundamentally enabled by the recent developments in nanomedicine, targeted medicine and complementary leading edge technologies.
Nanomedicine, the application of nanoscience and nanotechnologie to the benefit of the patient, has evolved from a science fiction topic in the late 20th century to a strong and growing research field in the last decade. Numerous groups are moving from in vitro and preclinical research to clinical and industrial translation. This talk will highlight the importance of an integrated approach to nanomedical research and development, will identify the need for a systematic understanding of nano-bio interactions as a sine qua non for further progress, will emphasize the importance of a integrative approach encompassing complementary technologies and approaches to the patient, and will discuss strategies to overcome the translation challenges, to achieve a benefit for patients and society, in developed and developing countries likewise.

THE PRINCIPLES OF KNOWLEDGE BASED MEDICINE AND THE ROLE OF NANOMEDICINE

PATRICK HUNZIKER

Cardiovascular disease is the key cause of death in the western world and its prevalence is rapidly increasing in developing countries.
As it is a major cost driver, major advances in prevention, early diagnosis and cost-effective therapy of this disease is needed.
The current guidelines to cardiovascular management are based on the “evidence-based” flavor of medicine based on average results in large cohorts of patients and limited personalization.
The presentation gives an overview and examines the need for personalization and for management strategies based on knowledge about individual patients and disease course, an approach termed “knowledge-based”.
Nanomedicine and targeted medicine offer new options for improved insight into individual variations of the disease process and elucidation of optimal targets for a given patient, and yields new tools for better monitoring of the disease process along the time line of disease.
Finally, it is claimed that eradication of atherosclerosis is potentially feasible in the forseeable future, a development that would have a major impact on healthcare systems globally, and therefore justifies a significant boost in research and development support towards this goal.

SUPER-RESOLUTION INTERFERENCE MICROSCOPY FOR NANOPARTICLES 3D MEASUREMENTS

PAVEL IGNATYEV, CEO, AMPHORA Laboratories LLC, Moscow, Russia

The technology of Modulation Interference Microscopy (MIM) brings together design, optical, methodical and algorithmic solutions to develop super-resolution microscopes for 3D imaging and metrology. Three generations of microscopes based on the MIM technology have been developed so far for biomed studies and material science.
In this study, we demonstrate essential principles of the MIM technology, and its applications for liposomes and chitosan nanoparticles investigations, as well as interaction visualizing of metal oxides nanoparticles with cancer cells. Resolution of MIM based microscopes (0.1 nm vertical and 10–100 nm lateral) was demonstrated in the course of experiments in HOPG structures and in targets developed for lateral resolution tests.
The results of MIM applications in the biomed area, particularly, for studying nanoparticles, blood cells, cancer cells and viruses indicate that it has strong potential for use in clinical diagnostics.

ASSESSING GENERIC COMPLEX DRUG PRODUCT EQUIVALENCE - FDA PERSPECTIVES

WENLEI JIANG

In the United States (US), generic drug products evaluated as (1) safe and effective, (2) pharmaceutically equivalent, (3) bioequivalent, (4) adequately labeled, and (5) manufactured in compliance with Current Good Manufacturing Practice (CGMP) regulations are considered therapeutically equivalent and expected to have the same clinical safety and efficacy profile as the reference listed drug (RLD).
With pharmaceutical and regulatory sciences advancement, complex drug products having supra-molecular structures or containing an active ingredient with a distribution of molecular weight were developed and approved. Examples of complex drug products include liposomes, iron-carbohydrate complex drugs, protein bound drugs, mixture of naturally occurring substances, and others. Controversies exist regarding whether generic applicants are able to demonstrate therapeutic equivalence of generic versions of complex drug products. Regulatory opinions also differ in terms of approaches/requirements to demonstrate complex product equivalence. FDA has provided product specific guidance on the generic drug development path for some complex drug products.
In this presentation, challenges to demonstrate sameness/equivalence of complex drug products will be outlined. Case examples to illustrate scientific considerations in FDA complex drug product equivalence guidance development and product review will be presented. In addition, FDA pre-market research to support guidance development and post-market surveillance activities to monitor the safety and efficacy of approved generic complex drug products will be discussed.

LIGHT/ROS-RESPONSIVE NANOVESICLES FOR ANTICANCER AND ANTIINFLAMMATION THERAPY

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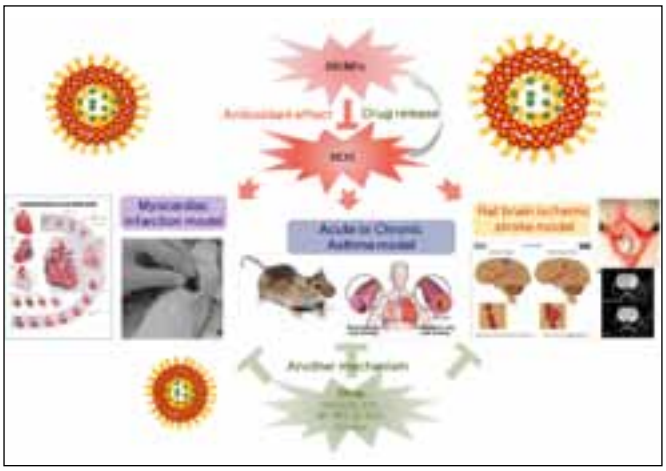
External or internal stimuli-responsive drug delivery systems have been of great interest in that drug molecules are to be released to the site of a disease in a controlled manner, thereby improving efficacy but minimizing toxicity. Here we report a dual light/ROS stimuli-responsive bilirubin nanovesicles (BNVs) for potential anticancer and anti-inflammation therapy. Bilirubin is a final metabolite of heme and functions as a potent antioxidant by scavenging ROS in our body. More interestingly, bilirubin can undergo photo-mediated isomerization to more water-soluble compounds. To use bilirubin for biomedical applications, it was first converted to pegylated bilirubin, which undergoes self-assembly to yield uniform nanovesicles with size of ~120 nm. In this lecture, preparation, characterization, and stimuli-induced disruption behavior of BNVs will be presented. Furthermore, as proof-of-concept, therapeutic efficacy of BNVs on a mouse colitis model will be also presented.



TARGETED POLYMERIC NANOMEDICINES FOR THE DELIVERY OF ANTI-INFLAMMATORY BIOLOGIC PROTEINS

NAZILA KAMALY

Inflammation is an essential biological response that is required for tissue homeostasis after injury or infection. Chronic inflammation however is destructive, can lead to tissue damage, and is a hallmark of many diseases such as arthritis, cardiovascular disease, and cancer. New potential therapeutic targets in addressing diseases associated with unresolved inflammation and their underlying mechanisms are now being investigated and understood, making therapeutics which dampen inflammation and enhance resolution of considerable interest - in particular those which can achieve this in a controlled manner with minimal host collateral damage.
This talk will present investigations into the development of targeted anti-inflammatory controlled-release polymeric nanomedicines for the treatment of inflammation driven diseases including atherosclerosis and colitis. These findings support the concept that defective inflammation resolution plays a role in these chronic conditions, suggesting a new form of therapy and shift in thinking from antagonistic to agonistic approaches for the treatment of these types of disease. The nanoengineering, characterization and in vivo biological investigations of polymeric nanoparticles containing a payload of biologic drugs including a potent biomimetic peptide and the anti-inflammatory cytokine IL-10 will be presented.

FRONTIER OF INTEGRATED INTRACORONARY STRUCTURAL-NANOMOLECULAR IMAGING OF HIGH-RISK PLAQUE

JIN WON KIM, M.D., Ph.D., F.A.C.C.,Korea University Medical Center, Guro Hospital Cardiovascular Research Center

With the advances in molecular biological techniques, a growing body of evidence has provided key mechanistic insight into atherosclerosis and revealed that coronary atheroma inflammation is a critical mediator of plaque complications, and demarcates high-risk plaques which accounts for sudden cardiovascular events. Once recruited to the atheroma, monocytes differentiate into lesional macrophages and ingest oxidized lipoproteins, leading to a foam cell phenotype. Inflammatory cells, chiefly macrophages and T cells, elaborate destabilizing matrix metalloproteinases (MMPs),

cysteine proteases, and serine proteases that can degrade extracellular matrix, collagen and elastin in protective fibrouc cap. Inflammation also induces smooth muscle cell (SMC) death, causing uneven thinning and/or rupture of the cap. Molecular imaging techonology targeting at a specific biological process of atherothrobmosis yields complementary new informations at the molecular levels not available with conventional imaging.

The recent advent of molecular sensing with intravascular near-infrared fluorescence (NIRF) technology using an injectable CatB protease activatable nanoagents demonstrated the feasibility of one-dimensional optical fiber-based NIRF catheter to image the biological pathways related to plaque instability in coronary-sized vessels. However, the point sampling nature of the fluorescence detection inherently limited this approach. To address this need, our group have developed the fully integrated catheter system having both OCT and NIRF molecular imaging in a single fiber based on the OCT clinical platform. This integrated OCTI-NIRF could simultaneously image arterial anatomy and arterial molecular detail. This highly translatable fully integrated structural-molecular imaging offers the synergistic potential to identify the high-risk plaque characterized by abundant inflammation and thin-cap fibroatheroma (TCFA).

MUCUS PERMEATING NANOCARRIERS FOR THE DELIVERY OF PROTEINS

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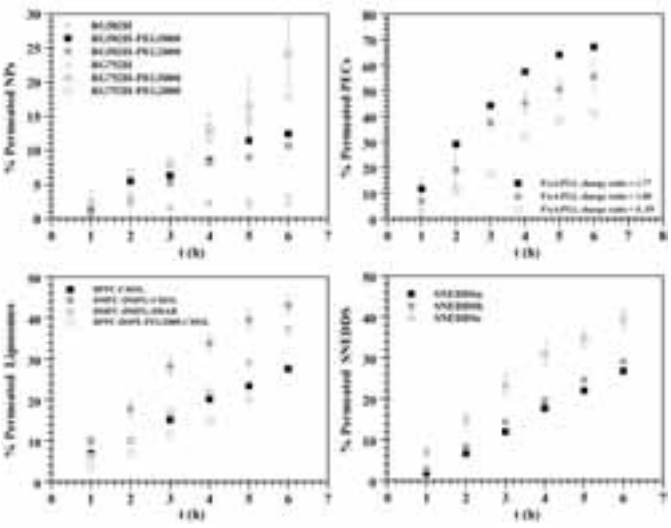
Macromolecular drugs (e.g., peptides, proteins, etc.) have the unique ability to tackle challenging diseases but their structure, physicochemical properties, stability, pharmacodynamics, and pharmacokinetics place stringent demands on the way they are delivered to a specific site/tissue in the body. At present, protein drugs are usually administered parenterally, but this route is less desirable and poses problems of oscillating blood drug concentrations. Moreover, their short biological half-lives necessitate in some cases multiple injections per week causing considerable discomfort to the patients. Nanocarrier-based drug delivery systems can diminish the toxicity of biopharmaceutics, improve their bioavailability and make possible their administration via less-invasive routes. To date various types of nanocarriers have been developed for the mucosal administration of macromolecular drugs. Apart from oral vaccines which target Peyer's patches that are not covered by a mucus gel layer, mucosal nanoparticulate delivery systems have to permeate the mucus gel barrier in order to reach the epithelium. More specifically, an ideal mucosal nanoparticulate delivery system should exhibit an enhanced permeation rate through the mucus gel layer thus allowing the delivery of the therapeutic payload to the epithelium. Additionally, it should exhibit a sustained drug release profile and sufficient protection towards enzymatic degradation of the drug, thus, resulting in increased bioavailability of macromolecular drugs. However, none of the available nanocarrier-based drug delivery systems is capable of efficiently permeating the mucus gel barrier.

The mucus gel layer consists of highly entangled mucus glycoproteins and, depending on the type of mucosa, varies in thickness from 0.5 μm (in ocular surface) to 100 μm (in large intestine). Small molecules can easily move through the three-dimensional network of mucus glycoproteins, whereas, the mucus layer is almost impermeable to macromolecules and nanoparticles. Current mucus penetration technologies, aiming at overcoming the mucus gel barrier, result in the disruption of the entire mucosal tissue which is highly undesirable from a toxicological point of view. Thus, alternative mucus penetration strategies (e.g., controlled release of mucolytic agents, immobilization of proteolytic enzymes, thiomer based nanoparticles exhibiting pH reactivity, zeta potential changing systems, self-nanoemulsifying drug delivery sustems, nanocarriers simulating the shape of Helicobacter pylori, etc.) focusing on the local and selective disruption of the mucus layer, need to be developed, aiming at facilitating the local transport of nanocarriers and allowing them to deliver their therapeutic payload to the epithelium or to be uptaken by the epithelial cells.

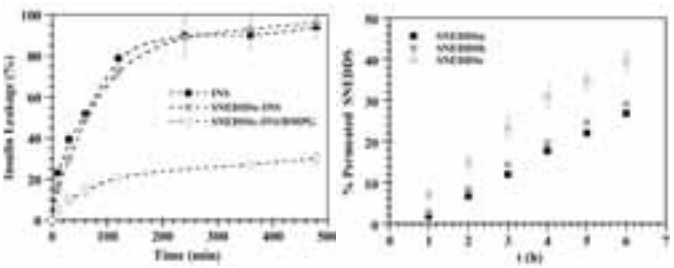
This paper addresses the development of mucus permeating nanocarriers according to the aforementioned strategies. More specifically, nanoparticles with "slippery" surface (i.e., poly(lactide-co-glycolide)-polyethylene glycol (PLGA-PEG) NPs, and polyelectrolyte complexes (PECs) consisting of polyacrylic acid (PAA) and poly-L-lysine (PLL) or polyarginine (PArg)) and liposomes loaded with the mucolytic agent 4-mercaptobenzoic acid (4MBA) were prepared. The PLGA-PEG NPs were prepared by the method of double emulsion following the synthesis of PLGA-PEG copolymers via a carbodiimide reaction between PLGA (e.g., RG502H, RG752H) and various types of functional PEG (e.g., NH2-PEG-NH2 (MW 3 kDa), CH3O-PEG-NH2 (MW 2 and 5 kDa)). The effect of PLGA (i.e., MW, lactide:glycolide ratio) and PEG (i.e., MW, functional groups) type on the conjugation efficiency of PEG as well as on the properties of the produced NPs (i.e., average size, mucus permeability) was examined. Polyelectrolyte complexes (e.g., PAA/PLL, PAA/PArg) were synthesized by ionic complexation. The effect of various process parameters (e.g. PAA / polycation charge ratio, polyelectrolyte concentration, PAA molecular weight, mixing order) on the PECs size and zeta potential was examined. Pegylated and non-pegylated liposomes containing 4MBA were prepared by the hydration/extrusion method. The effect of the liposome composition (i.e., lipids combination and molar ratios) on the properties of the produced liposomes (i.e., average size, encapsulation efficiency of the mucolytic agent, release profile of 4MBA from the liposomes, mucus permeability) was examined. For the development of SNEDDS, three oil/surfactant/cosurfactant combinations and 27 weight ratios of oil, surfactant and cosurfactant for each combination were evaluated with the aid of ternary phase diagrams indicating the self-nanoemulsifying region of each combination. One weight ratio from each combination (i.e., SNEDDSa, b, c) was selected for further studies. The ability of the PLGA-PEG NPs, PAA/PLL complexes, 4MBA loaded liposomes, and SNEDDS to diffuse through fresh porcine intestinal mucus was experimentally assessed.



Diffusion studies of (a) PLGA-PEG NPs, (b) PECs (CPAA = 0.1 w/v %), (c) 4MBA loaded liposomes and (d) SNEDDS in porcine intestinal mucus. Particles/liposomes/SNEDDS were incubated for 6h at 37°C. Results are presented as mean ± SD (n = 3). Error bars are plotted for one set of data for clarity reasons.

Additionally, state-of-the-art mucus permeating nanocarrier-based systems for controlled delivery of insulin are presented and critically assessed (e.g., SNEDDS, PECs, etc.). More specifically, a novel mucus permeating SNEDDS formulation for oral insulin delivery, incorporating a hydrophobic ion pair of insulin/dimyristoyl phosphatidyl

glycerol (INS/DMPG) was developed. The effect of the initial insulin concentration on the protein loading was also evaluated. With respect to PECs, complexes of insulin and polyelectrolytes (i.e., PAA, PLL) were prepared by ionic complexation. Ternary complexes (i.e., (PAA+INS)/PLL, (PLL+INS)/PAA) were also formed. The drug loaded nanocarriers were characterized with respect to particle size distribution, zeta potential, protein loading and release, storage stability, cytotoxicity and ability to protect the therapeutic protein from enzymatic degradation by intestinal enzymes.



(a) Leakage of insulin (INS), insulin that has been mixed with SNEDDS (SNEDDSc-INS) and insulin associated with SNEDDS in the form of the hydrophobic complex INS/DMPG (SNEDDSc-INS/DMPG); (b) Insulin release profile from ionic complexes (PB 7.4 50 mM and 0.1 mM EDTA, 37°C). Results are presented as mean ± SD (n = 3).

ALDOXORUBICIN: CLINICAL UPDATE OF AN ALBUMIN-BINDING PRODRUG OF DOXORUBICIN

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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. The acid-sensitive hydrazone linker present in Aldoxorubicin allows doxorubicin to be released either extracellularly in the slightly acidic environment often present in tumor tissue or intracellularly in acidic endosomal or lysosomal compartments after cellular uptake of the albumin conjugate by the tumor cell. Aldoxorubicin emerged as a clinical candidate due to a high water-solubility, a high plasma stability in its albumin-bound form, its profound acid-sensitive properties, and due to superior efficacy in meanwhile nine murine tumor models and a favorable toxicity profile including significantly reduced cardiotoxicity.
Phase 1 and Phase 1b studies (in soft tissue sarcoma) have shown that the MTD of Aldoxorubicin is 260 mg/m^2 (doxorubicin equivalents) which is a 3.5-fold increase over the MTD of doxorubicin. Due to the promising results in sarcoma patients with Aldoxorubicin and considering that doxorubicin is the only single agent approved in this indication with a poor outcome, CytRx Corporation has completed a 123-subject, 31-center global Phase 2b clinical trial in first-line soft-tissue sarcoma. Patients with advanced soft tissue sarcomas were administered either 260 mg/m^2 of Aldoxorubicin (doxorubicin equivalents) (83 subjects) or 75 mg/m^2 of doxorubicin (40 subjects) every 3 weeks for up to 6 cycles. In an intent-to-treat analysis, the investigator-assessed median progression free survival (PFS) was 8.4 months for Aldoxorubicin patients versus 4.7 months for doxorubicin patients (p = 0.0002), while the blinded central lab review revealed that median PFS for Aldoxorubicin patients was 5.7 months versus 2.8 months for doxorubicin patients (p = 0.018). The overall response rate as determined by the investigators was 21.7% for Aldoxorubicin subjects (2.4% complete response and 19.3% partial response) versus 5.0% for doxorubicin subjects (0% complete response and 5.4% partial response). As assessed by blinded central lab review, 23.8% of Aldoxorubicin subjects had a partial response while 0.0% of doxorubicin subjects exhibited any objective response. Median overall survival was 16.0 months for Aldoxorubicin-treated patients versus 14.4 for doxorubicin treated patients. For treatment-naive patients, representing 90% of the patients in the clinical trial, median overall survival was 16.0 months for Aldoxorubicin-treated patients versus 14.0 for doxorubicin treated patients. Aldoxorubicin is thus the first single agent to surpass doxorubicin as a first-line treatment for soft tissue sarcoma.
At present, Aldoxorubicin is being evaluated in six clinical trials: A Phase 1b trial in combination with ifosfamide in patients with soft tissue sarcoma, a Phase 1b trial in combination with gemcitabine in subjects with metastatic solid tumors, a Phase 2 clinical trial in HIV-related Kaposi's sarcoma, a Phase 2 clinical trial in patients with late-stage glioblastoma, a global Phase 2b clinical trial in platinum-refractory small cell lung cancer in comparison to the clinical standard topotecan, and a global phase 3 study in second-line soft tissue sarcoma under a Special Protocol Assessment granted by the U.S. Food and Drug Administration. The Phase 3 registration trial was initiated at the beginning of 2014 and is designed to compare the efficacy of aldoxorubicin for patients with soft tissue sarcoma whose tumors have progressed following chemotherapy to treatment with five optional anticancer agents. This talk will give an overview of the clinical progress with Aldoxorubicin which is the first albumin-binding prodrug that has reached an advanced clinical stage.

HOW EXOSOMES TARGET TUMOR CELLS – LESSONS LEARNED FROM NATURE.

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Exosomes are nature's nanocarriers that transport biological information in humans. Exosomes were first described in the 1980's by Johnstone et al. (1) and were defined as vesicles formed in the endosomal compartments (multivesicular endosomes) which then get secreted into the extracellular space to serve as nano-rafts carrying biological information between cells. Hence, they play a central role in intercellular communication (2). Exosomes have been found to originate from various types of cells in the body, including stem cells and fully differentiated cells. Their most important feature as compared to the endosomes is that the extracellular leaflet of the plasma membrane is fully preserved as extracellular part of the exosomes.
It follows from the mechanism of exosome formation that the content of exosomes contains mainly cytosol derived molecules, such as miRNA, mRNA, proteins, peptides, enzymes (1,3-5) and, as confirmed recently, also dsDNA (6-8). Details about the content and the composition of exosomes can be found on the web (http://www.exocarta.org/) provided by Mathivanan and Simpson (9).
Their structural properties, origin and functions are making them interesting objects for the diagnosis of diseases, such as cancer, and also, as innovative tools for drug delivery. The proteins attached to the lipid bilayer of exosomes originate from the plasma membrane which is preserved its original orientation. They cover a broad spectrum of immune-modulating and cell recognizing molecules that are either common, ubiquitous proteins or cell-type specific proteins. The former group includes cytoskeletal proteins, such as actin and tubulin, membrane transport and fusion proteins (annexins and Rab proteins), integrins and proteins belonging to the heat-shock family (immune activators such as Hsp70, Hsc70 and Hsp90) 21). The cell-type specific proteins include MHC class-I and class-II proteins, which present antigens, tetraspanins (CD63, CD81, CD82, CD9 and CD86) which are involved in cell-cell contacts and in

selective binding to certain target cells (10). As discussed later in detail, the surface exposed proteins have different roles including the targeting of exosomes to specific cells and modulating the immune response via activation or suppression. Additionally it has to be mentioned that the fact that endogenous exosomes are made from fragments of the plasma membrane in the preserved original orientation means that they inherit also the glycome, the glycocalyx from the originating cells with its innate immune tolerance. The interaction of exosomes with the immune system has been one of the focal points of interest; nevertheless their "stealth" properties helping to avoid adverse immune reactions are still not fully understood. The potential of exosomes of creating stealth nanoparticles that are better tolerated by the immune system than the presently available synthetic drug delivery systems represent a promising new approach in nanomedicine.

Exosomal properties which are on one hand promising in terms of stealth drug delivery system unfortunately enable them to play also a crucial role in disease development and progression. Exosomes play a central role in the manifestation and progression of several diseases, as well as in drug resistance, therefore mapping their functions in intercellular communication is essential in understanding the pathomechanism of these medical problems.

Several recent publications discuss the function of exosomes in oncogenesis, tumor cell exchanges and metastatic activities of tumors. Exosomes can transfer oncogenic materials which affect organization of tumor cells and progression of a tumor. Intercellular communication can happen through the delivery of genetic information in the form of microRNA that is delivered from tumor cells to normal or pathological cells (11). Exosomes are pivotal in shielding tumor cells from the immune system, and at the same time inducing inflammatory and angiogenetic responses helping tumor cell adhesion and metastatic growth (12).

In infections by different pathogens (viral, bacterial, fungal and parasite) exosomes play a key role in several areas. The pathomechanism of oncogenesis after specific viral infections, such as Epstein-Barr virus, has been recently connected to viral RNA carried by exosomes, creating an intercellular pathway for genetic information passage, leading to tumor formation (13). In HIV infection replication of the virus is helped by the presence of exosomes derived from HIV expressing cells (14). Exosomal delivery of virus components and proteins are essential for disease progression in Human T-lymphotropic virus type 1 infections (15). The pathomechanism of parasite infections in some cases involves exosomes, where the vesicles can induce adhesion of the pathogen (16).

In neurologic disorders exosomes can be important factors. Exosomal communication between microglia and neurons of the brain is a way of the central nervous system to modulate the pathology of amyotrophic lateral sclerosis, which is the most common and most aggressive form of adult motor neuron degeneration (17). Exosomes can be a reason behind drug resistance in neurological diseases and cancer, an example of this can be found in the treatment of multiple myeloma (18).

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KADCYLA: REALIZING THE PROMISE OF ANTIBODY-DRUG CONJUGATE TECHNOLOGY IN TREATING SOLID TUMORS.

JOHN M. LAMBERT

Oncologists viewed monoclonal antibody technology with great optimism when the technology was first developed since they offered the promise of targeted elimination of tumor cells without the systemic toxicity associated with chemotherapy. However, despite considerable effort spanning over three decades of clinical research, application of monoclonal antibody technology has had only modest success at improving treatment outcomes in patients with solid tumors. In general, the immunological mechanisms for cell elimination induced upon antibody binding to cell surfaces have not proven effective against solid tumors without some other mechanism for enhanced potency.

Enhancing the cancer cell-killing activity of antibodies through conjugation to highly potent cytotoxic "payloads" to create antibody-drug conjugates (ADCs) offers a strategy for developing anti-cancer drugs of great promise. As with many simple ideas, its successful execution has proved to be challenging. Early ADCs exhibited side-effect profiles similar to those of "classical" chemotherapeutic agents, and their performance in clinical trials in cancer patients was generally poor. However, with the recent clinical development of ADCs utilizing highly potent cytotoxic agents as "payloads" designed specifically for antibody-targeted delivery, interest in the ADC field has been reinvigorated. With the approval of brentuximab vedotin for treatment of Hodgkin lymphoma in 2011, and the approval of ado-trastuzumab emtansine for the treatment of HER2-positive metastatic breast cancer in 2013, it has become apparent that ADC technologies utilizing potent tubulin-acting agents are able to generate highly active, well-tolerated, anticancer agents that fulfill the long-awaited promise of ADC technology.

Ado-trastuzumab emtansine (T-DM1; Kadcyla®) is an ADC that

combines the anti-tumor properties of the humanized anti-human epidermal growth factor receptor 2 (HER2) antibody, trastuzumab, with the maytansinoid, DM1, a potent microtubule-disrupting agent, joined by a stable linker. Upon binding to HER2, the conjugate is internalized via receptor-mediated endocytosis, and an active derivative of DM1 is subsequently released by proteolytic degradation of the antibody moiety within the lysosome. Initial clinical evaluation led to a phase III trial in advanced HER2-positive breast cancer patients who had relapsed after prior treatment with trastuzumab and a taxane, which showed that T-DM1 significantly prolonged progression-free and overall survival, and with less toxicity than lapatinib plus capecitabine. In 2013, T-DM1 received FDA approval for the treatment of patients with HER2-positive metastatic breast cancer who had previously received trastuzumab and a taxane, separately or in combination, the first ADC to receive full approval based on a randomized study. Several more ADCs, including those developed with ImmunoGen's maytansinoid technologies, have shown encouraging efficacy in clinical trials in both solid tumors and hematologic malignancies. In creating effective, well-tolerated, ADCs, each element in its design, from target selection, selection of the antibody, the cytotoxic "payload", and the linker, is important, and will be exemplified in the presentation in the context of the development of ado-trastuzumab emtansine.

MIRVETUXIMAB SORAVTANSINE, AN ANTIBODY-DRUG CONJUGATE UTILIZING A MAYTANSINOID PAYLOAD WHICH TARGETS THE FOLATE RECEPTOR FOLR1

JOHN M. LAMBERT

A majority of ovarian and non-small cell lung cancers overexpress folate receptor α (FRα). Mirvetuximab soravtansine is an anti-FRα antibody-drug conjugate (ADC), consisting of an anti-FRα antibody coupled to a highly cytotoxic maytansinoid that induces cell cycle arrest and cell death by targeting microtubules. From screening a large panel of anti-FRα monoclonal antibodies, the antibody moiety of the ADC was selected by virtue of its activity in antigen-selective delivery of maytansinoid payload into FRα-positive cells in vitro, and in eradicating FRα-positive human xenografts in mice. Various linker/maytansinoid combinations were evaluated, and a conjugate with the N-succinimidyl 4-(2-pyridyldithio)-2-sulfobutanoate (sulfo-SPDB) linker, and with $N^{2'}$-deacetyl-$N^{2'}$-(4-mercapto-4-methyl-1-oxopentyl)-maytansine (DM4), exhibited the most potent antitumor activity in several FRα-expressing xenograft tumor models, and was selected for development (denoted as IMGN853). Currently, mirvetuximab soravtansine (IMGN853) is in phase I clinical evaluation in patients with cancers likely to express FRα (especially, ovarian and endometrial cancers), and recent findings in the ongoing clinical development of IMGN853 as a novel targeted therapy for patients with FRα expressing tumors will be presented.

THERANOSTIC TISSUE ENGINEERING: MR IMAGING OF USPIO NANOPARTICLE-LABELED CELLS AND SCAFFOLDS

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INTRODUCTION

Non-invasive imaging holds significant potential for implementation in tissue engineering. It can e.g. be used to monitor the localization and function of tissue-engineered implants, as well as their remodeling and resorption. Thus far, however, efforts in this area of research have largely focused on the use of USPIO-labeled cells, colonizing the scaffolds, to indirectly image the implants. Reasoning that direct scaffold labeling might be I) more beneficial, enabling imaging also in case of non-cellularized implants; II) more informative, enabling the visualization and quantification of scaffold degradation; and III) easier to translate into the clinic (as cell-free materials are less complex from a regulatory point of view), we have developed concepts and contrast agents for non-invasive MR imaging of collagen-based scaffolds and PVDF-based vascular grafts, and we have evaluated these constructs both in vitro and in vivo.

METHODS

USPIO nanoparticles were embedded into collagen scaffolds and PVDF-based textile materials using several different techniques. The textile materials were spun into fibers via melt-spinning, and vascular scaffolds were knitted and characterized using MRI (Figure 1A-D). They were then molded with a mixture of fibrinogen, fibroblasts and smooth muscle cells, and placed in a bioreactor, in which they were colonized with endothelial cells for 21 days under physiological flow conditions. The resulting materials were subsequently implanted into mice and sheep, and longitudinally monitored.

RESULTS

The USPIO-labeled collagen scaffolds and PVDF-based vascular grafts could be sensitively detected using T1-, T2- and T2*-weighted MRI. The collagen scaffolds were employed to provide proof of principle for monitoring scaffold degradation [1]. The tissue-engineered vascular grafts were surgically implanted into sheep, as a shunt between the carotid artery and the jugular vein. As exemplified by Figure 1E-G, they were shown to be readily detectable (as compared to non-labeled grafts), biocompatible (using FDG-PET and ex vivo microscopic validation) and functional (using MR angiography) [2].

CONCLUSIONS

These findings demonstrate that the labeling of collagen- and PVDF-based scaffold materials with USPIO nanoparticles is straightforward and safe, and enables the non-invasive assessment of their in vivo localization and function using MRI. Such theranostic constructs and concepts are considered to be highly useful for facilitating the production, performance and translation of tissue-engineered implants.

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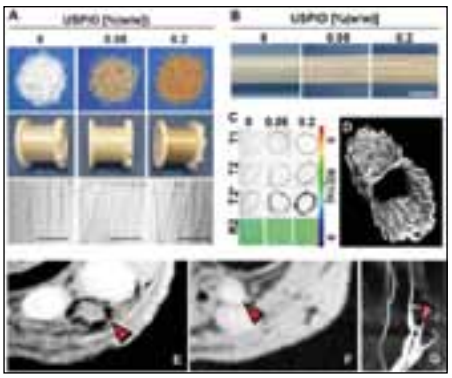

Figure 1: USPIO-labeling of PVDF pellets (A), knitting of MR-imageable vascular scaffolds (B-D), and in vivo MR imaging of labeled (E) and non-labeled (F) tissue-engineered vascular grafts in sheep, confirming proper perfusion (G; MR-ToF-angiography).

OPEN ACCESS FUNDING AND SUBSCRIPTION JOURNALS

KARIN E. LASON

Lately, Open Access publishing has become subject to more critical reflection beyond its initially noble idea of universal knowledge dissemination. Predatory Open Access journals and sinking quality of once high impact Open Access publications are used as rationales for keeping to traditionally subscription based journals. This talk would like to provide a critical overview of the ongoing discussion of established and vanguard publication models. Pros & cons of Open Access funding and subscription based as well as hybrid models are reflected herein.
You are invited to witness the revelation of the surprisingly simple secret behind it all.

RADIOLABELED EXOSOMES: ENDOGENOUS NANOMEDICINE

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Exosomes and microvesicles, collectively called as extracellular vesicles, are excreted by bacteria or mammalian cells and are proposed to be used as vaccine delivery system or drug delivery system, respectively. Cell therapy is also supposed to be replaced by exosome therapy. The future in vivo use of exosomes depend on the proper exosomal preparation and successful targeted delivery. Targeted delivery can be confirmed by fluorescence or bioluminescence imaging of exosomes labeled before or after exosome preparation from the donor cells. These methods are easily used in rodents but cannot be used in large animals or humans. Radiolabeling offers the opportunity to visualize and trace the biodistribution of exosomes in large animals or probably in humans and preparation of exosomes in mild conditions should enable the easy tracking of the whereabouts of intact exosomes administered in vivo.
As for radiolabeling, membrane labeling is also possible with ^{111}In oxine or NOTA-^{68}Ga (or NOTA-^{64}Cu), however, if the exosomes are disintegrated in vivo and the fragmented membranes are still attached with NOTA-^{68}Ga (or ^{64}Cu), the in vivo biodistribution will represent the whereabouts of the fragments and not the intact exosomes.
Labeling intact exosomes was implemented using ^{99}mTc-HMPAO. Macrophage-derived exosomes were incubated with lipophilic ^{99}mTc-HMPAO, intact exosomes took up ^{99}mTc-HMPAO, and ^{99}mTc-HMPAO would have been transformed to hydrophilic ^{99}mTc-HMPAO by the action of intra-exosomal glutathione. The radiochemical purity of ^{99}mTc-HMPAO-exosomes reached more than 90% and exosome specific protein (CD63) persisted after ^{99}mTc-HMPAO labeling. ^{99}mTc-HMPAO-exosomes showed high serum stability (90%) and on SPECT/CT images ^{99}mTc-HMPAO-exosomes were taken up in liver but not in brain. We proposed that the intact exosomes could be traced using ^{99}mTc-HMPAO-exosomes. Feasibility of depth imaging and quantifiability as well as track-ability of intact exosomes in vivo shall promise the future clinical application and also the use for mechanistic study of would-be successful targeted delivery of exosomes or exsome-mimetic nanovesicles.

ALBUMIN BASED DISEASE TARGETING DIAGNOSTIC/THERAPEUTIC NANO-PLATFORM – THE PROOF OF CONCEPT

YUN-SANG LEE

Human serum albumin (HSA, 66.5 kDa and 5~7 nm) is one of the most powerful drug delivery systems (DDS) among numerous nanomaterials, because it has extremely high stability, ready availability, biodegradability, and lack of toxicity and immunogenicity, which make it an ideal nanocarrier for drug delivery. Traditionally, radiolabeled HSA has been used for in vivo blood pool imaging in nuclear medicine field for several decades. Tc-99m-labeled-HSA is retained in the blood pool and can show us vascular structures of our body as well as distributed to the liver at early time point (10 min after IV injection). The liver uptake of Tc-99m-labeled-HSA is quite unusual if we think about the albumin's nature. The biological half-life of HSA is known to be more than 20 days in our body, and the denatured or structurally modified HSA is decomposed at the hepatocyte in the liver. Therefore, we hypothesized that the physico-chemical stress (high or low pH, high temperature, organic solvent, oxidizing or reducing agent) can shorten the biological half-life of HSA. This is the start point of our research.
We designed all procedure for the modification of HSA should be along with the physiological pH (7~7.4) and without using any oxidizing or reducing chemicals. For this purpose, we adopted the Cu-Free Click Chemistry to the modification or radiolabeling of HSA, and finally we could elongate the biological half-life of HSA to more than 12 hrs. And also, we evaluated that azadibenzocyclooctyne (ADIBO) or azide (N_3) modified HSA, which is called 'clickable HSA', could be used disease targeting diagnostic/therapeutic nano-platform.

NANOCARRIERS FOR NONINVASIVE TRANSCUTANEOUS IMMUNIZATION VIA THE HAIR FOLLICLES

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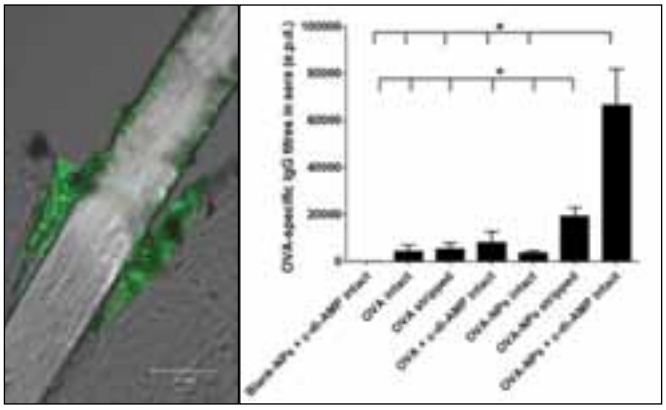
INTRODUCTION

As a result of intensive, mainly safety-driven research regarding the skin penetration of nanomaterials (e.g. used in sun screens) during the past 10 years, penetration oft the intact stratum corneum (SC) can meanwhile been ruled out. Rather surprisingly, however, this research has also revealed that nanoparticles (NP) accumulate in hair follicles and skin folds as well as penetrate deeper into hair follicles than much smaller molecules, the depth and extent of penetration depending on the size of particles and also the application of some massage (1,2,3). Therefore the dermal application of nanomedicines may still hold perspectives for some specific applications, of course provided they can be made from safe and biodegradable materials.
Transcutaneous immunization (TCI) refers to the needle-free application of vaccines across the skin. However, many of the current strategies for TCI (e.g. micro-needles, gene gun, PowderJect, and skin abrasion), reduce the protective SC barrier for a significant time to facilitate the absorption of the vaccine, making them suboptimal for certain applications, such as mass vaccination campaigns in countries having critical hygienic conditions (4). In contrast, transfollicular vaccination aims to reach the perifollicular antigen presenting cells without impairing the SC barrier. Transfollicular delivery based on NP-based formulations had been already investigated, not both for antigenic proteins and for DNA vaccines (5,6). However, this route is usually studied after pretreating the skin via various methods such as waxing (hot or cold), plucking the hairs, and stripping (tape or cyanoacrylate ["Superglue"] stripping). Because of such pretreatment, upper layers of SC are partially removed, making it unclear to what extent the antigen penetrates via the hair follicles or across the permeabilized SC. In this context, we recently studied the potential of transfollicular delivery of ovalbumin (OVA) using polymeric NPs but without compromising the SC barrier by any pretreatment for the purpose of noninvasive TCI, eventually by combining the nanotechnology approach with some innovative adjuvantation.

RESULTS AND DISCUSSION

NPs were prepared by a double emulsion method using poly(lactide-co-glycolide) (PLGA) or chitosan and polyvinyl alcohol as stabilizer. The mean size of OVA-loaded PLGA and chitosan-PLGA (Chit- PLGA) NPs was ≈170–180 nm with a monodisperse size distribution (polydispersity index < 0.2) with negative surface charge for PLGA NPs and positive surface charge for Chit- PLGA NPs. OVA was protected from cleavage or aggregation inside the NPs and retained its biological activity to 74% (PLGA) and 64% (Chit-PLGA) The microscopical evaluation and follicular delivery efficiency of NPs were measured in intact pig skin based on the differential stripping technique and compared with OVA solution (7) However, no significant differences were observed in the follicular uptake of PLGA and Chit-PLGA The Figure left shows the distribution of fluorescently labeled NPs in the hair follicle after application to excised pig ears. The NPs accumulated in the follicle openings, covering the hair as well as invading into the follicular duct. An adoptive transfer experiment was performed to verify the usefulness of the noninvasive transfollicular immunization route in vivo. In brief, two days before immunization, the flanks of C57BL/6 mice were shaved, and one day prior to immunization CFSE-labeled naïve nonactivated OVA-specific CD4+ T cells were injected into the tail vein of the shaved mice. Then, 200 µg/60 µL of LPS-free OVA in Chit- PLGA NPs plus 2 µg of c-di-AMP as adjuvant, were applied topically under slight massage. Proliferation of adoptively transferred OVA-specific CD4+ T cells was measured by CFSE dilution in the draining lymph nodes and secondary lymphatic organs, indicating that by this transfollicular immunization approach it is possible to deliver antigens, thereby stimulating antigen-specific T cells. (7)



Figures: Left: Microscopic evaluation of NP distribution in hair follicles after application to excised pig ear skin. (Adapted from Ref 8) Right: Systemic humoral immune responses as indicated by OVA-specific IgG serum titer in sera in C57BL/6 mice (n = 5) after four vaccinations with different OVA- formulations via intact and tape stripped skin. (Adopted from Ref 9))

In a next study (9), the impact of formulation composition i.e. antigenic solution or antigen-loaded nanoparticles with or without the adjuvant bis-(3',5')-cyclic dimeric adenosine monophosphate, (c-di-AMP) on immune response was investigated. Female C57BL/6 mice (n = 5) were immunized on days 0, 14, 28 and 42 by applying 60 µl of different formulations containing 200 µg of LPS-free OVA topically to an area of 2.25 cm^2 of either intact or tape stripped skin. The results of this confirmed the ability of nanoparticle based vaccine formulations to deliver antigen across the intact skin via the follicular route (see Figure right) , but at the same time demonstrate the necessity to include adjuvants to generate efficient antigen-specific humoral and cellular immune responses.

Finally, we evaluated the strength and type of immune response elicited after administration of surfactant based inverse micellar sugar glass nanoparticles (IMSG NPs) by the transfollicular route and also compared it with intranasal and intradermal route, co-encapsulatig c-di-AMP as adjuvant (10). The study showed enhanced stability and encapsulation efficacy of the antigen when encapsulated in IMSG NPs in comparison to polylactic-co-glycolic acid (PLGA) and chitosan-PLGA NPs. Moreover, by transfollicular delivery, IMSG NPs showed enhanced follicular uptake in comparison to OVA solution or OVA-loaded chitosan-PLGA NPs. While the immune response stimulated after intranasal administration was negligible, significant humoral and cellular responses were observed after immunization via transfollicular and intradermal route, but again the effect wasbound to the presence of the adjuvant.

CONCLUSION

Our present data highlight the feasibility and the potential of transfollicular immunization (TCI) across the intact skin, but also the need for optimized nanocarriers and adjuvants.

ACKNOWLEDGEMENTS

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NANOATHERO – STATE OF THE ART AND FIRST RESULTS OF THIS FP7 - EU PROGRAMME NANOMEDICINE FOR TARGET-SPECIFIC IMAGING AND TREATMENT OF ATHEROTHROMBOSIS

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Atherothrombotic diseases, regardless of the anatomical localization, remain the main causes of morbidity and mortality with clinical manifestations of angina, heart attack and stroke. There is a need for new approaches for early diagnosis and im-proved

therapies. This is the focus of NanoAthero, 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.
NanoAthero aims both the imaging and the treatment of thrombus and plaque. i) Nanosystems will be used for delivery and improved efficacy of drugs for plaque and stroke treatments in humans. ii) New imaging agents will allow molecular imaging of key processes and early adverse events using clinically available imaging modalities. The NanoAthero consortium is a unique opportunity to extend the frontiers of knowledge on atherothrombosis management. NanoAthero aims to demonstrate the preliminary clinical feasibility of the use of nanosystems for targeted imaging and treatment of advanced atherothrombot-ic disease in humans. 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 collaborative European consortium (Figure - 16 partners from 10 countries - see http://www.nanoathero.eu/).
The NanoAthero project gathers together chemists, engineers, pharmacists, biologists, toxicologists, ethicists and clinical key leaders from RTOs, hospitals, SMEs and a large pharmaceutical company to prove that the benefit of the use of nanoparticle technologies can be measured in a clinical setting. NanoAthero partners have already patented and provided some proofs of the efficiency of different nanodelivery systems and ligands for use in imaging or therapies. NanoAthero project integrates several key elements: GMP production, the initiation of clinical investigations in patients at high cardiovascular risk, including 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 and targeting agents were studied and evaluated in vitro and in vivo (Almer et al, 2014; Su-zuki et al. 2015). Several combinations are under intense study within the consortium. Using GMP liposomal nanoparticle (Lobatto et al., 2015), the first clinical studies of liposomes encapsulating prednisolone in atherosclerosis were already ob-tained (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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CHARACTERIZATION OF DNA/POLYMER NANOPARTICLES AND APPLICATION FOR PRECISION IMMUNOTHERAPY

JULIANNA LISZIEWICZ

Immunotherapies arerevolutionizing cancer treatment by activating T cells that attack tumor cells. A fraction of patients survived over 5 years, but most did not respond and experienced toxicities due to non-specific T cell activations.
We have technologies that likely improve the safety and the specificity of immunotherapies:
1. We use computational immunology to identify the peptide targets on the surface of the tumor cells that are frequently recognized by T cells. Computational immunology is an enabling technology of precision immunotherapy, specifically the interpretation of genetic sequence data for identification of peptide targets that drive the T cell killing of tumors. We found that survival benefit of patients treated with Ipilimumab can be predicted by the number of peptide targets derived from Melanoma Driver Antigens.
2. We design tumor-targeted T cell therapies by encoding the peptides into a plasmid DNA to be processed by the patient's own cells and activating T cells. For example, we showed in silico that DNA designed for targeted melanoma immunotherapy can activate T cells to attack an average of 20 peptide targets (PEPIs) on tumors.
3. We complex the DNA with a polymer and obtain a nanomedicine suitable for immunotherapy. Langerhans cells are professional antigen presenting cells that function is to activate T cells. We developed a stable DNA nanomedicine formulation that specifically delivers the DNA into Langerhans cells. Two published clinical trials proved (i) potent activation of T cells and (2) safety comparable to placebo.
In conclusion: precision cancer immunotherapy is enabled by new diagnostic and delivery technologies. Tailoring immunotherapies to the molecular signature of patients and their tumors improves the efficacy and safety of these novel therapies.
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CHARACTERIZATION OF DNA/POLYMER NANOPARTICLES AND APPLICATION FOR PRECISION CANCER IMMUNOTHERAPY

Julianna Lisziewicz, Eniko Toke, Levente Molnar, Ferenc Horkay, Jack Douglas, Orsolya Lorincz, Eszter Somogyi, Zsolt Csiszovszki, Jozsef Toth, Kata Pantya and Franco Lori

Four immunotherapies that recently approved by FDA revolutionized cancer treatment by providing over 5 years survival benefit to

a fraction of patients. These drugs activated T cells that attacked tumor cells. However, most cancer patients did not respond to immunotherapies and experienced life threatening toxicities due to non-specific T cell activations.
Our technologies likely improve the safety and the specificity of immunotherapies:
1. We use computational immunology to identify the peptide targets on the surface of the tumor cells that are frequently recognized by T cells
2. We design tumor-targeted T cell therapies by encoding the peptides into a plasmid DNA to be processed by the patient's own cells to activate T cells
3. We deliver the DNA into epidermal Langerhans cells using a polymer that complex the DNA into a nanomedicine

Computational immunology is an enabling technology of precision immunotherapy, specifically the interpretation of genetic sequence data for identification of antigenic targets that drive the T cell killing of tumors. We found that survival benefit of patients treated with ipilimumab can be predicted by the number of peptide targets (PEPIs) of T cells derived from Melanoma Driver Antigens.

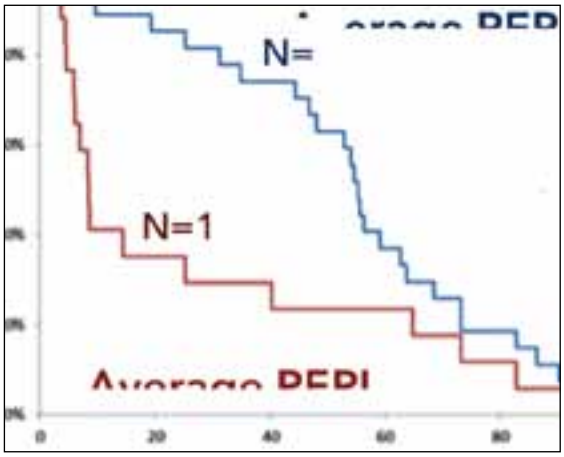

Fig.:1. Patient's PEPI counts predicts long-term survival of patients treated with immunotherapy. Cohort: 44 melanoma patients treated with 10mg/kg Ipilimumab. Average PEPI counts were computed from sequences of both Melanoma Driver Antigens and HLA class I of the patients. Data demonstrates that PEPITM counts predict clinical outcome of melanoma immunotherapy.

Plasmid DNA of our Tumor-Targeted Melanoma T Cell Therapy (peMU-12) can activate T cells that recognize an average of 20 peptide targets (PEPIs) on the tumor. and act in combination to kill the cancer. We have tested the efficacy of peMU12 in silico on 162 melanoma patients and found that it can activate melanoma-specific T cells in every patient. PEPI biomarker-driven patient selection enables the clinical development of precision immunotherapy for the subpopulation of cancer patients likely respond to this treatment.
Langerhans cells are professional antigen presenting cells that function is to activate T cells. We developed a DNA nanomedicine formulation with a mannosylated polyethyenimine that specifically deliver the DNA into epidermal Langerhans cells. The main characteristics of the DNA/PEIm nanomedicine are the following:
[1] Stability that is based on the chemical characteristics of the components
[2] Specifically target the DNA into epidermal Langerhans cells after topical administration
[3] 4 clinical trials proved (i) potent activation of antigen-specific T cells and (2) safe comparable to a placebo treatment [3,4,5]
In conclusion: precision cancer immunotherapy is enabled by new diagnostic and delivery technologies. Tailoring immunotherapies to the molecular signature of the patients and his tumors improves the efficacy and safety of these novel therapies.
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ARTIDIS® – USING THE NANOMECHANICAL SIGNATURE TO PREDICT DISEASE COURSE"

MARKO LOPARIC

The nanomechanical testing of soft biological tissues was relatively underdeveloped as most available products are optimised for testing hard materials (e.g. bone, tooth) but not soft rough samples such as cartilage or breast tissue. This is because the established equipment is adapted from industrial material testing (e.g. metals and alloys, etc.). Testing of soft biological samples represents a significant challenge for indenter-based setups since fundamental changes of the experimental systems are required. Therefore to achieve optimal accuracy and compatibility for the testing of soft biological materials we have devised an ARTIDIS® ("Automated and Reliable Tissue Diagnostics") apparatus to measure the nanomechanical properties of native living tissues. is able to directly detect and differentiate both local and regional differences in the mechanical properties of various tissue biopsies. The suitability of our system includes numerous improvements and adjustments such as tip geometry, cantilever spring constant, sample holder design, mechanical models used for stiffness analysis, perfusion/heating systems, bright field and fluorescence optical control, optimal approach and levelling of the sample, software solutions etc. Moreover, by employing the nanometer-sized tip that allows exploration at molecular length scales, we are able to extract nanomechanical profiles of native tissues that are not presently accessible to other available products. In particular, emerging evidence indicates that the mechanical properties of cancer cells play a critical role in defining cancer invasion and metastasis. To study the disease-relevant mechanobiology of cancer at the nanometer scale, we have measured the nanomechanical properties of cells (and the surrounding matrix) within the tumor microenvironment using ARTIDIS. By obtaining tens of thousands of force measurements over unadulterated human breast tumor biopsies, we found that malignant tumors give rise to characteristic stiffness profiles in comparison to benign or healthy tissue. Interestingly, a similar "soft" phenotype was found for hypoxic cancer cells in both primary tumors and secondary lesions - strongly implicating the role of cancer cell "softness" in promoting metastasis (Plodinec, Loparic et al., 2012). As a result, soft peaks may serve not only as mechanobiological markers for the diagnostics of cancer but can have a prognostic value as well. Last but not least, we would like to emphasize that the ARTIDIS technology is not limited to breast and cartilage tissues and we have thus far, optimized the method for lung, prostate, colon, kidney and liver samples.

PHARMAKOKINETIC ON A MICROSCALE: UNDERSTANDING THE INTRACELLULAR NANO-PARTICLE DRUG DELIVERY PATHWAYS BY COMBINING PROTEOMICS WITH MOLECULAR BIOLOGY

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Rational design of nanocarriers for drug delivery approaches requires an unbiased knowledge of uptake mechanisms and intracellular trafficking pathways. Ways to investigate these processes using a quantitative proteomics approach as well as molecular biology are discussed (see Fig. 1). Isolation of intracellular vesicles containing superparamagnetic iron oxide polystyrene nanoparticles and methods how to dissect their protein composition by label free quantitative mass spectrometry will be presented. As an example how this can yield new insights the proteomic snapshot of organelle marker proteins indicating an atypical macropinocytic-like mechanism used for the entry of nanoparticles is demonstrated. We show that the entry mechanism is controlled by actin reorganization, atypical macropinocytic signaling and ADP-ribosylation factor 1. Additionally, this proteomics approach demonstrated a central role for multivesicular bodies and multilamellar lysosomes in trafficking and final nanoparticle storage. This is confirmed by confocal microscopy and cryo-TEM measurements. By quantitatively analyzing the protein composition of nanoparticle-containing vesicles, we demonstrate a methodoloy how to clearly define the routes of nanoparticle entry, intracellular trafficking and the proteomic milieu of a nanoparticle-containing vesicle. Finally also the degradation process of proteins adsorbed to nanoparticles was visualized.

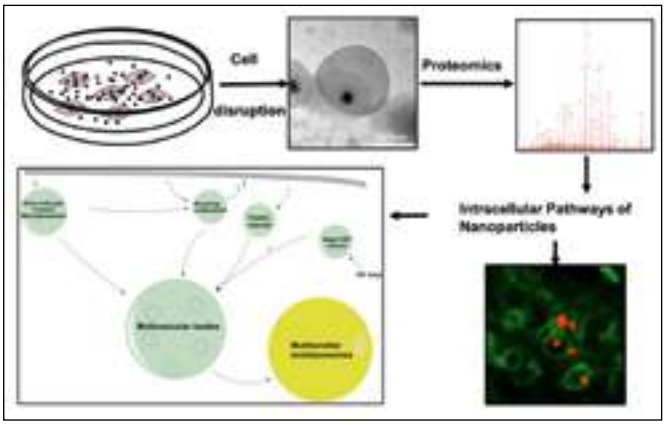

Fig 1: Methodology to investigate intracellular trafficking

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LATEST INSIGHTS IN MEDICAL IMAGING BASED ON NANOPARTICLES

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Medical imaging is the technique and process used to create images of the human body (or parts and function thereof) with the goal of enabling early diagnosis or reveal and examine diseases. Consequently, nanotechnology in medical imaging reefers to the use of nanoparticles (NPs) as contrast agents for in-vivo diagnosis in combination with different medical imaging modalities like Computed tomography (CT), ultrasound (US), magnetic resonance imaging (MRI), positron emission tomography (PET) and single-photon emission computed tomography (SPECT).

The increasing attention in the development of multifunctional nanomaterials is principally due to NPs versatilities offered over the conventional agents. In fact, the availabilities of several surface chemistries, unique magnetic properties and tunable energy absorption and emission properties make the nanoparticles an exciting opportunities on the whole imaging techniques.

Nevertheless the translation to nano-sized system as carrier of imaging agents is still rather complicated. In contrast to the considerable amount of papers published on the use of nanoparticles for functional and molecular imaging, there are only a very few nanoparticle-based contrast agents which really seem relevant from a clinical point of view. Although SPIO and USPIO have been approved for clinical use in the past, currently they are no longer available on the market as intravenous imaging agents.

The current clinical contrast enhanced imaging applications are based on relatively fast procedures in which, after intravenous administration of a small molecule based contrast agent, the patient is imaged in a quite short time. On the contrary, the use of nano-sized agents increases the time required to complete a diagnostic procedure, particularly when the analysis of a wash-in/wash-out curve in a pathological tissue is needed. Moreover the uptake of a nano-sized contrast agent could be the result of several mechanisms, where both active and passive interactions and different localizations in the tissue, are mixed together contributing to the whole signal.

It is also important to stress the relevance of toxicity or adverse reactions occurrence in the usage of contrast agents because, differently from therapeutics, the level of practical acceptance of toxicological events is very low for a diagnostic procedure.

Nevertheless also taking all the above into consideration, there are several possible imaging applications where a nanoparticle-based contrast agent can be used after a fine tuning of its pharmacokinetic, biodistribution and elimination properties. However more than for diagnostic purposes, the imaging-guided therapy applications seems to offer better opportunities for clinical translation, particularly in the contest of imaging-guided surgery where nano-colloids are already in clinical use for sentinel lymph node detection/resesection. In this context the whole application scenery changes as the target product profile is completely different. The aim of this talk is to analyze which are the main challenges in the engineering of nanoparticles for medical imaging application in order to address the development of nano-sized system for clinical purpose. To do this, the main advances and drawbacks of preclinical and clinical nanoparticles are evaluated with particular attention to their physico-chemical features aimed to control pharmacokinetics improving in vivo targeting properties and safety.

BEYOND CHOLESTEROL – NEW CARDIOVASCULAR BIOMARKERS

HARALD MANGGE

Although brought into connection with obesity, dyslipidemia and higher age, myocardial infarction (MI) and stroke are also seen in young, slim, and even sportive persons. Nevertheless, irrespective of heterogeneous phenotypes, MI and stroke are always caused by a so called vulnerable atherosclerotic (AS) plaque. An improved early diagnosis and treatment of this vascular lesion 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, C-reactive protein, and interleukin-6 may be increased, but are insufficient for a useful preventive diagnostic assessment. More specific biomarkers (e.g., troponin, copetin, natriuretic peptides, growth differentiation factor-15, 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 (months to years) that could be used to treat the patient is wasted. An improved management of this dilemma may involve a better detection of variations in degrees of immune inflammation in plaques by using new biomarkers in blood and/or within the lesion (molecular imaging).
Macrophage and T-cell polarization, innate- and adaptive immune responses (e.g., The Toll-like receptors 2, 4, 7), are involved in this critical process. New biomarkers include Pentraxin 3, Calprotectins S100A8/A9, Myeloperoxidase, Adiponectin, and chemokines. 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. Furthermore, we present herein for the first time nitric oxide coated albumin particles tagged with gadolinium as a possible theranostic tool to detect AND stabilize critical AS lesions. Under the scope of the EU project NanoAthero this approach is currently examined for a development to the human pipeline.

CATEGORIZATION, CLASSIFICATION & CLUSTERING (3C) STRATEGY TARGETING PERSONALIZED HEALTHCARE

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The goal to provide individualized tailored treatment to patients in terms of efficacy and adverse events, depends on the ability to define the "disease signatures". A term that became a premise in focusing therapeutic targets, precision medicine and prediction of disease, but depends on the comprising, analysis and translation of all relevant personal data. It may include all the micro and macro factors of the human body functioning in its physical, mental and community environment, such as: age, gender, clinical tests, biological markers, lifestyle, medical history, genetics, imaging etc. The new sophisticated and advanced technologies are enabling the capturing of these parameters through profound insights on the human body and it's functioning, in various levels and degrees of sensitivity, providing "Big Data" initiated from various sources while utilizing different techniques and tools. The goal is to analyse simultaneously all these identified and captured factors, including their interplay, translated to "disease signatures" towards responsible personalized, preventive and predictive medicine applied in the clinics.
Thus, it creates the need to bridge barriers between "Big Data" such as the simultaneous analysis of different types of data, sometimes related to a few number of people (many parameters, few records) – defined as "Small Data", tackling over fitting as well as database leakage (Brown et al 2012), replicability (Rosenblatt, Vink, Benjamini-2013) and reproducibility barriers. For example, hospital databases suffer from leakage and replicability barriers, as being constructed for specific purposes with limited availability of healthy records and different diagnostic tests that the healthy and sick people passed.
Furthermore, other challenges are:
- Expert knowledge is valuable but current diagnosis might be misleading
- Compensatory mechanisms obscure the linkage between biological markers (i.e. imaging, pathology and genetics) and disease manifestation, are difficult to discover.
- Big Data: Big potential but increased chance of capturing irrelevant markers.

Thus to address these challenges new technologies and targeted tools are being developed, among them an approach developed in our group that will be presented. The goal is to meet the growing sensitivity and specificity needs in representing the medical data variance and its relevance and contribution to personalized medicine.
A 3C- Categorization, Classification & Clustering- strategy, developed as part of the Medical Informatics in the Human Brain Project Flagship, targeting to utilize and converge the medical expert knowledge, the disease manifestations and the potential biomarkers towards personalized prediction and treatment.
The methodology includes the three steps: Categorization, Clustering and Classification, based on supervised and unsupervised algorithms.
Step 1 - Categorization of variables into three types: (1) disease diagnosis as assigned in the electronic health record (EHR), by the medical expert (2) Clinical measurements reflecting the patient's condition and functionality. (3) Potential biological markers,
(proteins, imaging, etc.) Proposing predictive value for disease risk, deterioration or for severity. Step 2 - Feature selection and Clustering. Following the categorization an unsupervised learning creates sub-classes representing new disease diagnosis classes based on the differences in the manifestation of the disease. The Clustering step is preceded by feature selection of the clinical measurements.
Step 3 - Classification including potential biomarkers. This step is seeking for relations between identified potential biomarkers and each of the homogenous clusters using hierarchical decision trees, or other rule based analysis. This step is preceded by feature selection of the potential biomarkers, as done for the clustering.
A preliminary feasibility study of the "3C strategy" was applied successfully to the Alzheimer's disease Neuroimaging Initiative (ADNI) cohort, identifying and suggesting 10 sub-classes, rather than the 5 assigned in the ADNI data set – AD (Alzheimer disease), EMCI, LMCI (Early & Late Mild Cognitive Impairment), SMC (Significant Memory Concern) and CN (Normal).

AMYPOSOMES®: MULTI-FUNCTIONAL LIPOSOMES FOR THERAPY OF ALZHEIMER'S DISEASE WAITING FOR CLINICAL TRIALS

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With the long-term complications of aging, AD presents an enormous problem in terms of morbidity, mortality and economic burden, where by 2050 an estimated 10 million people are expected to suffer from AD, to say only in Europe. The total estimated worldwide cost of dementia was US$604 billion in 2010, where 70% of the costs occurred in Western Europe and North America. This figure included costs attributed to informal care (unpaid care provided by family and others), direct costs of social and hospice care (provided by community care professionals) and the direct costs of medical care (the costs of treating dementia and other conditions in primary and secondary care). If dementia-care were a country, it would be the world's 18th largest economy. It has been forecasted (http://www.alz.org/documents_custom/trajectory.pdf) that, if a treatment that delays the onset of Alzheimer's by five years be-

came available in 2025 in the USA, in the first year Medicare would save $3 billion. In 2035, Medicare savings would total $67 billion. Medicaid savings would grow from $1 billion in 2026 to $38 billion in 2035, compared to the current trajectory. Over the course of the 10-year period, the cumulative savings for federal and state governments would be $535 billion. Similarly, if a hypothetical treatment were available in 2025, families affected by Alzheimer's and other dementias would see an immediate decrease in their out-of-pocket spending. In 2026, individuals living with Alzheimer's and other dementias and their families would spend $2 billion less on their costs of care. In 2035, these savings would grow to $44 billion.
Although the cause and progression of AD are still not well understood (with the exception of genetic forms), the amyloid hypothesis is dominant and widely accepted. Accordingly, accumulation of Aβ peptide (an hydrolytic fragment of Amyloid Precursor Protein) in the brain, eventually deposited as senile plaques (a main hallmark in AD), evokes inflammatory response, synaptic dysfunction, neuronal death and neurodegeneration. Formation of neurofibrillary tangles containing tau protein is proposed to result from an imbalance between Aβ production and clearance. Therefore, Aβ has served as a target in recent years for approaches in AD therapy, Although many Aβ-centric therapies have been attempted, they all failed and no efficacious therapy is available yet.
Objectives. Following a project started with the FP7 NAD (Nanoparticles for therapy and diagnosis of Alzheimer Disease, 2008-2013) Project, we have rationally designed and patented multi-functional liposomes (Amyposomes ®)1-3 targeting the brain and able to 1. promote disaggregation of brain Aβ assemblies, 2. reduce brain Aβ burden, 3. restore memory, 4. prevent pathology progression. Amyposomes are liposomes composed of a matrix of sphingomyelin/cholesterol functionalized with a dodeca-peptide synthesized by modification of the receptor-binding domain of apolipoprotein-E, for purposes of blood-brain barrier targeting, and with phosphatidic acid (PA) for the purpose of Aβ binding.
Amyposomes were administered for 3 weeks (I.P., 3 times a week; 2.6 mg total lipids/injection) to an AD mouse model (APP/PS1 Tg mice aged 10 months). At the end of the treatment, mice were submitted to the novel object recognition memory test (NORT). Then, mouse brains were collected and analyzed through histology and biochemistry for Aβ deposition (plaques visualized with anti Aβ antibodies and total Aβ assayed by ELISA). The same treatment was also administered to APP23 mice aged 15 months (a single transgenic AD model) and plaque deposition was followed by PET imaging with [11C]-PIB and by histology.
Subsequently, different doses of Amyposomes (0.4 mg total lipids/injection; 2.6 mg total lipids/injection or 15 mg total lipids/injection; 3 injections/week for 3 weeks) were administered to APP/PS1 mice in order to fine tune an optimal dosage.
Results. Administration of Amyposomes decreased total insoluble brain Aβ1-42 (-33%), and the number and total plaque area (-34%). Also Aβ oligomers were reduced (-70.5%). Plaque reduction was confirmed in APP23 mice by PET imaging with [11C]-PIB and by histology. The reduction of brain Aβ was associated with its increase in liver (+18%) and spleen (+20%). Notably, the treatment also restored mouse impaired memory to normal.3
Subsequently, different doses of Amyposomes were administered to APP/PS1 mice (Fig.1). The effect on memory amelioration was obtained with Amyposomes at all the doses tested.

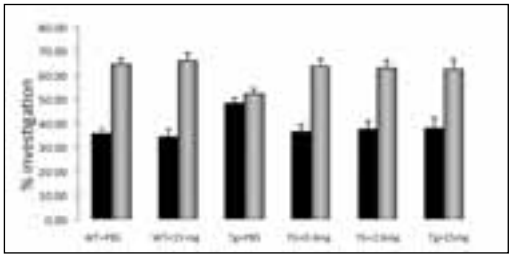

Fig.1: Effect of treatment with different doses of Amyposomes® , evaluated by Novel Object Recognition (NOR) test on Alzheimer Mouse models.
APP/PS1 Tg mice and WT controls (10 animals/group) were injected i.p. with different amounts of Amyposomes (0.4/2.6/15.0 mg total lipids/injection) 3 times/week for 3 weeks.

Conclusion. These data suggest that Amyposomes destabilize brain Aβ aggregates and promote peptide removal from the brain and its peripheral clearance, while at the same time . This all-in-one multitask therapeutic approach can be considered as a new candidate for AD treatment.
University of Milano-Bicocca is establishing a Spin-off company for the exploitation of the IP connected to Amyposomes with the aim to complete the preclinical development, to achieve the IND filing, and to carry out Clinical studies of Phase I and Phase II.
The NewCo can develop a product that fulfills the increasing needs of Pharma and Biotech companies.
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2. Patent "Liposomes active in-vivo on neurodegenerative diseases (in particular Alzheimer's disea-se)" US Patent n. US 8,877,236 of 04.11.2014 ; International Patent Application n. PCT/EP2013/001660 of June 05, 2013 - US patent application for "Continuation" n. US 2015/0017235 of January 15, 2015
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PEPTIDE NANOFIBER COMPLEXES WITH SIPLK1 FOR THE NEUROSURGICAL TREATMENT OF GLIOBLASTOMA MULTIFORME: TARGETING TUMOR PROLIFERATION

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The use of nanomedicine to complement existing therapies for the treatment of patients diagnosed with progressive or recurrent GBM is of great interest, because of the poor outcomes of the current therapy strategies.
Glioblastoma (GBM), a grade IV astrocytoma, is the most aggressive form of brain tumour, giving a life expectancy of 5 years in less than the 5% of the newly diagnosed patients1. Despite extensive research and clinical trials, only 3 drugs are currently FDA approved for the treatment of GBM (temozolomide, carmustine and bevacizumab)2.
RNAi is a relatively recent technology that can be used to inhibit the function of individual gene products. siRNA-based therapeutics have been increasingly explored in various disease settings, with the common need to localize biologically active double-stranded siRNA sequences into the target cell cytoplasm.
The local RNAi treatment of malignant glioblastoma (GBM) using peptide nanofibers (PNFs) in complex with siRNA has been investigated in the present study.
Polo-like kinase 1 (PLK1), a serine/threonine-protein kinase, is an early trigger for G2/M transition during the cell cycle, highly expressed in glioma tissue in comparison to normal brain tissue3. The inhibition of PLK1 using small molecule inhibitors has been shown to induce arrest of the cell cycle to the mitotic phase and to induce mitotic catastrophe, effect that translates in vivo in inhibition of tumor growth and radiosensitization of the treated cells with no effect on normal cells such as normal lung fibroblasts4. Combination therapy of PLK1 inhibitors and temozolomide has also shown a synergistic effect on GBM cell lines and the inhibition of PLK1 seems to have an effect also on the inhibition of cell invasion5.

In this study we present a strategy to locally target drug resistant glioma cells using a novel needle-shaped vector for the delivery of siPLK-1 to selectively target tumor proliferation.
PNFs are formed starting from palmitoyl-GGGAAAKRK peptide amphiphile containing basic aminoacids, Lys-Arg-Lys, that confers a positive charge to the fibers, as previously reported6. Complexation of the siRNA with the PNFs results in condensation of the siRNA around the fiber by electrostatic interaction. PNF:siRNA constructs are internalized by cells and achieve gene silencing in brain tissue when administered by stereotactic injection6. To test the hypothesis of peptide nanofibers as delivery systems for localized RNAi treatment of GBM we decided to use gene silencing to target PLK1 and inhibit GBM tumor proliferation. Cancer cells, such as U87MG luc2, have a high division rate, thus silencing of PLK1 should result in cell death and it would have therapeutic potential in the treatment of brain cancer. Treatment of U87 MG luc2 and glioblastoma-derived neural stem cells with PNF:siPLK1 complexes results in cell death in comparison to control treated cells. These findings supported our hypothesis that positively charged PNFs complexes could be used for sequence-specific silencing of gene expression by delivery of short interfering RNA.
The therapeutic potential of PNF:siPLK1 complexes has been assessed in vivo in a xenograft model of GBM. Athymic nudes mice were implanted with 2x105 U87 MG luc2 cells in the right striatum and tumor progression was measured before and after treatment using bioluminescence imaging by mean of an IVIS camera.
Animals receiving administration by intracranial injection in the tumor core of PNF:siPLK1 complexes, 14 days after tumor implantation, had an improved median survival time in comparison to untreated animals and control treated animals.
Taken together our data are a proof-of-principle that PNF:siRNA complexes can be considered useful tools for the localized genetic intervention in the treatment of GBM. Our findings with siPLK1 indeed could be extended to other relevant targets for the management of brain cancer therapy.

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ADRESSING COMPLEX DRUG SIMILATITY

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There is a global interest in developing a rational, science-driven approach to the process of making generic versions of nanomedicines and non-biological complex drugs (NBCDs) available and affordable, without compromising on safety or efficacy in comparison to innovator products. However, the unique development issues and inherent regulatory challenges of establishing therapeutic equivalence for such products have significantly hindered progress. As reflected in recent regulatory science initiatives by the FDA & EMA, there is increased expectation that 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 an overview of the current challenges in NBCD and nanomedicine product development and the opportunities this has created. It will highlight recent advances in development of physicochemical and in vitro characterization methods for use in guiding formulation strategy and demonstrating product equivalence, and discuss observed trends in how physicochemical parameters influence nanoparticle biocompatibility and toxicity. Funded by NCI Contract # HHSN261200800001E.

AQUEOUS SYNTHESIS OF IRON OXIDE NANOPARTICLES SUITABLE FOR HYPERTHERMIA TREATMENT

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The full potential of nanoparticles' clinical use is still hindered mostly by complex and often non-reproducible synthesis methods. Additional problems are the use of toxic molecules in the preparation, or the inability of available nanoparticles to simultaneously fulfill all the physicochemical and biological requirements. Among all the nanoparticle types, one of the most studied are superparamagnetic iron oxide nanoparticles (SPIONs), which are regardless of the size suitable as MRI contrast agents, but the remaining obstacle for theranostic applications is their low heating efficiency, and thus the main focus of our research was to optimise SPIONs for hyperthermia treatment. Theory predicts that maximal heating would be achieved for the narrowest possible particle size distribution around 18-20 nm. Hence, few synthesis routes giving SPIONs around this size were reported (mostly hydrothermal, HT, synthesis) but with the drawback of using organic solvents, toxic molecules and/or complex processes with issues such as reproducibility, repeatability and scaling (all necessary requirements for real clinical applications). All these issues could be avoided by co-precipitation (CP) synthesis which unfortunately produces SPIONs' sizes well below the required ones, but with narrow size distribution. Hence, we studied the influence of all parameters of CP synthesis (pH, concentration, Fe^{+3}/Fe^{+2} ratio, temperature and time of Fe^{+2} oxidation, etc.) on SPIONs' size. On the other hand, we studied as well influence of the same parameters (plus few cupping agents) on the SPIONs' size during the HT synthesis. Finally, we developed a novel synthesis route with parameters optimized in previous studies at the temperature sufficiently low to avoid rapid particles growth and control over SPIONs' size. Moreover, all this was performed in aqueous medium without the use of any toxic molecules as capping agent, any organic solvent or solvent exchange. As we aim for real applications, the synthesis with optimal conditions was repeated more than 100 times with different dishes, different

people and different batches of chemicals to assess the reproducibility and repeatability, confirmed by characterization of randomly chosen synthesis' samples. The primary particle size (as a mean of 500 manually measured particles' Feret diameters from transmission electron microscopy, TEM, micrographs), hydrodynamic size (measured by dynamic light scattering), crystalline size (deduced from X-ray diffraction (XRD) measurements) and ζ-potential at pH 4 for the SPIONs synthesised by novel route were obtained. As expected, longer heat treatment and higher temperatures resulted in larger particle size. The representative TEM micrographs of the SPIONs obtained under the different synthesis conditions are given in Fig. 1. In order to understand particles' growth mechanism, we have studied particles by HR TEM revealing coalescence of initially formed small (~8nm) nanoparticles (their TEM micrograph is given in left panel of Fig. 1). Further aging resulted in crystallization producing rectangular highly crystalline nanoparticles, while even longer aging caused broadening of particle size distribution and further particle's growth through Ostwald ripening (right panel of Fig. 1).

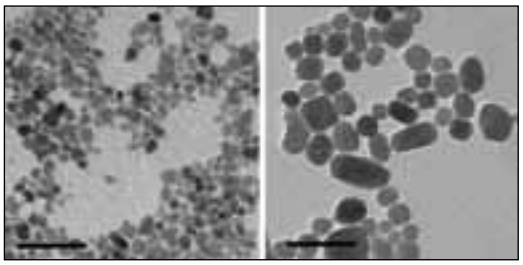

Fig.1: Transmission electron microscopy micrographs showing the SPIONs synthesized under the different synthesis conditions allowing control over the primary particle size. The scale bars on the micrographs is 50nm.

Beside the granulometric characterisation, the phase composition was determined by XRD confirming the expected crystallographic structure of the samples, with better crystallinity for longer heat treatments. Due to the large size of SPIONs obtained for the longest heat treatment, crystalline structure of this sample was additionally analysed in details at the Swiss-Norwegian beam-line (ESRF, Grenoble). It was found that these particles consist of one phase – maghemite, γ- Fe_2O_3. The average domain size is close to isotropic and is in agreement with the primary particle sizes measured from TEM micrographs.

For theranostics applications and especially for hyperthermia treatment, it is crucial to control magnetic properties of the materials, and therefore, detailed characterization of magnetic properties was done for all synthesized SPIONs. AC susceptibility measurements have been performed as a function of temperature for several frequencies and as a function of frequency at 300K. The first dependence allows to determine freezing temperature at which the high jump of the susceptibility occurs and the blocked nanoparticles (ferromagnetic) can rotate following the field in what is called Brownian relaxation. For higher temperatures, the out-of-phase susceptibility is linearly dependent on the temperature, since most of the nanoparticles are still blocked when the ferrofluid defrosts. Above the blocking temperature, the out-of-phase susceptibility dramatically increases due to rotation of blocked ferromagnetic nanoparticles with the magnetic field (Brownian relaxation), whereas below the blocking temperature, the relaxation process occurs by rotation of the magnetic moments (Néel relaxation). This Brownian relaxation is strongly dependent on temperature for a given frequency, and it is also dependent on frequency for a fixed temperature. Thus, AC susceptibility measurements have also been performed as a function of frequency at 300K. Since the maximum of the out-of–phase susceptibility should correspond to the maximum of the specific loss power (SLP), even before measuring SLP values we could see for which synthesis conditions we obtained SPIONs with the highest values of out-of–phase susceptibility for measured frequency range from 10Hz to 10kHz, meaning that we could expected the highest SLP values in that frequency range for the same SPIONs samples.

The measured magnetization as a function of magnetic field strength (M(H) curves) showed the expected behaviour for iron oxide nanoparticles of their size with a saturation magnetization close to bulk values (about 73-76 emu/gFe_2O_3 for maghemite). In fact, for only one synthesis condition SPIONs had saturation magnetization higher than maghemite bulk values suggesting the possible presence of magnetite phase in this sample. The hysteresis curves at 250K, in frozen state, and 300K, in liquid state, were measured for all samples of SPIONs, and from these measurements were extracted the corresponding saturation magnetizations, coercive fields and remanent magnetizations at both temperatures.

In order to evaluate the potential of each synthesized SPIONs for hyperthermia, the SLP values were extrapolated from the measured heating curves by using: SLP = (Cp_{H2O}/m_{Fe2O3})*(dT/dt), where mFe_2O3 is the mass of SPIONs added in the measurement vessel and CpH2O is the specific heat of water. The highest SLP values obtained in this way was ~127 W/gFe_2O3. Since SLP values calculated in this way assume perfectly adiabatic system, the obtained SLP values strongly depend on the (goodness of) adiabatic condition of measurement (meaning depends on the equipment), and thus SLP values were measured with 3 different equipments (one home made and 2 commercial). In addition, we measured the SLP values as a function of particle size, particle concentration, magnetic field and frequency. Moreover, as the SLP strongly depends on the frequency f and the magnetic field H, and therefore, instead of SLP, intrinsic loss power (ILP) was introduced as SLP divided by $f*H^2$. However, it was afterwards shown that some nanoparticles, like maghemite exhibit strong size-dependent magnetic permeability, meaning that instead of calculating H one could use B to obtain "nonstandard" ILP values which was calculated to be around 2 W/($g_{Fe2O3}T^2$Hz) for our particles (which is among the highest reported values [1]).

We confirmed, as well, the suitability of our particles with different sizes and concentrations as a diagnostics MRI contrast agent, and we found that T2 relaxation time increases with increasing SPIONs size.

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A POINT-OF-CARE DIAGNOSTIC PLATFORM FOR INFECTIOUS DISEASES BASED ON CENTRIFUGAL MICROFLUIDICS (DISCOGNOSIS)

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People in sub-Saharan Africa, but also in many tropical regions, suffer dramatically from infectious diseases, a vast majority of which develop fever as a clinical symptom. Most of the cases are attributed to malaria, a disease that is reported in 207 million cases globally in 2013, leading to approximately 627,000 deaths [1].

Although malaria is one of the major causes of fever, a high proportion of patients suffer from non-malaria febrile illnesses. These may be bacterial infections (such as typhoid fever or pneumonia) or viral infections (such as dengue), which require totally different treatment. However, the fact that the presented symptom is the same renders diagnosis and, subsequently, the treatment, extremely challenging as false diagnosis may take place in up to 30% of the examined cases [2].

Current diagnostic methods are often unsuitable for providing an accurate answer to the nature of the causative pathogen: (i) the microscopy blood smear tests are only applicable in case of malaria; (ii) the Rapid Diagnostic Tests (RDTs) are cheap and easy-to-use immunochromatographic tests but detect only one disease per test, while their sensitivity and specificity are often in dispute; (iii) culture methods are specific but too time consuming and require experienced personnel.

Within this context, the DiscoGnosis project aims to develop a Point-of-Care (PoC) platform for the detection of malaria and other infectious diseases with similar symptoms, namely dengue, typhoid fever and pneumonia. Due to the multiple causative pathogens targeted (parasites, viruses and bacteria) the diagnostic tool will assist the healthcare providers to select the most suitable drug (antimalarial, antibiotic) for efficient treatment.

The diagnostic platform performs a combined DNA/RNA and protein-based diagnosis on a single cartridge in order to specify the nature of the fever-causing pathogen. The cartridge is a centrifugal microfluidic disc (the LabDisk, Fig.1) that integrates all biochemical components in order to achieve a fully-automated analysis from sample (50µl whole blood) to answer (real-time amplification curves). Microfluidic "unit operations" are designed and interfaced in order to transfer the assays from bench to the disc and fully automate the processes (e.g., sample inlet, extraction and purification of nucleic acids, mixing with amplification reagents, aliquoting and amplification/detection, are all functions that take place on the disc). Pre-storage of liquid reagents in stickpacks (Fig.1) allows full integration of the aforementioned processes [3]. The amplification takes place by means of loop-mediated isothermal amplification technology (LAMP), which is faster than PCR (typical time-to-positive 20 min). A true sample-to-answer analysis of Salmonella Typhi and Paratyphi spiked in whole blood has been achieved on disc in ~40 min (time includes in situ sample preparation without manual steps). The cartridges are manufactured via microthermoforming of polymer foils, a technology that is routinely used in blister package production and is adapted to microscale features. The foil nature of the cartridges enables the scalability of the production in a "line" (and batch) format, i.e., in a sequence of steps: thermoforming; surface modification/processing, filling with dry/liquid reagents; sealing; cutting and packaging.

The validation will take place in Medical Center Nyankunde, D.R. Congo (real-time patient recruitment) and Institut Pasteur in Dakar, Senegal (using biobanked samples). The generic nature of the platform renders it adaptable to different diagnostic panels defined by the end-users' needs.

Further information about the project is available at www.discognosis.eu.

ACKNOWLEDGEMENTS

DiscoGnosis is supported by the European Commission through the objective FP7 ICT-2011.3.2 and under Grant Agreement No. 318408. All consortium partners are also greatly acknowledged.

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Fig.1: The LabDisk and Reader. The sample (50µl whole blood inserted in NA.6) is treated with buffers (pre-stored on disc in pouches called "stickpacks" in NA.1-NA.5) for pathogen lysis, DNA/RNA extraction (presence of magnetic beads) and purification. The purified (eluted) nucleic acid is pumped from NA.9 to NA.11 where it rehydrates a lyopellet, which contains amplification enzymes, dNTPs. The mixture is then aliquoted (via the microfluidic module NA.12.1) to the reaction chambers (NA.12.2) where the pre-stored primers initiate the LAMP amplification, which is detected in real time via fluorescence.

METABOLOMICS OF POLYCATION CYTOTOXICITY: MECHANISTIC ASPECTS

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Polycations such as polyethylenimines (PEIs) are among the most efficient non-viral transfectants, but the underlying mechanisms of PEI architecture- and size-dependent cytotoxicity still poorly understood. Through an integrated analysis of PEI and PEI polyplex trafficking at live- and single-cell levels, global proteomic fingerprinting and metabolomic profiling we show that PEI-induced cytotoxicity is dynamic, multifaceted, and occurs through different modes of cell death processes (apoptosis, necrosis, programmed necrosis, and authophagy). PEI in an architecture and size-dependent manner destabilizes plasma membrane and mitochondrial membranes differently and with consequences on mitochondrial dynamics, motility, oxidative phosphorylation and energetics, glycolytic flux and redox homeostasis that ultimately modulate the switching of cell death pathways. These integrated approaches have provided a rapid approach for mechanistic understanding of multifactorial and multifaceted PEI-mediated cytotoxicity, and could form the basis for combinatorial throughput platforms for improved design and selection of safer polymeric vectors for transfection purposes. On the basis of these approaches, we have further designed libraries of PEI-based polymeric nanosystems as simple, safe and versatile vehicles for cell targeting.

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miRNA-INHIBITORS FOR CANCER STEM CELL ERADICATION

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Cancer stem cells have been discovered as rare subpopulation in solid tumors with properties similar to stem cells, such as ability for self-renewal and asymmetric cell division. Except for high tumorigenic potency, cancer stem cells display drug and radiation resistance and propensity for metastatic spread. According to the present concepts, recurrent drug resistant metastatic cancer is due to cancer stem cells escaping primary treatments, so that intense efforts are presently undertaken to discover novel drugs that are able to eliminate cancer stem cells.

miRNAs are master regulators in stem cells and cancer via their ability to post-transcriptionally regulate large sets of genes. Antisense nucleic acids (miRNA-inhibitors; also: antimirs or antagomirs) can efficiently inhibit the action of these master regulators. We performed a genome-wide miRNA-inhibitor screen directly comparing activity in breast cancer versus tissue-matched normal breast stem cells. The screen identified a total set of miRNA-inhibitors with prospective selective killing activity for breast cancer stem cells. Initial downstream analyses of the miRNA-inhibitors will also be summarized.

EVALUATION OF NANOPARTICLE PEGYLATION: QUANTITATIVE AND QUALITATIVE DETERMINATION

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INTRODUCTION

Polymeric nanoparticles of poly(alkyl cyanoacrylate) (PACA) have been extensively studied as drug carriers for targeted drug delivery, and are currently in phase III clinical trials. One of the main challenges when using nanocarriers for drug delivery is the immediate recognition and fast uptake by the mononuclear phagocyte system, limiting the amount of nanoparticles that reach their target site. PEGylation - the coverage of PEG (polyethylene glycol) on the particle surface - is by far the most commonly used strategy for escaping the mononuclear phagocyte system. However, in spite of its wide use there is no general agreement on the optimum PEG chain length or coverage-density to avoid clearance by the immune system and extend the circulation time. In fact, although the majority of the studies found on nanoparticles utilize PEGylation, few papers describe the actual quantification of PEG and very few have associated this directly with cellular uptake, biodistribution or blood circulation time. One of the main reasons for this is the shortage of straightforward characterization techniques to assess the coverage density.
We have made PEGylated PACA nanoparticles with various types and lengths of PEG, using a one-step synthesis method. PEGylation of the nanoparticles was evaluated using both direct and indirect methods. We further studied how different PEGylation affected protein adsorption, cellular uptake and blood circulation time.

METHODS

PACA nanoparticles were synthesized in one step by preparing oil-in-water emulsions consisting of an alkylcyanoacrylate, co-stabilizer and a fluorescent dye, in an acidic aqueous medium containing surfactant. The anionic polymerization was initiated by adding PEG-surfactants to the emulsion, resulting in PEGylated NPs. The PEGs used were of different type and length, also affecting the strength of association to the nanoparticle surface.
Evaluation of PEGylation was done either by direct measurements using 1H-nuclear magnetic resonance (NMR), thermogravimetric analysis (TGA) and TOF-SIMS/XPS, or by indirect measurements studying zeta-potential, protein adsorption and hydrophobicity of the nanoparticle surface.
The effect of various PEGylation strategies was evaluated by studying the cellular uptake of nanoparticles in various cell lines (endothelial cells and cancer cells) and by measuring the blood circulation time in mice.

RESULTS AND DISCUSSION

Direct measurements of PEG using 1H-NMR, TGA and TOF-SIMS/XPS gave the same trends, indicating that these methods are all useful for comparing PEGylation of different nanoparticles. As expected, zeta-potential values became less negative upon increasing the amount of PEG (Fig 1).
However, direct and indirect methods for determining PEGylation did not always correlate well, meaning that the amount of PEG is not necessarily related to the effectiveness (stealth effect). In fact, blood circulation time of PACA nanoparticles in mice showed that particles with high MW PEG, although less densely covered, had the longest circulation half-life (Fig.2). The blood circulation time correlated well with protein adsorption on the nanoparticle surface. Particles that showed least adsorption of proteins had the longest blood circulation time.

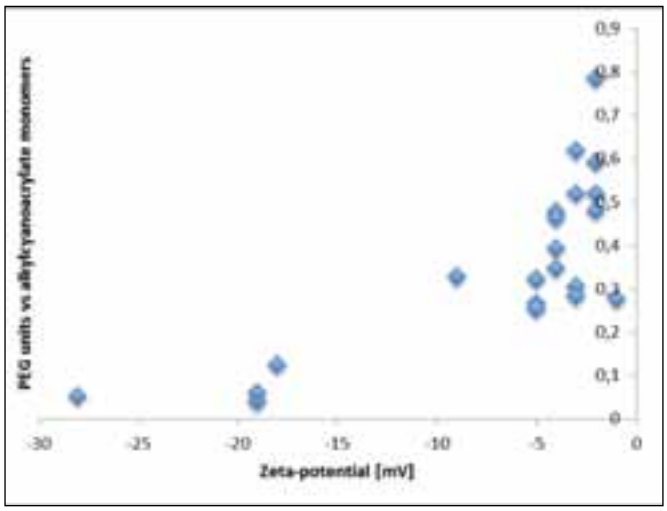

Fig. 1: Relative amount of PEG vs alkylcyanoacrylate monomers as a function of zeta-potential for PACA nanoparticles.

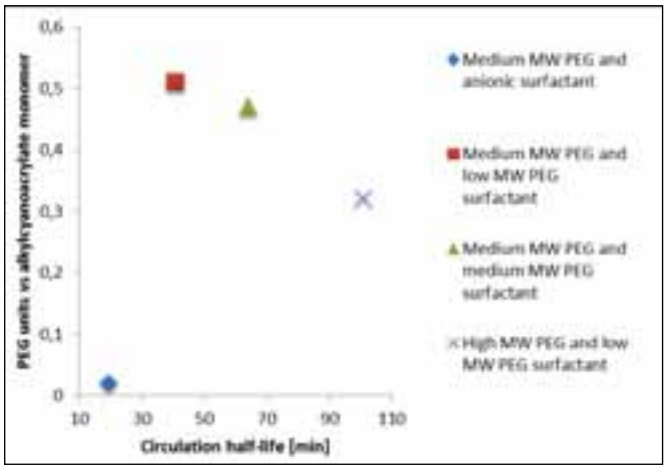

Fig. 2: Relative amount of PEG vs alkylcyanoacrylate monomers as a function of circulation half-life of PACA nanoparticles in mice.

The cellular uptake of PACA nanoparticles was also affected by the type and amount of PEG, and did not necessarily correlate with the PEG density. Further, the cellular uptake varied significantly between the different cell lines.

CONCLUSION

There is a common assumption that high density of PEG is necessary to reduce protein adsorption and give longer circulation time. We have shown that not only the amount of PEG, but rather the PEGylation strategy used (type and length of PEG) has significant effect on the particle efficiency both with regard to cellular uptake and clearance by the immune system. Importantly, we have shown that an evaluation of the PEGylation strategy can, and should be, performed by various complementary methods when designing a nanocarrier system.

DRUG DELIVERY AND TISSUE TARGETING WITH ULTRA-SMALL GOLD NANOPARTICLES

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Midatech's core technology relates to glycan-coated gold nanoparticles (GNPs), a class of self-forming nanoparticles comprised of a noble metal atom core to which an organic layer of carbohydrates or glycans are attached via gold-sulphur bonds. The carbohydrate residues stabilize the metallic core (passivation) and make the particle both water-soluble and biocompatible. During the self-formation process other ligands can be attached to the gold core and these will be interspaced between the glycans. This process results

in the single-step synthesis of multi component particles that can deliver multiple copies of a drug with targeting efficiency.
The physical and chemical properties of GNPs are important. The effective hydrodynamic diameter of a GNP is ~3.5 nm (gold core is about 100 atoms of gold and 1.4nm in diameter), which conceivably is smaller than any other delivery vehicle used in medical treatment today and is about the size of a small globular protein. This small size creates several critical qualities for Midatech's GNP-based drugs:

- They are able to pass through the normal pore sizes of blood vessels, and circulate via interstitial flow to normal and diseased organs, to allow delivery to a large number of disease sites
- The GNP's are quickly cleared from the body by excretion in the urine without the need for metabolism thereby increasing the elimination of the product
- Midatech is using gold core GNPs in all its development programmes due to the superior biological properties of gold

Midatech is currently developing its platform technology to target cancer cells with nanoparticle chemotherapeutic agents. The multivalent nature of the gold nanoparticles allows to couple both the anti-tumor agents and various targeting molecules specific for cancer cells on the same nanoparticle.
The targeting agent seeks out specific tumours or diseased organs, using "velcro type" adhesion, and due to the unique uptake properties of small nanoparticles, the nanoparticle drug combination is able to enter cancer cells to deliver its chemotherapeutic payload exactly where it is required.
At present, three oncology projects (ovarian, liver and brain) are being pursued based on similar principles and using similar chemotherapeutic agents with different targeting agents.
First results of in vivo targeting and efficacy studies in human tumour xeno-transplanted mouse models will be presented.

INDUSTRY CASE STUDY #1 IRON NANOPARTICLES

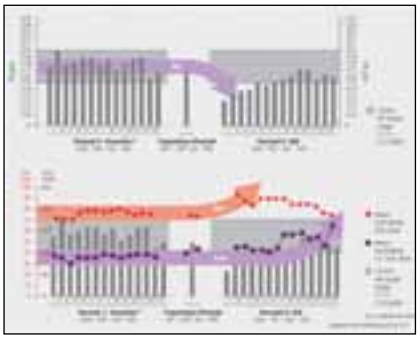
STEFAN MÜHLEBACH

i.v. iron colloidals are widely used nanomedicines to treat iron deficiency. They are indicated when there is a clinical need for rapid iron supply (e.g. in very low ferritin patients) and where oral iron therapy is ineffective (e.g. in inflammatory bowel disease) or not tolerated (gastrointestinal discomfort). i.v. iron carbohydrate colloidals are synthetic complex drugs composed of a polynuclear iron (III) oxohydroxy core stabilized by a shell of different mono-, di-, oligo- or polysaccharides defining the (nano-) sized structure and morphology impacting pharmacokinetics, pharmacodynamics including toxicity, and immunogenicity upon injection into the body (fig.1).

Fig. 1: Nanocolloidal iron sucrose: a typical non-biological complex drug (NBCD) of about 35-60 kD molecular size (molecular weight range).

The in-vitro and in-vivo characteristics of such polydisperse NBCDs, consisting of non-homomolecular structures are defined by a multi-step manufacturing process and the appropriate tight control. NBCDs cannot be fully characterized by physicochemical tests alone. Although iron nanomedicines have been used successfully and extensively since a long time, tolerance of higher doses and also the rare but potentially severe hypersensitivity reactions vary between chemically different carbohydrate moieties indicating the necessity to evaluate and compare the in vitro and in vivo profile. Therefore, such complex products and their follow-on versions need quality, non-clinical, and clinical assessments to prove comparability. There might be unknown small differences between such NBCDs versions with clinical significance. The totality of evidence is needed to show similarity and finally therapeutic equivalence of an originator and a nanosimilar follow-on version.
This was only realized when the first iron sucrose similars were approved using the generic paradigm by authorities in Europe not realizing the colloidal (nanomedicine) character of these NBCDs eventually key for comparability and performance of these medicinal products.
In 2011 a retrospective-prospective study in hemodialysis patients with well controlled and stable hemoglobin values revealed clinically meaningful differences between the originator iron sucrose and an iron sucrose similar (Fig.2).

Fig. 2: Switching form originator (Venofer®) to an iron sucrose similar (FerMylan®) in 75 HD patients (Rottembourg et al. Nephrol Dial Transplant 2011;26:3262–3267)



Also non-clinical data in non-anemic rats showed differences between originator and iron sucrose similar products indicating changed biodistribution and tissue targeting resulting in different pharmacodynamic effects (Toblli JE et al. Drug Research 2009; 59: 176-190). Based on such evidence EMA published several reflection papers on i.v. iron colloidals indicating data requirements for follow-on versions (2011-2015). EMA concluded in a stepwise and weight of evidence approach to show highly similar pharmaceutical quality, non-clinical (biodistribution), clinical (PK, safety, efficacy) characteristics, including a suited post marketing surveillance/risk management plan to assess hypersensitivity or iron overload. Only fulfilling such an extensive comparability exercise finally allows to conclude on therapeutic equivalence, a prerequisite to use such NBCD nanomedicines follow-on versions as substitute or interchangeable medicinal products. This also indicates that similar is not equal and that a regulatory approach although highly desired is not yet defined and not existing for a harmonized global approach to guarantee safety and efficacy for such NBCDs developed with reference to a nanocolloidal i.v. iron sucrose formulation widely and safely used in different patients since many decades.

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EX VIVO IMAGING OF BRAIN TISSUE IN HEALTH AND DISEASE

BERT MÜLLER, Biomaterials Science Center, University of Basel, Switzerland)

Combining the current prospects of X-ray imaging, spatially resolved X-ray scattering and sophisticated data analysis, the goal of visualizing the human body down to the atomic level could become reality. We have investigated the nanostructures within the human brain with the focus on myelin sheaths and determined their abundance and orientation with micrometer precision [1]. Here, we demonstrate the complementary character of conventional histology and spatially resolved, small-angle X-ray scattering. We further demonstrate the power of the single-grating-based phase-contrast tomography of the human hippocampus [2], and the in-line and grating-interferometry phase tomography of human cerebellum [3] to visualize individual cells and their internal structures. These activities bridge the current gap between the imaging modalities of hospitals and the nanometer-scale solid-state physics.

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INTERACTION OF DENDRITIC CELLS WITH FOOD-BORNE NANOPARTICLES

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Consumers ingest large amounts of food-borne nanoparticles (NPs) for example in the form of SiO_2 (E551) or TiO_2 (E171), and it is expected that new emerging nanotechnology applications (for example $FePO_4$) will dramatically enhance oral exposure to NPs by intentional or accidental incorporation into food, beverages or supplements. In view of these developments, it becomes necessary to characterize the interaction of NPs with the intestinal immune system because any derailment of the gut-associated immunity may precipitate immune-mediated pathologies like inflammatory bowel disease. After oral uptake, NPs encounter steady-state intestinal dendritic cells, which are the key “decision makers” in the immune response as they discriminate innocuous antigens from potentially harmful pathogens. Steady-state dendritic cells avoid reactions against beneficial commensal bacteria or normal food constituents, but induce inflammation and immune responses to counteract invasions by pathogens. NPs could potentially interfere with this delicate balance between tolerance and activation of intestinal immunity. Therefore, a new hazard inherent to food-borne NPs is their possible ability to interact with steady-stated dendritic cells and alter their critical function in fine-tuning inflammation and immunological outcomes.
Under homeostatic conditions, dendritic cells develop from hematopoietic progenitors of the bone marrow under the direction of FMS-like tyrosine kinase 3 ligand (Flt3L) and Flt3L receptor signaling. In vivo, Flt3L is required to generate steady-state dendritic cells that reside in lymphoid organs like the spleen and also migrate into peripheral tissues of the skin, lung and intestine. Here, murine bone marrow cells were cultured in vitro in the presence of Flt3L. The resulting steady-state dendritic cells were incubated with food-grade NPs consisting of E171, E551 and $FePO_4$. The endpoints analyzed include cellular uptake demonstrated by flow cytometry, transmission electron microscopy (TEM) and energy dispersive X-ray spectroscopy (EDX), dendritic cell viability, release of pro-inflammatory cytokines (particularly IL-1β) determined by immunoassays as well as dendritic cell maturation assessed by changes of the surface marker repertoire (up-regulation of CD40, CD69, CD86; down-regulation of CD62L). We found that food-grade silica NPs (E551) induce distinctive and dose-related activation patterns in steady-state dendritic cells. Interestingly, silica NPs are able to elicit a release of the inflammatory cytokine IL-1β without preceding dendritic cell priming. However, prior activation by an inflammatory cocktail containing GM-CSF and IL-3 (generating inflammatory dendritic cells) further enhances their response. On the other hand, steady-state dendritic cells are refractory to NPs consisting of E171 or $FePO_4$. These findings support the hypothesis that specific NPs may interfere with the function of the gut-associated immune system and our in vitro assay provides a screening system to test whether NPs considered as food ingredients or food contact materials are able to activate steady-state dendritic cells.
This project is funded by the Swiss National Science Foundation in the frame of NRP64.

HUMAN IMMUNE SYSTEM RECONSTITUTION IN THE MOUSE FOR BETTER SAFETY/TOXICITY PROFILING AND PREDICTIVE TRANSLATIONAL EFFICACY

PATRICK NEF, PhD, CEO & President TransCure bioServices, Archamps (France)

A new approach toward the profiling of any preclinical drug candidate or medical device for its level of safety or toxicity potential will be presented. In vivo pharmacology models with the human immune system (HIS) functions fully reconstituted in immunodeficient mice are emerging as more relevant and predictive than traditional approaches. An example of a highly toxic drug in human (FIAU, a nucleoside analog), but 100% safe in traditional rodent and non-rodent models will be presented, as the toxicity was similar to human using the HIS models with a humanized liver. More and more, the HIS models are improved, and provide valuable options to select the best preclinical candidate based on human efficacy and predictability. Illustration of a better efficacy assessment with anti-HIV, or anti-inflammatory Bowel Disease, or anti-cancer preclinical candidates will also be presented. The reconstitution of a full human immune system response in mouse models represents a new approach toward selecting the best pre-clinical candidates for safer and more efficacious clinical trials and ultimately marketed products. The early assessment of safety and efficacy allows for the de-risking of expensive clinical trials. We will suggest the use of HIS models for the proper in vivo immuno-safety assessment of any nanoparticle/device or of any preclinical drug candidate.

QUANTIFICATION OF NANOPARTICLE UPTAKE AND DEGRADATION BY CELLS AND ITS CORRELATION TO BASIC PHYSICOCHEMICAL PARAMETERS

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Some common sense statements about the interaction of colloidal nanoparticles with cells are given. While detail of this interaction strongly depends on the precise properties of the nanoparticles, the type of cells, conditions of incubation, etc. still some basic universal common sense dependences exist, which will be outlined. In general small, elongated, positively charged, soft particles are incorporated in vitro by cells to a higher extend than big, flat, negatively charged, stiff particles. After in vivo incorporation the environment of the nanoparticles drastically changes. In particular the nanoparticle may be exposed to different enzymes such as proteases. This may lead to changes in the surface chemistry of the nanoparticles. Consequences for active versus passive targeting will be discussed.

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UTILIZING RNA FOR IN VIVO DRUG DISCOVERY AND POTENTIAL THERAPEUTICS

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RNAi is a ubiquitous and highly specific, endogenous, evolutionarily conserved mechanism of gene silencing. Since RNAi was shown in mammals almost 10 years ago, the prospect of harnessing RNAi for human therapy has developed rapidly. The RNAi machinery probably exists in all cells and mark a new path to silence genes.
RNAi can be activated by expressing short hairpin RNA (shRNA) with viral vectors, or by incorporating small interfering RNAs fragments (siRNAs) into the cell cytoplasm. The later, eliminates clinical safety concerns associated with viral vectors. Therefore, siRNAs represent the most promising type of RNAi-based therapeutics currently advancing in preclinical and clinical trials. Despite the promise as a new form of medicine, the prime challenge facing the translation of siRNAs into clinical practice is delivery. The efficiency by which synthetic siRNAs cross the plasma membrane and enter the cytoplasm is usually very low. Unmodified 'naked' siRNAs are subject to rapid renal clearance and are degraded by RNases, shortening their half-life in vivo. Furthermore, siRNAs could stimulate the immune system by being recognized via Toll-like receptors, thus provoking interferon responses, causing cytokine induction, and activating coagulation cascades. These can cause global suppression of gene expression as well as aberrant immune activation, generating off-target effects and misinterpreted therapeutic outcomes.
Novel nanotechnologies utilize an interdisciplinary approach to generate nanocarriers and create new opportunities for targeted therapies. Unlike conventional therapies, targeted nanocarriers, have the potential to offer more effective treatment with significantly reduced adverse effects through precise interactions with membrane receptors by including targeting moieties that can direct drugs in a cell- or tissue-specific manner. The carriers allow the delivery of large amounts of therapeutic payloads per recognition event, the capacity to deliver multiple therapeutic agents simultaneously, and the potential to overcome physiological barriers. Despite the progress in engineering siRNA delivery platforms to the liver, delivery of RNAi payloads for other organs such as brain tumors and to hematological cells remains challenging and less characterized.
The examples we will detail in the presentation will focus on an aggressive brain tumor (GBM) and systemic delivery of siRNAs to leukocytes.
Glioblastoma Multiforme (GBM) is one of the most infiltrating, aggressive and poorly treated brain tumors. Progress in genomics and proteomics has paved the way for identifying potential therapeutic targets for treating GBM. Yet, the vast majority of these leading drug candidates for the treatment of GBM are ineffective, mainly due to restricted passages across the blood brain barrier. Nanoparticles have been emerged as a promising platform to treat different types of tumors, due to their ability to transport drugs to target sites, while minimizing adverse effects. Herein, we devised a localized strategy to deliver RNA interference (RNAi) directly to GBM site using hyaluronan (HA)-grafted lipid-based nanoparticles (LNPs). These LNPs having an ionized lipid previously shown to be highly effective in delivering small interfering RNAs (siRNAs) into various cell types. LNPs surface was functionalized with hyaluronan (HA), a naturally occuring glycosaminoglycan that specifically bind the CD44 receptor expressed on GBM cells. We found that HA-LNPs can successfully bind to GBM cell lines and primary neurospheres of GBM patients. HA-LNPs loaded with Polo-Like Kinase 1 (PLK1) siRNAs (siPLK1) dramatically reduced the expression of PLK1 mRNA and cumulated in cell death even under shear flow that simulate the flow of the cerebrospinal fluid compared with control groups. Next, human GBM U87MG orthotopic xenograft model was established by intracranial injection of U87MG cells into nude mice. Convection of Cy3-siRNA entrapped in HA-LNPs was performed and specific Cy3 uptake was observed in U87MG cells. Moreover, convection of siPLK1 entrapped in HA-LNPs reduced mRNA levels by more than 80% and significantly prolonged survival of treated mice in the orthotopic model. Taken together, our results suggest that RNAi therapeutics could effectively be delivered in a localized manner with HA-coated LNPs and ultimately may become a therapeutic modality for GBM.
Leukocytes continue to be among the most difficult targets for siRNA delivery due to the fact that they are resistant to conventional transfection reagents, and that they disperse in the body, making it difficult to successfully localize or passively deliver siRNAs via systemic administration. Modulating T cells functions by down regulating specific genes using RNA interference (RNAi) holds tremendous potential in advancing targeted therapies in many immune related disorders including cancer, inflammation, autoimmunity and viral infections. Hematopoietic cells, in general, and primary T lymphocytes, in particular, are notoriously hard to transfect with small interfering RNAs (siRNAs). Herein, I will describe a novel strategy to specifically deliver siRNAs to murine CD4+ T cells using targeted lipid nanoparticles (tLNPs). To increase the efficacy of siRNA delivery, these tLNPs have been formulated with several lipids designed to improve the stability and efficacy of siRNA delivery. The tLNPs were surface functionalized with anti-CD4 monoclonal antibody (mAb) to permit delivery of the siRNAs specifically to CD4+ T lymphocytes. Ex vivo, tLNPs demonstrated specificity by targeting only primary CD4+ T lymphocytes and no other cell types. Systemic intravenous administration of these particles led to efficient binding and uptake into CD4+ T lymphocytes in several anatomical sites including the spleen, inguinal lymph nodes, blood and the bone marrow. This resulted in the efficient silencing of the pan leukocyte surface marker CD45 in circulating and resting CD4+ T lymphocytes. Taken together, these results suggest that tLNPs may open new avenues for the manipulation of T cell functionality and may help to establish RNAi as a therapeutic modality in leukocyte-associated diseases.

NEW BETULIN-BASED NANOEMULSION EFFECTIVE IN EARLY MELANOMA: IN VIVO MOLECULAR CHANGES IN TREATED SKIN ASSESSED BY CONFOCAL RAMAN SPECTROSCOPY IN MICE MODELS

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KEYWORDS:
betulin nanoemulsion, early melanoma, confocal micro-Raman spectroscopy.

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 Raman spectroscopy techniques, based on the 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 laser Raman spectroscopy 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.

The novelty introduced in this paper relies on the direct, in-vivo comparative Raman micro-spectroscopy assessment of the early malignancy evolution in mice models skin tissue when a new betulin-based pharmaceutical nanoformulation is topically applied. Raman spectroscopy study was assisted by the toxicology evaluation. The Raman spectral changes were directly correlated with the skin pathology without additional statistical methods for discrimination. The molecular changes in skin tissue in vivo following the treatment with a chemical carcinogen 7, 12 dimethylbenzanthracene (DMBA) as initiator and 12-O-tetradecanoylphorbol-13-acetate (TPA) as cancer promoter were assessed by in vivo Raman micro-spectroscopy. Among various betulin formulations to overcome the poor solubility, a new nanoemulsion was prepared, physic-chemical characterised and applied on animal models to evaluated its effectiveness in early melanoma. The experiment is part of a large multidisciplinary approach where reproducible melanoma has been obtained in mice and new betulin-based pharmaceutical formulations with potential efficacy in treating skin cancer have been prepared and tested. In this study, the animal models developed a slow form of cancer, epidermal tumorigenesis and the therapeutically surveillance could be performed.

Betulin (lup-20(29)-ene-3, 28-diol, B) is a pentacyclic triterpene used for a long time for its promising medicinal properties, particularly its chemopreventive and antitumor activities [1, 2]. Since betulin has a poor solubility in water [2], we recently prepared and tested an oil-in-water nanoemulsion formulation for efficient delivery [3]. Nanoemulsions have been widely used as drug delivery vehicles for poorly aqueous soluble anticancer drugs for both parenteral and oral administration [3, 4]. They are composed of a wide variety of edible oils as internal oil phase and biocompatible phospholipids as emulsifiers. Nanoemulsions can incorporate significant amounts of drugs in the high volume fraction of the oil core and are found suitable for the delivery of poorly aqueous soluble drugs. Additionally, nanoemulsion formulations are easy to scale up, cost effective, and relatively stable when compared to many other nano-sized drug delivery systems, such as liposomes [3-5]. The nanoemulsion obtained here with the betulin triterpene incorporated drug as poorly soluble compound was topically applied on the skin of mice specimens treated with the DMBA carcinogen.

Four groups of mice specimens, one exhibiting an early phase of melanoma skin cancer, a group topically treated with the newly prepared betulin-based nanoemulsion, a solvent treated group, and a healthy one respectively, were employed aiming to assess the ability of the confocal Raman micro-spectroscopy technique for in vivo monitoring both the spectral changes associated with the early malignancy and the spectroscopic signature of the applied treatment in early melanoma. The main spectral region that allowed the diagnosis of the investigated skin pathologies and monitoring the applied treatment was identified in the 1200-1340 cm-1 range. According to the spectral changes, the early phase of skin cancer revealed high content of nucleic acids and lipids, while the betulin-treated skin revealed higher content of proteins as suggested by the plotted values of the amide III band area recorded from the rodents groups.

Betulin containing nanoemulsion formulation was prepared with uniform droplet size using high shear microfluidizer processor. Size distribution and TEM analysis (Fig. 1) showed that the spherical shaped nanoemulsion droplets range between 100 and 200 nm diameter. Zeta potential values for the blank and drug containing nanoemulsions were in the range of -23 to -40 mV.

Confocal Raman micro-spectroscopy was employed for the identification of the molecular changes which occurred at the skin level along with the applied pharmaceutical treatment. The signal recorded exhibits many similarities to the ones earlier reported for in vivo as well as ex vivo Raman investigations on both human and animal skin [6, 7].

A graphical display of the areas calculated for the Raman peaks observed in the 1240-1300 cm-1 spectral range in all specimens is shown in the Fig. 2. The smallest areas of these bands are characteristic to the melanoma corresponding spectra, while the betulin treated skin show a higher content, increasing the value toward the area of the healthy specimens. These results support our conclusions and show that the betulin nanoemulsion treatment had a beneficial effect on the skin treatment in the case of early stage melanoma. The specificity of the technique was also exploited to monitor the skin reaction to the new betulin formulation as a potent anti-cancerous drug candidate. Although the betulin detection in the nanoemulsion is perfectly hampered by the emulsion overlapping bands with the betulin characteristic Raman signal (Fig. 2), the vibrational Raman characterization suggests the emulsion character, based on the slight spectral differences between the blank and betulin containing nanoemulsion. The betulin treated mice group was differentiated from the others and specific spectral markers were found in the 1200-1340 and 1030-1130 cm-1 spectral ranges, where amide III band of proteins showed higher intensity than the nucleic acids bands. Concluding, based on the Raman in-vivo measurements as well as the histopathology evaluations, the ability of technique to provide direct evidence of the molecular changes associated with the therapy was proved. These changes were associated with the reduction of the inflamed area and malignancy inhibition, to support the effectiveness of the betulin nanoemulsion. These results create future perspectives in personalized nanomedicine and cancer therapy assisted by Raman spectroscopy techniques, as noninvasive, rapid and sensitive alternative for in vivo pathology evolution and pharmaceutical monitoring. A short results review on the betulin extraction from local natural sources, content evaluation, various formulations and effectiveness in skin dissease [8] will be also provided.

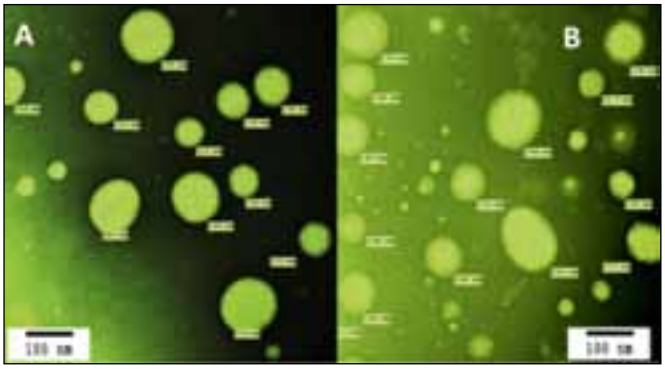
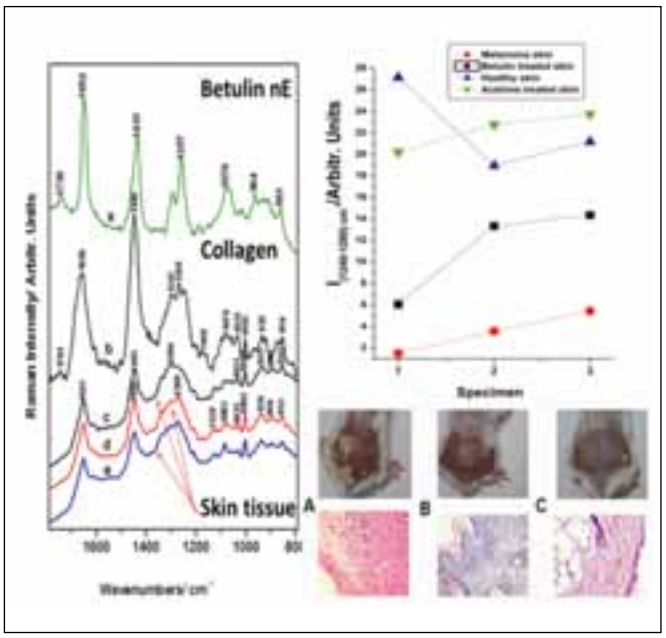


Fig. 1. Transmission electron microscopy (TEM) image of betulin-containing nanoemulsion (A) and of the blank one (B). The diameter size is indicated on each nanodroplet. Scale bar 100 nm.

Fig. 2. Left: Raman spectra of the betulin nanoemulsion (a), pure collagen (type I, powder, b) and of the in vivo skin tissue (skin surface (c), 350 µm skin depth (d) and 700 µm deeper (e). Right: Comparison between the calculated areas of the Raman bands in the 1240-1280 cm-1 spectral range assigned to the amide III of collagen observed in the spectra collected from each of the mice specimens. The spectra sequences were collected in 5 seconds. Laser line: 785 nm. Optical image of the skin lesions with (A) carcinogens application (DMBA/TPA), betulin nanoemulsion treated (B) and solvent treated (C) group and the corresponding histopathology images are shown in the bottom.



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NOVEL TARGETED MEDICINE TO MODULATE TUMOR-ASSOCIATED MACROPHAGES FOR THE TREATMENT OF BREAST CANCER

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BACKGROUND

Tumor-associated macrophages (TAMs), one of the most crucial cell types in the tumor microenvironment, contribute to the tumor growth and metastasis. These pro-tumoral macrophages show M2-like phenotype whereas their counterpart M1-like macrophages have been shown to inhibit tumor growth and survival. The signal transducer and activator of transcription 6 (STAT6) pathway is the downstream signaling pathway of IL-4 and IL-13, cytokines responsible for M2 differentiation. We hypothesized that inhibition of STAT6 pathway might be an interesting strategy to inhibit TAM (M2-like macrophage) differentiation and thereby their pro-tumorigenic activities. We therefore investigated the effect of an inhibitor for STAT6, AS1517499, on M2 differentiation and M2-driven tumor cell migration. Thereafter, we investigated the effect of AS on the tumor growth and metastasis in vivo in 4T1 tumor model. Furthermore, we developed a lipid nanoparticle formulation of AS and examined it in vitro on TAMs.

MATERIALS AND METHODS

Mouse RAW264.7 cells were differentiated into M2 type using IL4 and IL-13. The STAT6 inhibitor (AS1517499, AS) was evaluated for its effects on M2 differentiation using qPCR, Western blot (pSTAT6/STAT6) and arginase activity assays. Effect of M2 cells on the migration of mouse 4T1 breast tumor cells (ATCC) was investigated using M2-conditioned medium in 4T1 scratch assay. In vivo, 4T1-luc breast tumor mouse model was used to evaluate the effects of AS. In tumor-bearing female balb/c mice, AS was injected (20 mg/kg i.p.) twice a week and tumor size was measured. To detect the tumor cells in the local tumor and metastasis in different organs, animals were injected with luciferine and imaged in the IVIS Lumina II system before sacrificing. The lipid nanoparticle formulation of AS was prepared with DPPC:cholesterol lipids using an extruder. The lipid nanoparticles were characterized for their size, charge and drug release properties. In vitro, the AS lipid nanoparticles were examined for their effects on RAW cells for their M2-differentiaton.

RESULTS

Differentiation of RAW cells into M2-like phenotype was confirmed with induction of M2-specific genes (arginase-1 and mannose receptor-1) and arginase enzyme activity. Western blot analyses showed an upregulation of pSTAT6 in M2-differentiated cells compared to the control cells. Interestingly, treatment with AS (10, 100, 250 nM) dramatically inhibited the STAT6 phosphorylation in M2 cells with increasing concentrations. AS also significantly inhibited M2-induced genes and arginase activity. In contrast, AS did not show any inhibitory effects on M1 phenotype of RAW cells. Furthermore, we found that M2-conditioned medium strongly induced the migration of 4T1 tumor cells in vitro. Importantly, conditioned medium collected from AS-treated M2 cells did not induce these paracrine effects. In vivo, treatment with AS significantly attenuated the tumor growth by about >30% and the total tumoral luciferase activity by >50%. Luciferase activities in different organs indicated the reduced metastasis in the AS group. Furthermore, to improve the effects of AS in vivo by targeting them to TAMs, we successfully prepared the lipid nanoparticles of AS. The AS lipid nanoparticles were of about +130nm size and had encapsulation efficiency of 30% AS. The in vitro studies demonstrated that AS lipid nanoparticles could release AS and showed inhibitory effects on the M2 differentiation of RAW cells. In vivo studies to investigate the effect of AS lipid nanoparticles compared to empty lipid nanoparticles and free AS are currently being planned.

CONCLUSIONS

This study proposes that inhibition of STAT6 pathway is a vital approach to inhibit M2 differentiation (TAMs) of macrophages in the tumor microenvironment and thereby inhibit tumor growth and metastasis. Targeting of the STAT6 inhibitor could lead to improve therapeutic efficacy in vivo.

WHAT ARE THE PRIMARY REQUIREMENTS IN TOXICOLOGY AND IMMUNOLOGY? AN OVERVIEW

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Nanomedicine is the transition of nanotechnology into the clinic, ranging from the medical application of nanomaterials in medicinal products, nanoelectronic biosensors, targeted drugs and future applications of molecular nanotechnology. Current problems of nanomedicine involve the understanding the issues related to toxicity, environmental impact, immunological reactions of nanoscale materials as well as efficacy. The introduction will provide an overview of requirements related to toxicity and immunogenicity including the actual view of regulatory bodies on the subject.

EXPLORING BIO-INSPIRED NANOCOMPOSITES OF NANOGELS AND PULMONARY SURFACTANT FOR SMALL INTERFERING RNA DELIVERY TO THE LUNG

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One of the major bottlenecks impeding the clinical translation of RNAi-based therapeutics, is the lack of biocompatible nanocarriers enabling safe and efficient siRNA delivery in vivo. To date, the most widely studied siRNA delivery systems include polymer- and lipid-based nanoparticles.[1,2] In this context, our group also demonstrated the feasibility of cationic dextran nanosized hydrogels (nanogels) for siRNA delivery. These nanogels (NGs) encompass a high loading capacity for siRNA, are efficiently internalized by cultured cells and are able to induce a marked RNAi effect in vitro.[3,4] Unfortunately, polymer- or lipid-based siRNA-loaded nanoparticles often fail to surmount the numerous biological barriers en route to their intracellular target in vivo. In order to address the multifaceted drug delivery challenges, several research groups aimed to combine the unique strengths of both polymers and lipids within a single delivery vehicle, to obtain so-called lipid-polymer nanocomposites.[2] In addition, a growing interest exists in the implementation of bio-inspired materials in drug delivery vehicles. These materials are believed to possess specific features from which drug carriers could benefit, including an improved in vivo stability, biocompatibility, and intrinsic cell-targeting.

To evaluate the benefit of our siRNA-loaded nanogels (siNGs) for pulmonary applications, we first assessed the effect of natural lung-derived surfactants on the siRNA delivery capacity in lung epithelial and alveolar macrophage cell lines.[5] For this purpose, we applied surfactant preparations of animal origin that are in clinical use for the treatment of respiratory distress syndrome in premature infants. We could demonstrate that natural pulmonary surfactants play a crucial role in the cellular processing of siNGs in lung-related cell types, resulting in an improved cytosolic delivery of the encapsulated siRNA. Having established the synergistic effect of natural lung surfactant preparation on siRNA delivery, we next sought to combine the beneficial features of both nanogels and surfactant in a single delivery vehicle. To this end, a protocol was optimized to construct core-shell nanocomposites, composed of a biodegradable siNG core subsequently layered with a unilamellar pulmonary surfactant shell. Interestingly, we have found that the pulmonary surfactant shell improves the stability, potentiates intracellular siRNA delivery and enables the inclusion of targeting moieties to further boost cellular delivery performance (Figure 1).[6]

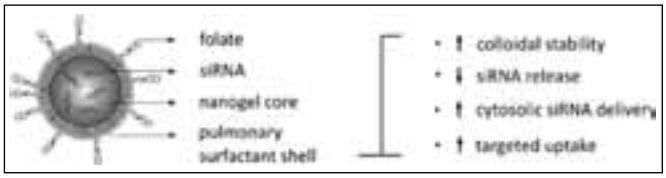

Fig.1: Schematic representation of bio-inspired nanocomposites, composed of a siRNA loaded nanogel core and a unilamellar pulmonary surfactant shell. The surfactant outer layer bestows the hybrid nanoparticles with many attractive features for (macromolecular) drug delivery.

Resident alveolar macrophages (rAM) are attractive targets for siRNA inhalation therapy. The latter is related to their prominent role in multiple respiratory disorders, such as cystic fibrosis, silicosis, asthma, tuberculosis, and chronic obstructive pulmonary disease, most of which are characterized by an underlying inflammation. Pharyngeal aspiration of surfactant-coated siNGs provoked only a mild inflammatory cytokine response and neutrophil infiltration in the bronchoalveolar lumen of BALB/c mice, but efficiently delivered siRNA to resident alveolar macrophages (rAM). The latter resulted in a substantial gene knockdown of the pan-leucocytic marker CD45 in rAM, which are notoriously difficult to transfect, with a relatively low siRNA dose (~1 mg/kg) (unpublished data).

In conclusion, we have developed a highly efficient, bio-inspired hybrid nanoparticle with promising features for siRNA inhalation therapy. Future research will be focused on evaluating this hybrid core-shell formulation for siRNA delivery in validated disease models of the lung.

ACKNOWLEDGMENTS

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RNA-LIPID NANOPARTICLES: A ROBUST AND POTENT TOOL FOR GENE KNOCKDOWN AND EXPRESSION IN PRIMARY NEURONS

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Genetic engineering is a powerful tool to study disease and investigate therapeutic pathways. However, there is an unmet need for a efficient, reproducible and scalable delivery tool to facilitate the use of RNA to manipulate gene expression in vivo. Here, we describe a solid-core lipid nanoparticle (LNP) system, developed with the microfluidics-based NanoAssemblr™ Platform, to deliver RNA into neurons in vitro and in vivo with high efficiency and low toxicity.

The NanoAssemblr™ microfluidic-based technology allows for a highly controlled nanoparticle formation process, which can be exploited for the rationale design of particle self-assembly. In the case RNA-LNP, this formulation process enables the formation of highly structured nanoparticles of controlled size with neutral surface charge at physiological pH. LNP are taken up by 96% of primary neurons within the first 4 hours of treatment through an ApoE mediated mechanism (Fig.1). Primary neurons exhibit > 85% gene knockdown, which is sustained for 21 days after LNP administration, even at a dose as low as 100 ng/ml of siRNA-LNP. No toxic effect is detected. By varying parameters on the NanoAssemblrTM

Instrument, LNP of different sizes were produced (Fig. 2) to investigate the influence of particle size on RNA LNP biodistribution in vivo (Fig. 3). Our data suggests that the NanoAssemblrTM Platform is a highly valuable platform for the development of complex nanomedicines such as RNA lipid nanoparticles with specific cellular uptake and pharmacokinetic profile.

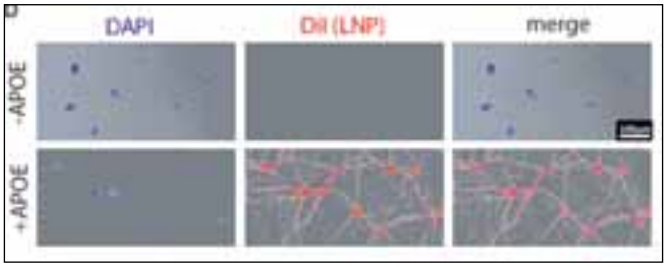

Fig. 1 siRNA LNP are taken up into primary neurons by an ApoE mediated mechanism.

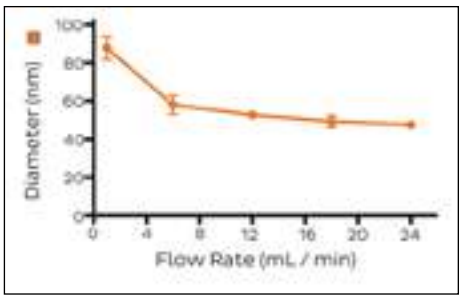

Fig. 2 Nanoparticle formulation with the NanoAssemblrTM Microfluidic Platform: particle size of RNA LNP can be fine tuned by changing instrument parameters such as the total flow rate of fluids in the microfluidic channel.

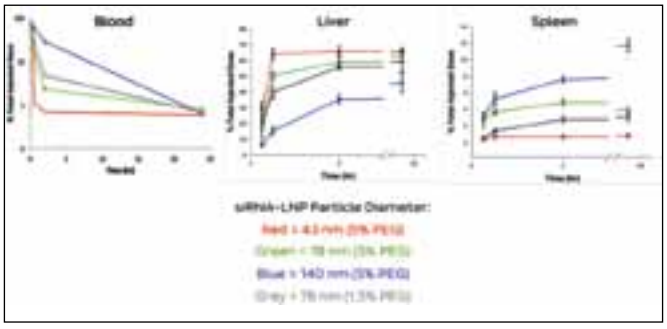

Fig. 3 Size matters. Particle size influences siRNA-LNP pharmacokinetics and biodistribution in vivo.

STANDARDIZED TOXICOLOGICAL ASSAYS FOR RISK ASSESSMENT OF COLLOIDAL NANOPARTICLES

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Nanoparticles are everywhere and mankind has been exposed to them for millennia. Nowadays exposure has even intensified due to the utilization of nanomaterials e.g. for intended use in medical applications. Consequently, risk assessment of nanoparticles is an issue of paramount global significance. Even though countless studies have been published addressing toxicological effects of nanoparticles, the outcome remains highly diverse and few general trends could be found so far. This is no doubt attributed to the high diversity of used materials, target cell lines and organisms but also originates from the lack of proper standards for toxicological trials. In this work, strategies for the standardization of nanotoxicological assays are presented1. Thereto, it is important to always adapt the used nanoparticles to the addressed exposure scenario. Particularly the impact of surface ligands is a critical issue, which may be more properly assessed when ligand-free standards obtainable by physical synthesis routes like laser ablation in liquid are used2, 3. Figure 1 (left) illustrates how the presence of surface ligands can compromise systematic toxicological assays. In addition proper dosing is critical. In contrast to commonly applied mass doses, surface dosing in reference to the relevant biological entity (cell number, organ surface) should be a gold standard for better comparability of toxicological trials. While this procedure is already well established in risk assessment of particles from the gas phase4, it is predominantly neglected in assays involving colloids, the species particularly relevant for medical applications. The severity of this issue is demonstrated in Figure 1 (right). Here dosing at varying particle sizes is demonstrated for mass doses frequently used in toxicological trials. These considerations clearly show that in case of small particles basically the surface area of the particles may exceeds the surface area of the cells by a factor of five, leading to doses far beyond any toxicological relevance.

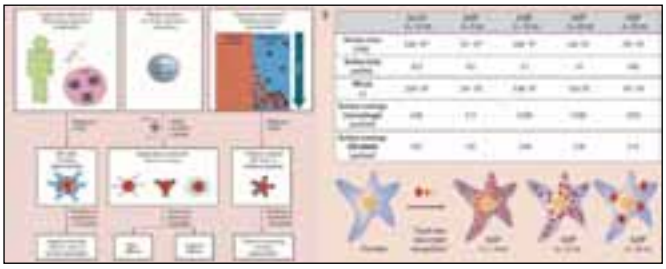

Figure 1: Left: General outline of the relevant nanoparticles required to systematically study toxicological effects in different exposure scenarios. Right: Table showing total particle numbers, particle SAs, number of nanoparticles per cell and SA coverage assuming full membrane accumulation for macrophages (diameter: 20 µm) and fibroblasts (diameter: 12 µm [2D]). The administrated mass dose was assumed to be constant at 10 µg/ml and a cell density of 106 cells/ml was presumed. These calculations are founded on totally spherical shapes for the nanoparticles and macrophages, while fibroblasts, as adherent cells, were assumed to be flat circular entities. The surface coverage was calculated using the projection of a spherical nanoparticle on a flat surface. The cartoon illustrates the surface coverage using fibroblasts as an example. #: Particle number; SA: Surface area. (Images and Figure captions were taken from Ref. 1)

Furthermore, the choice of an appropriate biological system warrants special attention. While the majority of in vitro trials are based on simple live-dead assays, functional toxicity could be more relevant as it allows detection of subtle adverse effects probably more suited for evaluation of in vivo settings. In this context, reproduction biology may be a key discipline as gametes are frequently available and relatively easy to handle while oocyte maturation5 and sperm functionality6 are highly sensitive processes where a single cell counts. Examples of toxicological trials conducted with reproductive cells are shown in Figure 2.

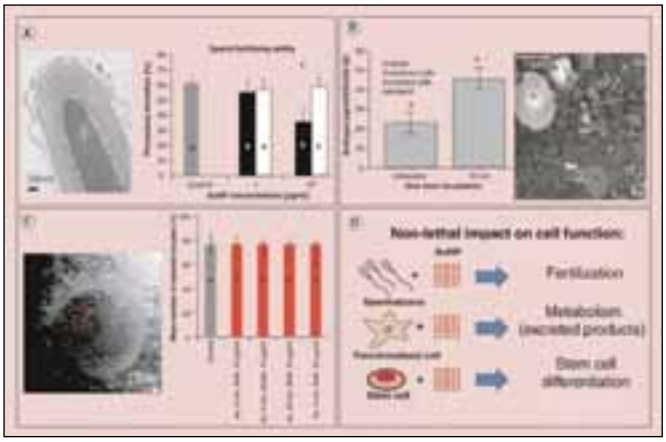

Figure 2: Biofunctional toxicity assays. (A) Sperm fertilizing ability/pro-

nucleus formation is affected by membrane-associated AuNPs. The presented mass concentrations of 1 and 10 µg/ml correspond to surface doses of 4.398 × 10-9 and 4.398 × 10-8 cm²/sperm, respectively. (B) Estrogen release of ovarian GCs is affected by AuNPs. (C) AuNPs do not affect oocyte maturation independent of particle size and surface functionalization, even though cell penetration is evident. (D) Cartoon illustrating how the function of spermatozoa (top), specialized cells (middle) and stem cells (bottom) may be influenced by nanoparticle addition. (Images and figure captions were taken from Ref. 1)

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THE ROUTE TO A PHASE I SAFETY, PHARMACOKINETIC AND PRELIMINARY EFFICACY STUDY OF CRIPEC® DOCETAXEL IN PATIENTS WITH SOLID TUMOURS

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Cristal Therapeutics is a pharmaceutical company developing innovative nanomedicines based on its proprietary CriPec® platform. Cristal Therapeutics' mission is to improve the therapeutic performance of new and existing drugs in various therapeutic areas. The application of CriPec® results in better efficacy and improved tolerability of drugs by an improved disposition in the body and controlled exposure to the sites of action.
CriPec® docetaxel is the company's lead product, designed for the treatment of various solid tumours, with gastric adenocarcinoma, endometrium and bladder cancer as potential indications of initial focus. In CriPec® docetaxel, the active molecule docetaxel is temporarily covalently bound within stabilised nanoparticles. The 65 nm-sized CriPec® docetaxel nanoparticles are designed to (passively) target the tumour tissue efficiently. Concomitant chemical hydrolysis results in release of only parent docetaxel in a controlled manner, and ensures a sustained exposure of the tumour to docetaxel.
The nonclinical efficacy profile of CriPec® docetaxel was evaluated in vivo in xenograft subcutaneous tumour mouse models, including gastric and breast cancer models. The maximum tolerated dose (MTD) of CriPec® docetaxel in nude mice (q7d3 injections) was shown to be at least 4-fold higher than of Taxotere, 125 mg/kg vs 30 mg/kg respectively. A single injection of CriPec® docetaxel (125 mg/kg) induced more profound suppression of both small as well as established MDA-MB-231 breast xenograft tumours when compared to Taxotere (30 mg/kg). Another study in small MDA-MB-231 breast tumours demonstrated the superior therapeutic efficacy upon equimolar (60 mg/kg) dosing of CriPec® docetaxel versus Taxotere.
In the NCI-N87 gastric cancer model, weekly i.v. doses of CriPec® docetaxel (100 mg/kg; 3 doses in total) also resulted in a more sustained suppression of tumour growth compared to Taxotere dosed at its MTD, viz. 25 mg/kg.
The efficacy findings in both breast as well as gastric models are consistent with the observed 60-fold higher tumour uptake (15 times higher when corrected for dose) of total docetaxel with CriPec® docetaxel dosed at (125 mg/kg) compared to Taxotere, dosed at (30 mg/kg) and with sustained presence of docetaxel in the tumour for at least 7 days after dosing.

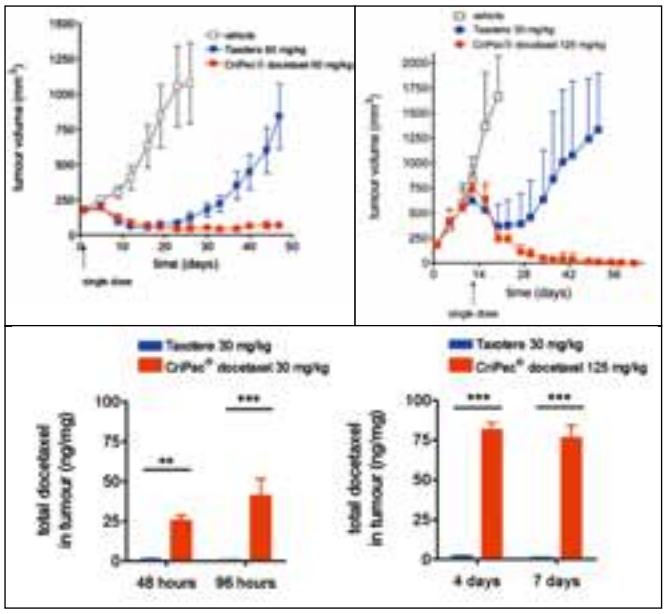

Fig.1: Efficacy and tumour accumulation of CriPec® docetaxel versus Taxotere in s.c. MDA-MB-231 breast xenograft-bearing nude mice. Left upper corner: single dose of either CriPec® docetaxel or Taxotere both at 60 mg/kg. Right upper corner: single dose of CriPec® docetaxel (125 mg/kg) or Taxotere (30 mg/kg) in established tumours. Left down corner: Total docetaxel levels in tumour following a single injection of 30 mg/kg of either CriPec® docetaxel or Taxotere. Right down corner: Total docetaxel levels in tumour following a single injection of 125 mg/kg CriPec® docetaxel or 30 mg/kg Taxotere. Data represent mean +/- SEM.

The nonclinical pharmacokinetic profile of CriPec® docetaxel is highly consistent in the various rodent studies performed and shows dose-linearity. CriPec® docetaxel has a higher Cmax, a much longer systemic circulation, a lower clearance and a smaller distribution volume when compared to Taxotere. All PK parameters clearly indicate that docetaxel is retained by the CriPec® nanoparticles upon circulation. Moreover, the preclinical safety and tolerability studies performed indicate that the MTD of CriPec® docetaxel is higher than Taxotere. In both groups, expected patterns of docetaxel toxicity and similar target organ toxicities were observed, but the toxicity to key targets was significantly less severe in CriPec® docetaxel-treated rats compared with Taxotere, despite an approximately 150% higher dosage. Equally important, the control arm with CriPec® empty demonstrated in the various safety studies no signs of any toxicity whatsoever.
Data to date support the clear potential of CriPec® docetaxel as novel nanomedicinal product in oncology with improved efficacy/safety balance compared to other taxane-based treatments. The first-in-human evaluation is scheduled to start end of May 2015. More specifically, a phase I/IIa study will be performed in patients with solid tumours in Leuven (Belgium) and Erasmus (Rotterdam). As the manufacturing of CriPec® docetaxel is already successfully upscaled, cGMP-grade clinical trial material is currently being prepared. In this talk, the most recent data will be presented.
In recent year, the CriPec® platform has also in general been significantly further developed. The large tuneability of the particle size and degradation profile is demonstrated, the broad applicability for various therapeutics agents is proven, as well as the controlled and selective approach to modify the surface with (targeting) ligands of choice. Consequently, next to CriPec® docetaxel, the CriPec® product portfolio comprises various early-stage products in several indications. Cristal Therapeutics develops these products independently, as well as in collaboration with other parties.

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NANOMEDICAL STRATEGIES AGAINST NEGLECTED DISEASES IN SOUTH AMERICA

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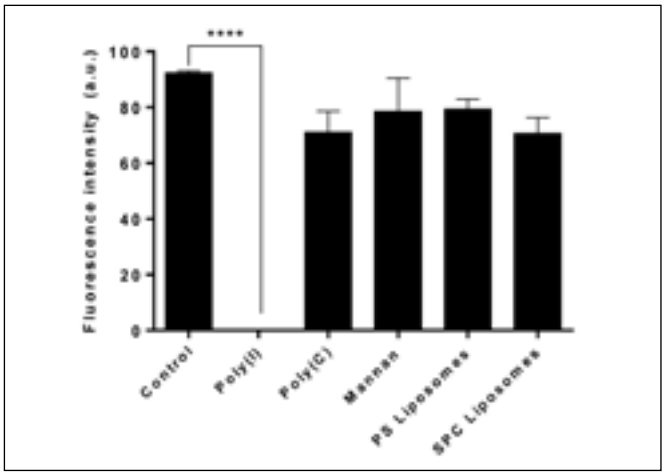

Fig. 1. ArchaeosomesHA are ligands of scavenger receptors (unpublished results)
Uptake of Rhodamine-Phosphatidylethanolamine (Rho-PE) labeled AHA (-22 mV Z potential) by J774A1 cells after 1 h incubation.
SPC liposomes: liposomes made of soyphosphatidylcholine
PS liposomes: SPC liposomes at 20% w:w phosphatidylserine

Chagas disease and the different clinical forms (visceral, cutaneous and mucocutaneous) of leishmaniasis are a major cause of patient suffering, with huge economical impact on the health systems of the South American society. Pharmaceutical companies lack of interest to develop new specific medicines against these neglected diseases. And despite a vaccine could reduce their economic and social costs, no commercial vaccines are available yet. However, the challenges posed by these diseases are probably beyond those imposed solely by the economy. The elimination of eukaryote intracellular parasites such as Trypanosoma cruzi and Leishmania spp requires of efficient drug targeting, and of drugs delivered in therapeutic amounts on infected cells, without causing host toxicity. This is the other reason why the treatments are long, of poor efficacy and overall, highly toxic. From another point of view, the poor immunogenicity of the newer safer vaccines based in recombinant proteins (useful against virus and bacteria) or DNA (succeeding in small animal models) as antigens, claims for new adjuvants that well could be in particulate form. In this scenario, a potential succeeding application of nanotechnological strategies against Chagas and Leishmaniasis, must be focused in purposes wider that those concerning to infectious diseases affecting southamericans. For instance, if the same nanoparticle platform used in antichagasic or leishmanicidal treatments were also useful in treatment or diagnosis of worldwide interest diseases, such as cardiovascular (in particular vulnerable atherosclerotic plaque treatment, diagnosis or theranostic) or chronic inflammatory diseases (lung, gastrointestinal tract, joints), the chances of being produced at industrial scale and of a further use against neglected diseases would be increased. The archaeosomesHA (AHA) are nanoparticles obtained from natural sources (hyperhalophile archaea lipids). Despite being liposome-like, the AHA have striking differences compared to liposomes, vesicles made of phospholipids extracted from animals, plants, fungi or bacteria. The AHA possess high colloidal stability (high z potential) and chemical resistance to acid hydrolysis, enzymatic attack, inertness against oxidation caused by heat or oxidizing agents.
The AHA can trap from low molecular weight drugs to proteins, to keep them protected against thermal stress, lyophilization, or chemical injuries. Besides, the AHA can be engineered to be administered by topical, mucosa or parenteral route. Recently, our research group has discovered exciting new properties of AHA that complement those of structural endurance: The AHA are ligands of cells expressing scavenger receptors and also display anti-inflammatory activity on cells stimulated with lypopolysaccharide (LPS), a TLR4 ligand. We found as well the uptake of AHA by macrophages occurs by different receptors than those engaged in the uptake of phosphatidylserine (a classical ligand of scavenger receptors involved in the uptake of apoptotic cells) liposomes (figure 1). These findings overcome the classical use of AHA as nanoparticulate adjuvants that we studied in the last few years, and suggest new uses for selective targeting to non infectiuos diseases mediated by active macrophages, dendritic cells and vascular endothelial cells expressing scavenger receptors. In this presentation we will show an uptdate on the current antichagasic and leishmanicidal preclinical nanomedical strategies employing the platform of AHA, highlighting the new opportunities of using AHA on non infectious targets.

INTERACTION OF SURFACE MODIFIED GOLD NANOPARTICLES WITH THE LUNG IMMUNE SYSTEM IN VITRO AND IN VIVO

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Due to their unique physicochemical properties, engineered nanoparticles (NPs) offer site-specific delivery and size-specific deposition and uptake, and were proven to be capable of modulating immune responses [1]. The lung is an attractive target organ for innovative immunomodulatory therapeutic applications by inhalation approaches using such NPs, given the vast surface where interactions between resident antigen-presenting cells (APCs), such as dendritic cells (DCs) and macrophages, and these NPs may occur [2, 3]. However pulmonary immune responses to engineered NPs with varying surface charge are poorly understood and the use of advanced in vitro and in vivo models is essential in order to analyse specific immunomodulatory pathways.
Gold NPs (AuNPs) coated with polyvinyl alcohol (PVA) with either positive (NH_3^+) or negative (COO^-) charge were reproducibly syn-

thesized and characterized [4]. The in vitro experiments were done applying a 3D model of the human epithelial alveolar barrier including epithelial and two immune cell types, i.e. macrophages and dendritic cells [5]. For in vivo experiments NH_2-PVA and COOH-PVA AuNPs were intranasally instilled in naïve mice. AuNP uptake by APC populations in the in vitro model as well as in the different lung compartments was assessed after 24h by flow cytometry. Following AuNP exposure, the antigen ovalbumin (OVA) uptake by APCs and in vivo OVA-specific $CD4^+$ T cell proliferation in lung draining lymph nodes (DLNs) were examined.

At concentration levels of 20µg/mL, there were no cytotoxic effects as measured by the Lactose Dehydeogenase (LDH) and the Annexin V/Propidium Iodide assays in both models. NH_2-PVA AuNPs showed in vitro the highest uptake in macrophages and epithelial cells, compared to the –COOH AuNPs. These results were confirmed in vivo, as macrophages and DC subpopulations preferentially captured NH_2-PVA AuNPs compared to their COOH counterparts. Uptake of both types of particles increased the expression of CD40 and CD86 in analyzed APC populations. Although OVA uptake by DCs and macrophages was unaltered following exposure to NH2-PVA and COOH-PVA AuNPs compared to PBS controls, NH2-PVA AuNPs, but not COOH-PVA AuNPs, induced enhanced proliferation of OVA-specific $CD4^+$ T cells in lung DLNs.

These findings underline the importance of appropriate surface modifications of NPs and indicate that particle surface charge, i.e. positive charge in our study, is a key parameter determining uptake by APC populations in the lung and down-stream immune responses. These results emphasize on the importance of carefully analyzing particle-cell interactions when developing novel innovative NP-based carriers for immune-modulatory treatments.

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CELLULAR UPTAKE OF NANOPARTICLES: 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). Nanoparticle-induced crosslinking of cell surface molecules may induce macropinocytosis that facilitates uptake of particles, and 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 (6), 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 (6), 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 (7). 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 (6). 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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EXTRACELLULAR VESICLES FOR DELIVERY OF RNA

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Over the last decade we have tried to deliver therapeutic RNAs using synthetic nanosized delivery systems. These synthetic delivery systems were composed of three subunits:

1. A cationic core to complex and protect the RNA,
2. A shielding layer that protects against opsonization and assures stability of the complex, and
3. A targeting ligand that should promote interaction with the target cell type.

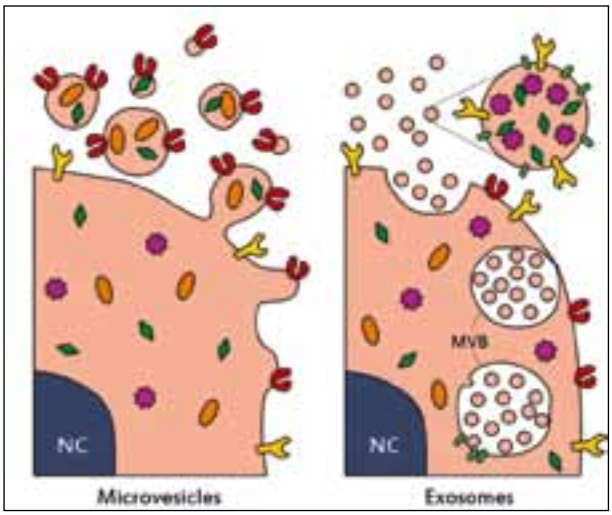

Figure 1. Extracellular vesicles can be distinguished based on the membrane of origin. Vesicles derived from the plasma membrane are known as microvesicles whereas the vesicles originating form multivesicular bodies (MVB) are known as exosomes. (NC=nucleus).

From our initial prototype based on Arg-Gly-Asp targeted, poly(ethylene glycol)-shielded, branched poly(ethylene imine)-

complexing polymer carrying siRNA against vascular endothelial growth factor receptor 2, we have explored alternatives for each subunit as well as cargo. For most applications the Arg-Gly-Asp targeting peptide and poly(ethylene glycol) shield proved superior to alternatives. For the poly(ethylene imine), linear versions of lower molecular weight grafted onto a biodegradable backbone were preferred as it reduced toxicity and improved delivery efficiency. This optimized polymer has been used to deliver anti-angiogenic miRNAs in a subcutaneous model of cancer as well as anti-invasive siRNAs against guanine exchange factors in a murine model of an orthotopically implanted glioblastoma tumor. In both models, successful silencing at the functional, cellular and molecular level can be observed.
Recently, we have also been looking into the delivery of RNA by extracellular vesicles (Figure 1). It is clear that we still need to learn the critical factors that determine successful transfer for these natural systems. However, in a collaborative study, we have observed transfer of mRNA and miRNAs in vivo between tumors, establishing these systems as promising candidates for future investigations.

INTEGRATING MOLECULAR MACHINES INTO NANOPARTICLES: A NEW DRUG-DELIVERY APPROACH

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Metastasis is the cause of 90% of cancer deaths. In many cases, by the time a primary tumor is detected, subsets of malignant cells have already disseminated to other locations in the body seeding the spread of the disease. Treating miniature lesions highlights the need for improving the accuracy of drug delivery systems.
Specifically, we are developing nanoparticles that target sites of cancer where they perform a programmed therapeutic task. These nano-systems utilize molecular-machines to improve efficacy and reduce side effects.
We developed lipid nanoparticles that are programmed to autonomously synthesize therapeutic proteins at a disease site. The particles can be can be remotely triggered to initiate drug production and release. The promise of these protein producing nanoparticles for treating cancer will be addressed.

DIAGNOSTIC BARCODED NANOPARTICLES FOR PERSONALIZED CANCER MEDICINE

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The field of medicine is taking its first steps towards patient-specific care. Our research is aimed at tailoring treatments to address each person's individualized needs and unique disease presentation.
A nanoparticle-based barcoded system for predicting the personalized therapeutic potency of drugs against cancerous lesions will be described. The drug screen is performed inside the patient's body and grants insights regarding drug potency with single-cell sensitivity.
The diagnostic system is based on 100-nm liposomes loaded with a drug and a corresponding unique DNA barcode. The technology enables identifying which drug or drug combination is optimal for treating the lesion. Proof of concept, from diagnosis to therapy, was performed on mice bearing triple-negative breast cancer tumors.

CANCER STEM CELLS AND PERSONALIZED CANCER NANOMEDICINE

SIMO SCHWARTZ JR., MD PhD

Cancer Stem Cells (CSC) are a subset of tumor cells responsible of tumor repopulation after chemotherapy. Moreover, CSC facilitate tumor cell survival in non-attachment conditions, tumor growth and metastatic spread. Since current therapies do not specifically target CSC, the overall survival of cancer patients is poor. In this scenario, the use of specific drug delivery systems and tailor-made nanomedicines to increase the dose of anticancer agents reaching tumor and metastatic sites rise expectations for more effective and less toxic treatments. However, active tumor targeting, using antibodies or peptides as director moieties, do not seem to significantly improve retention of delivered drugs or their overall efficacy in animal cancer models. Further, evaluation of active targeting in CSC populations is hampered by technical difficulties and questioning CSC targeting feasibility. Here, we report new fluorescent CSC models, in which tdTomato reporter vectors under the CSC specific (ALDH1A1) promoter are used for preclinical validation of poly[(D,L-lactide-co-glycolide)-co-PEG] (PLGA-co-PEG) micelles loaded with paclitaxel. We validate targeting against CD44 and EGFR receptors, in breast and colon cancer cell lines. Accordingly, active CSC targeting sensitizes CSC to paclitaxel based chemotherapy. Further, finding new molecular targets to eliminate CSC is an additional goal which will greatly improve patient survival. Inhibition of specific kinases seems to be effective in CSC and inhibit CSC growth and spread. SiRNA strategies can therefore be applied also together with active targeting as new selective cancer treatments.

POSSIBLE CLINICAL AP(IM)PLICATIONS OF DATA OBTAINED WITH AN APPARATUS THAT CAN MEASURE THE METABOLIC ACTIVITY OF CELLS CIRCULATING IN THE PERIPHERAL BLOOD (ONE BY ONE IF NECESARY)

PROF. DR. GIACINTO SCOLES, Department of Medical and Biological Sciences and University Hospital, University of Udine, Udine (I) and Biology Department, Temple University, Philadelphia, PA (USA)

At CYTOFIND DIAGNOSTICS we have developed an apparatus for counting Circulating Tumor Cells (CTCs)in very low cost and efficient manner. Our technique leaves the cells viable for further analysis and genomic characterization and is based on the WARBURG effect that states that a tumor cell has a much faster and efficient metabolism than a normal cell. We isolate the cells in pico-liter droplets of water in a microfluidic-prepared emulsion and measure by laser spectroscopy the acidity (Lactic acid) produced by exposing the cells for a few minutes to the presence of sugar.
At the meeting we shall present results obtained with patients for which a comparison with similar results obtained with the only FDA approved apparatus on the market which however runs slowly and leaves the cell fixated and therefore unable to be analyzed further (Cell-Search by VERIDEX)
We do not expect much from this comparison because the VERIDEX apparatus sees only Epithelial Cells while with our technique we count all cell types epithelial, mesenchymal and others provided that their metabolism be altered by the cancer . Furthermore we will present a few data and will discuss their importance of the use of a metabolism based technique to solve other clinical problems such as the quantification of the presence of the consequence of inflammation in the circulating peripheral blood and the exploration of changes in the metabolism of cells with and without cancer of the alteration of other biochemical processes.
This work was conducted in collaboration with F. Del Ben, M. Turetta, W. Huck, A. Piruska and D. Cesselli.

TARGETING SIGLECS WITH A SIALIC ACID-DECORATED NANOPARTICLE ABROGATES INFLAMMATION IN AN IL-10-DEPENDENT MANNER

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Sepsis is the single most frequent cause of death in hospitalized patients. Approximately 19 million instances of sepsis occur each year, resulting in mortality of approximately 8 million of these cases. In addition, severe sepsis is a leading contributory factor to Acute Respiratory Distress Syndrome (ARDS). At present there is no effective treatment for these conditions and care is primarily supportive. Murine Sialic acid-binding immunoglobulin-like lectin-E (Siglec-E) and its human orthologues Siglec-7 and -9 are immunomodulatory receptors found predominantly on hematopoietic cells. These receptors are important negative regulators of acute inflammatory responses, and may be important targets in the treatment of sepsis and ARDS. In this study, we describe a novel Siglec targeting platform consisting of poly (lactide-co-glycolide) acid (PLGA) nanoparticles decorated with a natural Siglec ligand: di(α2→8) N-acetylneuraminic acid (α2,8 NANA-NP). This nanotherapeutic induced efficient oligomerisation of murine Siglec-E through increased avidity, which the free ligand was unable to achieve. Furthermore, treatment of macrophages with these nanoparticles blocked the production of lipopolysaccharide (LPS)-induced inflammatory cytokines in a Siglec-E-dependent manner. Importantly, the nanoparticles demonstrated a striking therapeutic benefit in vivo in distinct murine models of inflammation. Moreover, we demonstrate the translational benefit of these nanoparticles on human monocytes and macrophages in vitro and in an ex vivo lung perfusion (EVLP) model of lung injury. Mechanistically we found that IL-10 induces Siglec-E expression. Furthermore we show that α2,8 NANA-NP augment the expression of this cytokine and that the therapeutic effectiveness of the nanoparticle is dependent on IL-10, revealing a novel role for this immunosuppressive cytokine. Collectively, these results have demonstrated the therapeutic ability of targeting Siglec receptors with a novel nanoparticle-based platform.

ANTIBODY AND ANTIBODY COMBINATIONS IN TREATING CANCER

MICHAEL SELA, Weizmann Institute of Science, Rehovot, Israel

I entered immunology research as a result of studies of polyamino acids as models of proteins.
The modification of proteins by peptidylation, and the synthesis of multichain polyamino acids permitted the creation of the first synthetic polypeptide antigens. This helped to elucidate the role of many variables in defining their role in immunogenicity (a notion which we introduced into immunological literature) and antigenic specificity.
The use of chemically defined multichain polymers and genetically defined inbred mice led to the discovery of the genetic control of immune response.
With Anfinsen we found out that the amino acid sequence of a protein (ribonuclease) defines its conformation, and there is no need to additional genetic information. For an antibody, its disulfide bridges could be opened only after its solubility was helped by poly-DL-alanylation. The modified antibody could regain all its activity, thus supporting the selective theory of antibody formation. Using synthetic antigens it was shown that an antibody can transconform an antigen.
An important use of synthetic copolymers of amino acids was the drug against multiple sclerosis, denoted Copaxone, which became the most frequent drug against this disease. Many years were spent – with Ruth Arnon – on the elucidation of its mode of action, and of the role of Copaxone in retarding demyelination and promoting remyelination.
Around 1970 I suggested to my post-doc Ron Levy to approach research on cancer by attaching chemically small chemotherapeutic drugs to antibodies – still polyclonal – against tumors. Daunomycin and Adriamycin were bound to antibodies with retention of both drug and antibody activities, and the products were cytotoxic. The attachment was preferably through a bridge, either dextran or polyglutamic acid. These were the first cases of trying to treat cancer specifically. Years later, Ron Levy was crucial in the clinical treatment of cancer with antibodies against CD20, which is found primarily on the surface of B cells.
Daunomycin covalently attached via a dextran bridge to specific antibodies against rat α-fetoprotein was more efficient than either daunomycin alone or a mixture of daunomycin and specific antibodies or a conjugate with horse immunoglobulin. The conjugates produced long-term survival, whereas the controls delayed only slightly tumor development. Similar studies on hepatomas were carried out using a polyglutamic acid bridge.
The immunotargeting could also be performed in two stages: The platinum drug was complexed to a carboxymethyl dextran – avidin conjugate and this was targeted to a biotin – monoclonal antibody.
In recent years, the realization of a synergistic effect of two anticancer drugs, an antibody and a small chemotherapeutic drug, or two antibodies to the same receptor, led to our studies on pancreatic carcinoma.
Due to intrinsic aggressiveness and lack of effective therapies, prognosis of pancreatic cancer remains dismal. We assumed (with Yosi Yarden) that agents EGFR and/or HER2 would effectively retard pancreatic ductal adenocarcinoma. Accordingly, two immunological strategies were tested in animal models: Firstly, two antibodies able to engage distinct epitopes of either EGFR or HER2 were separately combined, and secondly we tested pairs of one antibody to EGFR and another to HER2. Unlike the respective single monoclonal antibodies, which induced weak effects, both types of antibody combinations synergized in animals in terms of tumor inhibition.
Aptamers, oligonucleotides able to avidly bind cellular targets, are emerging as promising therapeutic agents, analogous to monoclonal antibodies. We selected from a DNA-library an aptamer specifically recognizing ErbB-2/HER2, a receptor tyrosine kinase, which is overexpressed in a variety of human cancers, including breast and gastric tumors. Treatment of human gastric cancer cells with a trimeric version (42 nucleotides) of the selected aptamer (14 nucleotides) resulted in reduced cell growth in vitro, but a monomeric version was ineffective. Likewise, when treated with the trimeric aptamer, animals bearing tumor xenografts of human gastric origin reflected reduced rates of tumor growth. The anti-tumor effect of the aptamer was nearly twofold stronger than that of a monoclonal anti ErbB-2/HER2 antibody (With Yosi Yarden).
Could aptamers one day replace antibodies in fighting diseases?

TOXICOLOGY BIOMARKERS: LIVER AND KIDNEY

MARTIN SHAW, Biosafety Specialist, TECOMedical AG, Sissach, Switzerland

Hepatotoxicity and nephrotoxicity are the two commonest causes of toxicity related drug failure. However, traditional biomarkers of liver and kidney injury are late, non-specific indicators of toxicity leading to compounds failing late in development with increased costs and wasted resources.
The application of biomarkers with known sites and mechanisms of release provides a means of reducing this problem, enabling toxicity to be identified earlier, with more accuracy and providing information on toxic mechanisms
This presentation will provide examples of their application.

RENAL TOXICOLOGY

The renal cytosol proteins alpha and pi Glutathione S-Transferases (GSTs) are sensitive and specific indicators of necrosis to the proximal and distal tubules respectively. They are released into the urine following injury to these parts of the nephron, providing sensitive and specific information on renal tubular injury, its site and kinetics.

Induced renal biomarkers, e.g. KIM-1 provide information as to the recovery process and, by their combination with the GSTs, detailed information on the onset, mechanism and resolution of toxic events can be obtained.

HEPATIC TOXICOLOGY

The hepatocyte specific protein Alpha GST is rapidly released at the onset of hepatocyte injury and it then rapidly disappears from the blood upon its resolution. Many studies have shown it to be a sensitive and specific indicator of hepatocyte necrosis and to provide excellent toxicokinetics.

Keratin 18 (K18) is a component of the cytosol skeleton. It is released during hepatocyte death, providing an indication of cell death from all causes.

During apoptosis, K18 is attacked by caspases producing caspase-cleaved K18 (ccK18). Its appearance in blood is a sensitive and specific indicator of hepatocyte apoptosis.

By the combination of hepatocyte biomarkers covering different mechanisms of cell death, increased information may be obtained covering the mechanism and kinetics of hepatocellular injury.

All the above mentioned biomarkers are being evaluated as part of the IMI SAFE-T programme for the qualification of renal and hepatic biomarkers.

A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL TO PREDICT THE SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES (SPIONS) ACCUMULATION IN VIVO

MARCO SICCARDI

Superparamagnetic iron oxide nanoparticles (SPIONs) have been identified as a promising material for biomedical applications. These include as contrast agents for medical imaging, drug delivery and/or cancer cell treatment. However, the distribution of SPIONs in the human body has not been fully characterized. Physiologically based pharmacokinetic (PBPK) modeling combines mathematical equations to describe the anatomical, physiological and molecular processes regulating pharmacokinetics, with in vitro data to simulate and predict the absorption, distribution, metabolism and elimination (ADME) of nanomedicine. The distribution and accumulation of SPIONs in organs were simulated taking into consideration their penetration through capillary walls and their active uptake by specialized macrophages in the liver, spleen and lungs. Novel in vitro experimental data describing uptake of SPIONs in primary monocyte-derived macrophages were integrated into this computational approach. The developed PBPK model was validated against in vivo pharmacokinetic data, and accurately described accumulation in rodent organs. After validation of the murine model, a similar PBPK approach was developed to simulate the distribution of SPIONs in humans.

The application of modelling techniques may have value in bridging between pre-clinical and human NP pharmacokinetics. The integration of in vitro assays for ADME processes can improve PBPK approaches, allowing clarification of how NP characteristics influence distribution patterns. Consequently, although there are current gaps in knowledge, PBPK models have the potential to support the design of NPs by providing a rational approach for the selection of NP candidates with optimal pharmacological properties.

COMPLEMENT ACTIVATION AND IMMUNE RECOGNITION OF SUPERPARAMAGNETIC IRON OXIDE (SPIO) NANOWORMS IN MICE VERSUS HUMANS

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There is an unmet need in efficient and safe contrast agents for magnetic resonance imaging of various pathologies. Dextran iron oxide nanoparticles are efficient contrast agents, but their clinical use is associated with significant immune reactions and propensity for clearance by liver and spleen 1. Complement is the system of 30+ serum proteins that represents the first immune barrier to invading pathogens 2. The hypothesis is that complement activation could be responsible for some of the immune effects of iron oxide nanoparticles. Superparamagnetic iron oxide (SPIO) nanoworms were synthesized from ferrous and ferric chlorides and 20kDa dextran using a modified Molday method 3. The nanoparticles exhibited high molar transverse relaxivity (r2) of ~400 mM-1 s-1 but activated complement in serum as evidenced by deposition of C3b fragment (200-500 copies per nanoparticle). Complement activation in mice was due to triggering of the lectin pathway, and in humans due to triggering of the alternative pathway 4. The nanoparticles exhibited highly efficient complement-dependent uptake by macrophages, neutrophils and monocytes. The mechanisms of complement activation were studied using sera deficient for main complement factors. There was no C3b deposition in mannose binding lectin (MBL)-deficient sera, suggesting that nano-worms triggered complement in mice predominantly via the lectin pathway due to the presence of dextran coating on the nanoparticles. In order to block complement activation, the dextran shell of nanoworms was further modified by a novel two-step procedure using crosslinking-hydrogelating agent epichlorohydrin 5. The treatment resulted in a complete blockade of dextran immunogenicity as measured by loss of recognition of anti-dextran antibody and Concanavalin A. As a result of dextran modification, there was a 95% decrease of binding and activation of lectin complement pathway, and 90% decrease in C3b/iC3b opsonization. The resulting nanoworms showed minimal recognition by leukocytes and liver macrophages in vivo. Moreover, the circulation half-life of nano-worms in mice was increased 60-fold. Due to the high T2 contrast, long circulating properties and minimal immune recognition, the reported contrast agents is a significant step in the development of specific and safe MRI probes for clinical use. Moreover, this study demonstrates that understanding the mechanisms of immune recognition and complement activation by nanoprobes can be very useful for rational design of improved nanomedicines.

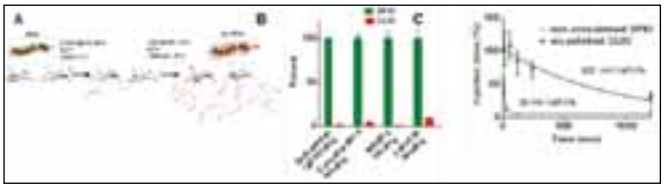

Figure 1: Synthesis and immunological properties of magnetic nanoworms. A. Dextran coat was modified with epichlorohydrin to generate hydrogel coated crosslinked CLIO nanoworms; B, the hydrogelation resulted in blockade of dextran immunogenicity (no binding of anti-dextran IgG), alteration of sugar structure (no binding of ConA), blockade of lectin pathway activation (no MASP-2) and mouse complement activation (decrease in C3b/iC3b); C, The hydrogel coating resulted in a prolonged half-life in mice from 10 min to 600 min.

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BIODISTRIBUTION AND EXCRETION OF BIODEGRADABLE VERSUS NON-DEGRADABLE NANOPARTICLES

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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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THE PROS AND THE CONS OF POLYMERIC MICELLES AS DELIVERY PLATFORM BY MINIMALLY-INVASIVE ADMINISTRATION ROUTES: MAKE IT THE STICKY WAY

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Polymeric micelles are nanostructures formed by the self-aggregation of copolymeric amphiphiles above the critical micellar concentration. Due to the flexibility to tailor different molecular features, they have been exploited to encapsulate a broad spectrum of hydrophobic drugs. Despite their proven versatility, polymeric micelles remain elusive to the market and only a few products are currently undergoing advanced clinical trials or reached the clinics, all of them for the cancer by the intravenous route. At the same time, polymeric micelles emerge as a promising platform for mucosal (non-parenteral) administration. Our group has extensively investigated the delivery of drug-loaded polymeric micelles by the oral, intranasal, ocular and inhalatory routes. However, standard polymeric micelles show relatively short residence times in the administration site and do not sustain the release of the encapsulated cargo. Aiming to optimize their performance for non-parenteral routes, polymeric micelles need to be reengineered (1). In this presentation, the approaches currently investigated in our laboratory will be discussed with emphasis on the hydrophobization of multifunctional polymeric templates.

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POLYMERIC NANOCAPSULES AS A PROMISING APPROACH FOR DRUG TARGETING TO BRAIN: STUDIES IN ALZHEIMER/SCHIZOPHRENIA MODELS

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Recently, various types of drug nanocarriers have been proposed as vehicles for delivering drugs to the brain tissue. Regarding Alzheimer disease, we demonstrated that the encapsulation of resveratrol in lipid-core nanocapsules improves, in vitro and in vivo, its effects against Aβ-induced neuroinflammation. In schizophrenia models, improvements of the relative bioavailability and antipsychotic effects (stereotyped behavior and prepulse inhibition impairment induced by apomorphine) have been observed after nose-to-brain delivery or ip administration of olanzapine-loaded nanocapsules to rats. Together, the results indicate that the use of polymeric nanocapsules is an effective tool to deliver drugs to the brain tissue by crossing the blood-brain barrier.
Acknowledgements: CNPq, FAPERGS, CAPES

ANTI-INFLAMMATORY LIPOSOMES IN ONCOLOGY

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Tumor-targeted delivery of corticosteroids using long-circulating liposomes was shown to exert potent antitumor effects in vivo. This study was aimed to apply this approach to prostate cancer treatment. Metastatic bone disease is a detrimental stage in prostate cancer for which no satisfying treatment options exist to date. Prostate cancer progression is strongly promoted by tumor-associated inflammation in which tumor-associated macrophages (TAM) play a prominent role by the secretion of inflammatory- and angiogenic factors. The latter induce tumor angiogenesis and these

chaotically-organized blood vessels are typically characterized by enhanced vascular leakage which can be exploited by nanomedicinal drug delivery systems (e.g. long-circulating PEG-liposomes). The long circulation time of liposomes and the leaky tumor vasculature leads to efficient localization and retention of these nanoparticles at the metastatic microenvironment (enhanced permeability and retention (EPR)-effect). After extravasation to the tumor microenvironment, liposomes are typically taken up by TAM.
In this study, we evaluated the antitumor efficacy of liposomally encapsulated dexamethasone, a glucocorticoid receptor-agonist with strong anti-inflammatory activity, in a preclinical in vivo model for prostate cancer bone metastasis (intra-tibial growth of luciferase-expressing PC-3M-Pro4 cells in Balb-c nu/nu mice). Liposomes were observed to localize efficiently at malignant bone lesions. Intravenous administration of both liposomal and free (non-encapsulated) dexamethasone displayed potent antitumor efficacy, suggesting that dampening the tumor-associated inflammation leads to inhibition of tumor growth. This is presumably mediated via silencing of pro-inflammatory TAM, as the expression of several pro-inflammatory cytokines (e.g. IL-6) was diminished in vitro upon treatment with liposomal and free dexamethasone. Interestingly, we found that the liposomal formulation of dexamethasone significantly outperforms administration of free dexamethasone. The superior uptake of liposomes by macrophages as compared to tumor cells in vitro suggests involvement of TAM in the effect of liposomal dexamethasone. It is important to note that liposomal dexamethasone was well-tolerated at therapeutically-active dosages. Taken together, our findings warrant clinical evaluation of liposomal dexamethasone in patients with advanced, metastatic, prostate cancer. Phase I clinical trials are now in preparation.
This study was supported by a grant from the NanoNextNL Drug Delivery programme 03D.01

TESTING FOR IMMUNE REACTIVITY AND IMMUNOGENICITY OF NANOMEDICINES

PROF. DR. MED. JANOS SZEBENI, Nanomedicine Research and Education Center, Semmelweis University, & SeroScience Ltd, Budapest, Hungary
Intravenous injection of a variety of nanotechnology enhanced (liposomal, micellar, polymer conjugated) and protein-based drugs (antibodies, enzymes) can lead to hypersensitivity reactions (HSRs), also known as pseudoallergic, anaphylactoid, or infusion reactions. The molecular mechanism of the consequent mild to severe allergy-like symptoms may differ from case to case and are mostly unknown. However, in many cases, a major cause, or contributing factor is activation of the complement (C) system. The clinical relevance of C activation-related pseudoallergy (CARPA) lies in its unpredictability and occasional severe or lethal outcome. Accordingly, there is increasing interest in laboratory assays and animal models that predict CARPA and may form the basis of future regulatory guidelines. The presentation will detail our strategies for the evaluation of reactogenic drugs and hypersensitive patients for the risk of CARPA.

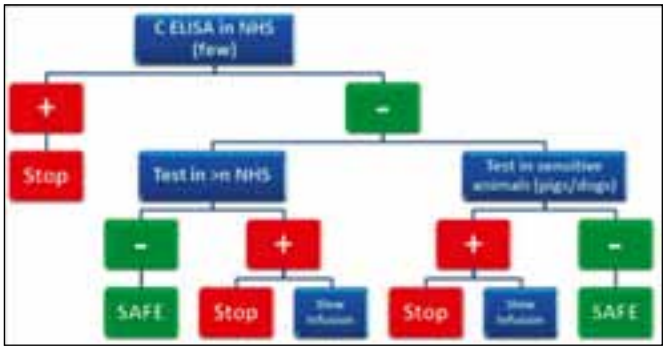

Fig. 1.: Flow chart for testing the risk of CARPA

As shown by the flowchart (Fig. 1) the test agent (drug candidate) is first incubated with a few normal human sera (NHS) to explore possible major C activation. C activation is measured by ELISA, most frequently the SC5b-9 test. If the result is positive, the agent is likely to carry a high risk for CARPA in vivo. As for the threshold for considering C activation as "major", an activation factor (for example a rise of sC5b-9 above baseline over 20–30 min incubation at 37 ◦C) of 5–10-fold may be realistic predictor of clinical reaction, as such rises (of sC5b-9) were shown to correlate with clinical symptoms of patients treated with Doxil. If the in vitro C assay in NHS is not showing C activation, based on the substantial individual variation of C response, testing in a much larger number testing in a much larger number of NHS (in the range of 10–100) can be recommended, and/or testing in sensitive large animals (pig and/or dogs) with bolus administration. The reactogenicity in these models can be quantitated, among others by using the cardiac abnormality score (CAS). In case of low reactogenicity (CAS score 1-2), the test agent may carry a small, but not negligible risk for CARPA in a small percentage of hypersensitive individuals. In case of strong reactivity (CAS = 3–5), the risk of CARPA is great(er). If none of the highly sensitive animal models indicate reactivity to any therapeutically relevant bolus doses, or the test drug does not cause C activation in a large number of NHS, it may be considered as CARPA-free, although obviously the experimental conditions need to be relevant and the tests technically valid. Our immunogenicity assays are standard antidrug antibody (ADA) ELISAs wherein we measure the rise of IgG against the drug, adhered to the ELISA plates as antigen. The presentation will show examples for positive tests, including the detection of anti-PEG antibody formation in rats treated with (empty) Doxil, and the detection of natural antibodies against paclitaxel in patients developing HSRs against Taxol.

INTERACTIONS OF NANO-TiO_2 WITH THE ORAL CAVITY DEPENDENT ON SIZE AND SURFACE HYDROPHILICITY

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Nano-titaniumdioxide (TiO_2) offers versatile material characteristics that are attractive for novel applications in different fields of medicine and industry [1]. During the use of such products, nano-TiO_2 is intended to, or may accidently, gets into the human body via the primary portal of entry – the oral cavity. Basic knowledge about the fate of such particles in the oral cavity/oral mucosa is mandatory to assess interactions at the nano-bio-interface.
This study was conducted to evaluate the penetration behavior, the intracellular distribution and potential toxic side effects of nano-TiO_2 in the oral cavity dependent on distinct particle properties [2, 3]. The influence of particle/agglomerate size was estimated using pigment-graded NM 100 (anatase), 7 nm NM 101 (anatase) and 22 nm NM 105 (80% anatase/20%rutile). Furthermore, the impact of surface hydrophilicity on the biological fate was determined using particles with the same crystallinity (rutile) and the same nominal size (22 nm), only differing in surface coating (hydrophobic NM 103 and hydrophilic NM 104). Physicochemical particle characterization was performed in biological relevant media (i.e., saliva, PBS) and penetration studies were conducted with a standardized porcine ex-vivo model. The localization of particles within cellular compartments gives hints to potential biological hazard. Thus, intracellular particle trafficking was performed with transmission electron microscopy (TEM)/energy filtered TEM (EFTEM) and confocal laser scanning microscopy (cLSM). Common endpoints of potential toxic effects (i.e., cell viability and membrane integrity) and the related intracellular mechanisms (i.e., generation of reactive oxygen spe-

cies (ROS), impairment of the mitochondrial membrane potential and mutagenic effects) were explored.
Physicochemical characterization studies demonstrated that nano-TiO_2 strongly agglomerated in physiological media. However, a significant amount (up to 10%) was present in the nanoscale range. NM 100 and NM 105 particles penetrated into the upper and the lower part of the oral mucosa, while NM 101 particles (smallest primary/agglomerated sizes) were only located within the upper region of the epithelium (Figure 1).

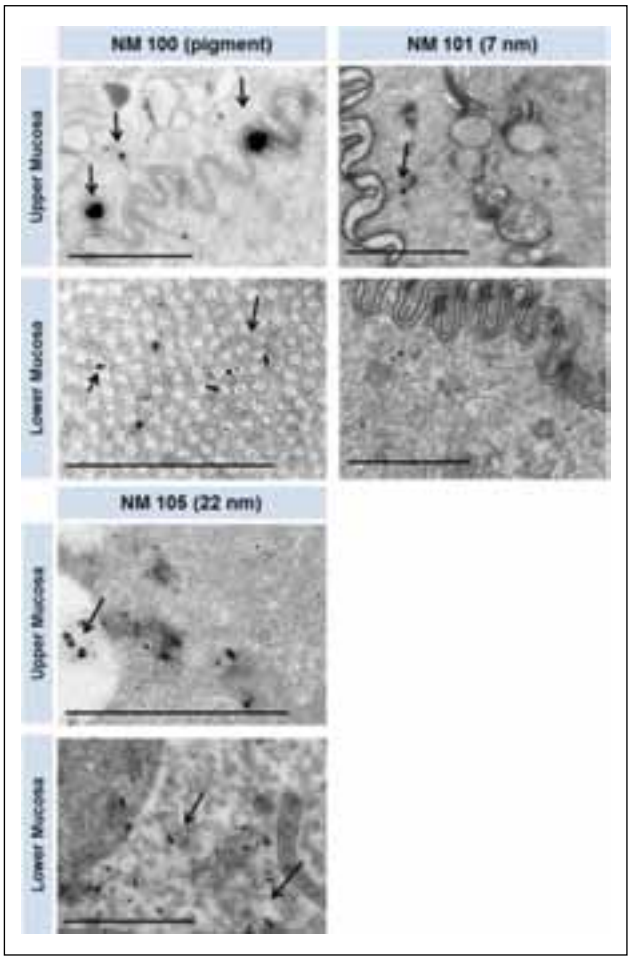

Figure 1: Nano-TiO_2 particles were internalized by the superficial cells of the oral mucosa. NM 100 and NM 105 particles were also found in the basal lamina and the connective tissue. NM 101 particles were only located in the upper epithelium. The black arrows indicate particles verified with EFTEM.

NM 103 and NM 104 particles penetrated into deep parts of the oral mucosa, indicating that the degree of the surface hydrophilicity exerted no influence on the penetration depth. However, particle trafficking studies revealed that hydrophobic NM 103 particles were closely aligned to the cell membrane and/or engulfed within vesicles (mainly endosomes/lysosomes), while their hydrophilic counterparts were freely distributed within the cytoplasm (Figure 2). The viability and the membrane integrity of the oral mucosa were not affected in a harmful manner. Moreover, nano-TiO_2 triggered no gene mutation in buccal epithelial cells. Nevertheless, a decreased mitochondrial membrane potential was detected for hydrophilic NM 104, associated with the generation of ROS after short-time incubation. In contrast, toxic side effects were low for hydrophobic particles since they were separated from the cytoplasm and not able to get in contact with intracellular structures.
It was demonstrated that nano-TiO_2, independent on physicochemical properties, interact with the oral mucosa. However, particle/agglomerate size of nano-TiO_2 determines the penetration depth, while the surface hydrophilicity contributes actively to the intracellular distribution.

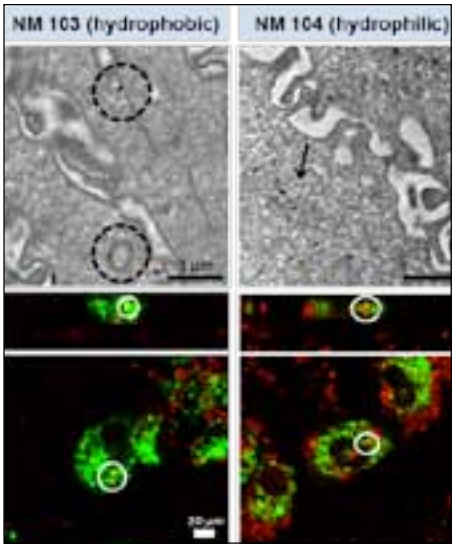

Figure 2: TEM images (upper panel) show that NM 103 particles were located in vesicular structures, while NM 104 particles were predominantly found non-membrane bound in the cytoplasm. The black arrows/dotted circles indicate verified TiO_2 particles. cLSM images (lower panel) revealed colocalization (yellow spots in circles) of NM 103 particles (red) with lysosomes (green). By contrast, NM 104 particles (red) could not be identified within the lysosomes (green).

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ANTIBODY MEDIATED TARGETED THERAPIES

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Some targeted therapy approaches which combine antibodies and payloads can be considered to be at the interface between biologics and nanomedicine. Antibodies having a molecular weight of 150.000 d (or even higher in case of designed bispecifics) are already molecules with multi-nanometer size. Conjugates or complexes of antibodies with payloads (including nanoparticles) generate larger entities. These may already fit into the category 'nanomedicine'.

DENDRIMER-BASED NANOMEDICINE: SYSTEMATIC CNDP ENGINEERING OF DENDRIMERS COMPARED WITH OTHER NANOPARTICLES

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Recent progress in the successful development of dendrimer-based cancer therapies (Starpharma DEPTM docetaxel conjugates) has demonstrated substantially improved efficacy and reduced toxicity with excellent tolerability and improved pharmacokinetics in Phase I Clinical studies.1 This progress has been possible largely due to unique structure controlled, physio-chemical features of dendrimers that allow one to predictively engineer all six-Critical Nanoscale Design Parameters (CNDPs), namely: (1) size, (2) shape, (3) surface chemistry,(4) flexibility/rigidity, (5) architecture and elemental composition.2 As shown recently,3 systematic engineering of these CNDPs provides an important pre-clinical strategy for optimizing critical nanoparticle properties such as nanotoxicity, biodistribution and pharmacokinetics (Figure 1a). With the exception of dendrimers, [S-1], most hard/soft nanoparticles do not possess

such structure controlled features suitable for successful CNDP engineering/optimization as shown in (Figure 1b). This lecture will briefly review those issues.

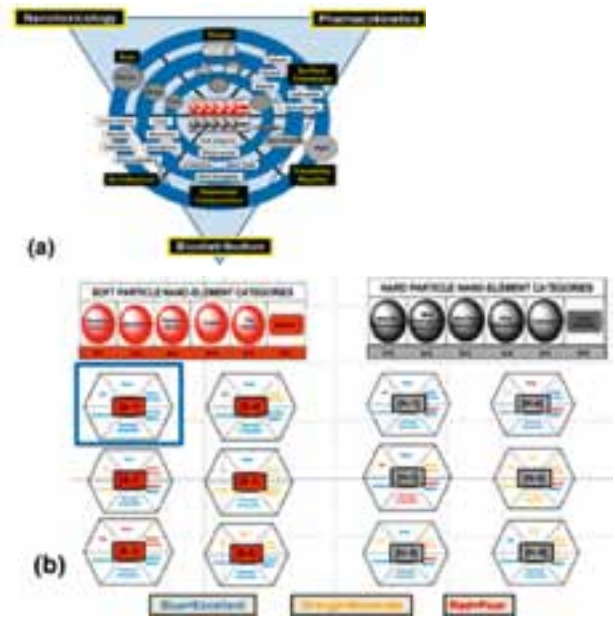

Figure 1.: (a) Soft and Hard Nano-element Categories (center). An overview of six-CNDPs that may be engineered to significantly affect nanotoxicology, pharmacokinetics and biodistribution parameters for all Soft and Hard Nano-element Categories in various nanomedical applications. (b) Soft and Hard Nano-element Categories (top). Comparison of ability to engineer the six-CNDPs in either Hard or Soft nanoparticles.

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MANIPULATING MEMORY ENGRAM CELLS TO ATTENUATE DEPRESSION IN MICE

SUSUMU TONEGAWA

Stress is considered a potent environmental risk factor for many behavioral abnormalities, including anxiety and mood disorders. Animal models can exhibit limited but quantifiable behavioral impairments resulting from chronic stress, including deficits in motivation, abnormal responses to behavioral challenges, and anhedonia. The hippocampus is thought to negatively regulate the stress response and to mediate various cognitive and mnemonic aspects of stress-induced impairments, though the neuronal underpinnings sufficient to support behavioral improvements are largely unknown. In our work, we acutely rescue stress-induced, depression-related behaviors by optogenetically reactivating DG cells that were previously active during a positive experience. A brain-wide histological investigation, coupled with pharmacological and projection-specific optogenetic blockade experiments, identified glutamatergic activity in the hippocampus-amygdala-nucleus accumbens pathway as a candidate circuit supporting the acute rescue. Finally, chronically reactivating hippocampal cells associated with a positive memory resulted in a rescue of stress-induced behavioral impairments and neurogenesis at time points beyond the light stimulation. Together, our results suggest that activating positive memories artificially is sufficient to suppress depression-like behaviors and point to DG engram cells as potential therapeutic nodes for intervening with maladaptive behavioral states.

XPLOITATION OF HIGH DENSITY LIPOPROTEINS FOR NANOMEDICINE

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Many clinical and epidemiological studies have shown the inverse relationship of high density lipoprotein (HDL) cholesterol levels with the risk of coronary heart disease (CHD) or diabetes mellitus type 2 (T2DM). HDL particles as well as protein and lipid components therein exert diverse anti-oxidative, anti-inflammatory, anti-thrombotic and cytoprotective effects in vitro and/or in vivo which suggest a direct anti-atherogenic and anti-diabetogenic role of HDL. In a prototypic HDL particle two to five molecules apolipoprotein (apo) A-I and about 100 molecules of phosphatidylcholine (PC) form an amphipathic shell in which several molecules of un-esterified cholesterol (UC) are imbedded and which surrounds a core of completely water-insoluble cholesteryl esters. Already molar differences in the content of apoA-I, PC, sphingomyeline, UC, and CE cause considerable heterogeneity of HDL in shape, size, and charge. This macro-heterogeneity is further increased by the presence or absence of quantitatively minor proteins or lipids, some of which may contribute to the pleiotropic functions of HDL. HDL particles carry more than 80 different proteins and 100's of lipid species. Most recently, even microRNAs were found to be transported by HDL. Many of these molecules are not passive cargo but biologically active and contribute to the pleiotropic and potentially anti-atherogenic and anti-diabetogenic properties of HDL. However, many potentially anti-atherogenic or anti-diabetogenic properties can be copied by artificially reconstituted HDL (rHDL). The principle form of these 8 to 10 nm large discoidal particles consists of wild type or mutant forms of apoA-I and PC at a molar ratio of about 1:100. They can be further modified by the addition of further lipids (for example sphingomyelin), or proteins, drugs (for example statins) or imaging molecules. Infusion of rHDL into animals revealed a strong enrichment of rHDL in atherosclerotic lesions which was exploited for imaging and local drug delivery. Several rHDL preparations have been obtained according to GMP standards and investigated in clinical phase II studies. In these studies, the infusion of rHDL was found to improve endothelial dysfunction, to reduce atherosclerotic plaque volume of coronary arteries, and to ameliorate glycaemia in patients with T2DM.

THE USE OF PHOSPHOLIPIDS AS NANOMATERIALS IN DRUG DELIVERY SYSTEMS

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Phospholipids are multipurpose excipients present in many established lipid nano-products and explored for future nano- delivery systems. In parenteral dosage forms, they can be found as liposome former in several types of liposomes (SUV, LUV, MLV, MVV), as emulsifier in oil-in-water emulsions and surfactant in mixed micelles. They are also suitable as wetting agent for nano-suspensions and as matrix forming excipient for solid dispersions and as surfactant in mixed micelles for oral use of poorly-water soluble drug substances. In the overall phospholipid excipient market, synthetic phospholipids play, compared to natural phospholipids (including hydrogenated and enzyme modified phospholipids), from number of drug products comprising these phospholipids, a very minor role. Their use is restricted to some parenteral liposomal products. If the use of synthetic phospholipids is an option, synthetic phospholipids using the GPC synthesis route should be used. At this way the natural stereochemical configuration is guaranteed and the extra production steps compared to natural phospholipids can be performed with minimal additional usage of solvents and chemicals. Because phospholipids are endogenous membrane compounds, they are biodegradable and biocompatible and possess a very broad safety profile. Phospholipid excipients fulfil the applicable regulatory requirements and have been approved by health authorities worldwide and are benchmark materials for other excipients used in nano-delivery systems.

PRECISE, REPEATABLE AND VERIFIABLE NANO-MEASUREMENT IS REQUIRED FOR NANOMEDICINE TO ADVANCE TO ROUTINE MEDICAL USE

HANS VAN DER VOORN

Nanomedicine using nanoparticles for drug delivery requires very high quality measurement to speed up development and to build confidence in the end product. All stages of development and trials need to have the particle system accurately characterized in detail to ensure that results between various trials can be properly compared. Many nanomedicine developers are still using outmoded measurement techniques that provide limited data that regulators cannot rely on, leading to expensive delays in implementation.
Traditional methods of describing particle concentration, size distribution and particle surface charge have been superseded by the routine availability of very precise data options. For example particle concentration now requires inclusion of the size range to which the concentration number applies. The use of a real number based size distribution is preferred over a dubiously derived average size. The use of zeta potential loses any real meaning is used with complex nanostructures so particle charge may be better described by electrophoretic mobility. Equally the distribution of charge across the particle set is of high interest in determining the extent to which particle surface functionalisation or particle interactions with biomolecules have occurred.

HOW IMMUNE CELLS WRESTLE WITH THEIR PREY

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To fight bacterial infections, it is not sufficient for our immune cells to just bind to pathogens. As pathogens evolved all kind of mechanisms to escape, from swimming away to protecting themselves within biofilms, macrophages prepared an arsenal of tools to hold on to their prey as they get prepared to take them up for phagocytosis. To explore how macrophages wrestle with their microscopic prey as it tries to hold on to surfaces or wiggles around to escape, we coated magnetic beads with molecules that mimic aspects of bacterial surfaces. When trapped in a magnetic tweezer system, we visualized the fight of macrophages with their prey and gained insights into the complexity of the mechanical aspects that are involved before a macrophage succeeds in picking up its prey. Understanding the mechanics by which immune cells clear sites of infections opens up new avenues for intervention.

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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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NANOPARTICLES TRANSPORT ACROSS THE HUMAN PLACENTA

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Nanoparticle exposure in utero might not be a major concern yet, but could become more important with the increasing application of engineered nanomaterials (ENMs) in consumer and medical products. Several epidemiologic and in vitro studies have shown that nanoparticles can have potential toxic effects [1-4]. However, nanoparticles also offer the opportunity to develop new therapeutic strategies to treat specifically either the pregnant mother or the fetus [5-6]. Previous studies mainly were performed in animal studies which cannot be extrapolated to humans because the anatomy and physiology of the human placenta are unique.
Therefore the motivation of the placenta – ENMs studies are to:

- determine the barrier capacity of healthy human placenta for ENMs
- study the accumulation, localization and potential effects of ENMs in placental tissue
- determine the influence of the physic-chemical properties of ENMs on the translocation rate
- identify and understand the underlying transport mechanism

We used the ex vivo human placental perfusion model [7] to analyze the transfer of plain and carboxylate modified polystyrene particles in a size range between 50 to 500 nm (Figure 1). We showed that fluorescent plain polystyrene particles with diameter up to 240 nm were taken up by the placenta and were able to cross the placental barrier without affecting the viability of the placenta explant 8]. In addition we showed that the transport of polystyrene particles in the fetal to maternal direction was significantly higher than for the maternal to fetal direction. Regardless of their ability to cross the placental barrier and the direction of perfusion, all polystyrene particles accumulated in the syncytiotrophoblast of the placental tissue [9].
Our results indicate that the syncytiotrophoblast is the key player in regulating nanoparticle transport across the human placenta.

The mechanism underlying this translocation is not mainly based on active, energy-dependent transport pathways.
These findings will be important for reproductive toxicology as well as for pharmaceutical engineering of new drug carriers.

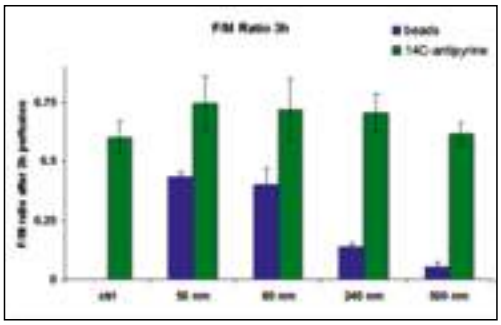

Figure 1: Size-dependent barrier capacity of the human placental tissue. The ration between fetal (F) and maternal (M) concentrations of ^{14}C-antipyrine (green bars) and polystyrene beads (blue bars) were calculated after 180 min of perfusion. The ^{14}C-antypyrine values remain unchanged, whereas the per-fusion rate of the beads showed size dependence. Data represent mean±SE of at least four independent experiments. adapted from [8].

The research leading to these results has received funding from the the Swiss National Foundation, (NRP 64 program, grant no 4064-131232).

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UNSOLVED PROBLEMS IN CANCER: OLD CHALLENGES AND NEW TOOLS

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Cancer continues to be one of the leading causes of death worldwide. The WHO expects the number of new cases to rise by 70% in the next two decades. Thus, in the years to come cancer prevention and therapy will likely remain in the focus of both policy-makers and care-givers.
In the last years, significant progress has been made in understanding molecular alterations that promote tumor development and progression. In particular, novel sequencing techniques have increased the pace of discovery of druggable targets. With the introduction of antibodies, tyrosine kinase inhibitors and other targeted agents, we have seen a multiplication of treatment options for many kinds of cancer. In addition, immune therapies have started to change the landscape of cancer therapy profoundly. They are effective in a range of different tumor types and the response tends to be longer than with other targeted agents. At the horizon, new classes of drugs such as second generation peptidomimetics hold the promise of targeting protein-protein interactions in vivo and thus may further increase the spectrum of druggable targets.
However, in spite of recent progress, cure rates of metastatic cancers have remained low. Tumor heterogeneity and clonal evolution are important reasons for the failure of anti-cancer therapies to induce long lasting remissions. Two of the most burning issues in cancer care are the following:

1. PREDICTION OF TUMOR RESPONSE TO TARGETED AGENTS.

Most new agents tested in early clinical trials show at least some signs of activity in patients. However, overall response rates are rather low and the difficulty lies in predicting who is going to benefit from a specific therapy. There is urgent need to develop innovative tools for the prediction of tumor response. One question is whether sequencing the tumor genome is enough to allow for prediction of tumor response. Can we identify the most aggressive clones of the malignancy and successfully foresee the impact of anti-cancer agents on those? Or do we need epigenetic, RNA- or phosphoprotein-based information? Do we need to treat all clones at the same time or is a sequential therapy as effective?

2. DELIVERY OF ANTI-CANCER AGENTS TO TUMOR CELLS.

Anti-tumor agents continue to have significant side-effects. This is also true for targeted agents. In order to increase the risk/benefit ratio, targeted delivery of therapeutic compounds remains an important goal. Shifting the balance of drug delivery from healthy tissue to cancer cells by a factor of 2 or 3 may already be enough to increase response rates and reduce side-effects dramatically.
In the last 20 years, the role of nanotechnology in the field of oncology has been increasing. Both diagnostic and therapeutic agents have found their way into clinical routine. However, this process has been slow. With regard to the above-mentioned challenges in oncology, nanotechnology may have more to offer.
In this presentation, I will highlight some of the most imminent challenges in oncology. In particular, I will address the question in which ways oncology may benefit from new developments in nanotechnology.

MULISTAGE DELIVERY OF SIRNA THERAPEUTICS

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Small interfering RNA (siRNA) holds great potential for the treatment of disease, as the activity of any desired gene can be suppressed. However, the therapeutic application of siRNA has been limited due to ineffective delivery in vivo. Here, an easy-to-use nanodelivery system was designed for safe and efficient systemic delivery of siRNA. The platform consists of a polycation-functionalized porous silicon-based multistage vector (MSV) that is loaded with siRNA through simple mixing. The porous silicon particle provides protection, improves biodistribution, and enables sustained release of polymeric siRNA nanoparticles, which subsequently undergo cellular internalization and lysosomal escape. Efficient suppression of target genes was achieved in vivo in the absence of detectable immunotoxicity or subacute toxicity. Collectively, these results indicate that the MSV can be used for safe and effective delivery of siRNA.

SMART NANOPARTICLES FOR ULTRASENSITIVE QUANTIFICATION OF CANCER MUTATIONS

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The ability of sensitive and specific detection of genetic biomarkers and mutations is key to human healthcare, allowing for non-invasive, earlier diagnosis of diseases, prediction of patients' responses to treatment and risk of disease relapse.1 In this regard, the polymerase chain reaction (PCR) based methods are widely used because of its great signal amplification power, and hence high sensi-

tivity. Such assays however, are typically slow, expensive and requiring a clean lab environment, making it less suitable for rapid, on-site detection. Furthermore, as a paradox of its great target amplification power, even a tiny amount of contaminants can be amplified into non-negligible signal, which can lead to false positives. Therefore significant efforts have been devoted to the development of alternative, PCR-free genetic sensing approaches.2 Despite great progress have been made over the past two decades, few techniques reported so far have the specificity, sensitivity, and multiplexing abilities required for cost-effective, accurate early diagnosis and prognosis of cancer via non-invasive means.2 In an attempted to address this challenge, we have recently developed an ultrasensitive DNA sensing approach by combining magnetic nanoparticle (MNP) based rapid, efficient target capture and enzyme based great signal amplification via a sandwich essay using a pair of capture/signal (c-/s-) DNAs conjugated to a MNP and enzyme, respectively. Hybridization between the c-/s- DNA pair with specific DNA target forms an MNP-duplex DNA-enzyme sandwich that is easily separated magnetically. After washing to remove any unbound species, specific enzyme substrates are added to trigger enzyme catalysed production of colorimetric and/or fluorescent products, acting as the readout signal for target DNA quantitation (Figure 1A).[3] This approach can simultaneously quantitate two different specific unlabelled DNA targets down to 100 fM via colorimetric readout. The assay is also robust and works effectively in complex media, e.g. 10% human serum. By exploiting the DNA duplex stability difference between the c-/s-DNA probes and the wild-type and cancer single-base mutants, we have successfully discriminated the cancer specific mutants from the wild-type gene associated with colorectal cancer by ~ 3 fold (KRAS codon 12/13).[3] More recently, we found its sensitivity can be further improved to10 fM by using a more sensitive fluorimetric readout signal (Figure 1B).[4]

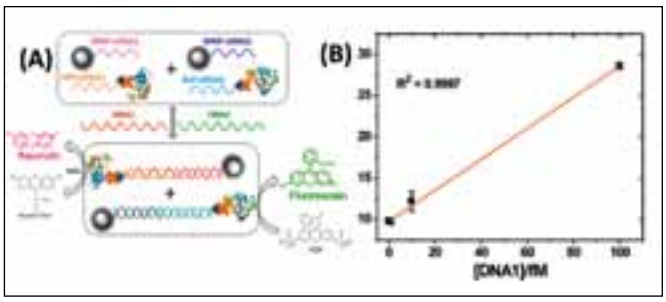

Fig. 1. (A) Schematic of the MNP-enzyme sandwich assay for simultaneous detection of two different DNA targets using two pairs of MNP-cDNA and sDNA-enzyme probes. (B) An example calibration curve of the MNP-enzyme assay for quantitation of fM DNA target (DNA-1) in 40 min via fluorimetric readout.

To further improve the signal discrimination between the wild-type and cancer mutants, we have developed a target-recycled ligation strategy. A pair of biotin-/phosphate- DNA probes is covalently ligated together by a Taq DNA ligase only templated by a full-match DNA target, but not a single-base mutant, forming a covalently linked biotinylated DNA product that binds strongly and specifically to streptavidin-linked enzymes (Figure 2A). After multiple cycles of denaturation, annealing & ligation to amplify the target DNA, where each full-match DNA produces a ligated product in each cycle, a MNP-capture DNA is then used to capture the ligated products via competitive hybridization followed by washing and enzymatic amplification (Figure 2A). This method can offer universally high discrimination (> 100 fold) between the single-base mutants and full-match target in all 16 possible mutation combinations (Figure 2B). Moreover, it can quantitate a specific cancer mutant in coexisting wild-type gene background down to 0.75% (Figure 2C), putting it among the very best reported in literature. Such sensitivity in gene mutation detection is comparable or better than most sequencing based techniques, including next-generation sequencing which can detect mutation at ca. 1-4%.[5]

Figure 2. (A) Principle of the MNP-enzyme sensor combined with target-recycled ligation strategy. (B) Discriminate the wild-type and single-base mutants for all 16 possible mismatch combinations, only the full-matched target-probes yield high signals. (C) Quantitate a cancer mutant in coexisting wild-type genes with a detection limit (LOD) of 0.75%.

More recently, we have further developed more powerful signal amplification strategies using poly-enzyme tagged nano-beads which enables the successful detection of target DNA down to 10 aM. We have further developed the on magnetic nanoparticle-ligation strategy which extends the detection of cancer specific mutants in the presence of co-existing wild-type background down to ~0.1% level.[5] These most recent developments will also be discussed in this presentation.

We thank the Leeds Cancer Research UK Centre, University of Leeds and the Wellcome Trust (UK) for funding this work.

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MECHANOSENSITIVE VESICLES FOR TARGETING CRITICALLY STENOSED ARTERIES

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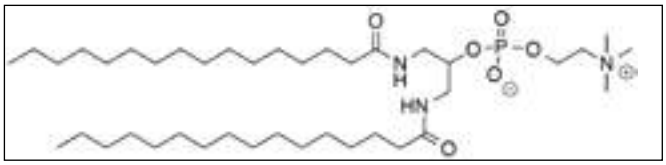
There is a need for special drug formulations to treat acute heart attack patients, as up to 50 % of the patients die before they reach the hospital environment. This is also due to the lack of a low-technology drug delivery approach that could be administered easily at the patient's home allowing for rapid reperfusion of the heart muscle with oxygen.
Targeted drug delivery is an attractive option for a heart attack drug formulation. Due to the lack of a specific biomarker expressed on the surface of a stenosis, chemical and biological targeting is impossible. At the site of the stenosis, however, the artery is narrow. This leads to a rapid increase of the wall shear stress by an order of magnitude or more. This purely physics-based wall shear stress trigger should be used for specific stenosis targeting. A nanocontainer is needed that opens on the increase of shear stress.(1)
Vesicles formulated from the artificial 1,3-diamido phospholipid Pad-PC-Pad are mechanosensitive ¬– the vesicles release their cargo if they are exposed to elevated shear stresses. Pad-PC-Pad vesicles filled with a vasodilator such as nitroglycerine can therefore become ideal candidates for the physics-based drug delivery approach.(2)
Hydrated Pad-PC-Pad forms inter-digitated bilayer membrane sheets with additional lateral stability imposed through amide-amide hydrogen bonding. Vesicles formulated from such stiff mem-

branes are non-spherical and show facetted geometry. The edges between the flat vesicle faces are highly curved and must therefore contain membrane defects. These defects are attenuated by the application of shear forces and lead to the mechanosensitivity.(3)



Structure of the 1,3-diamidophospholipid Pad-PC-Pad. The main features are the increased spacing between the fatty acyl chains compared to natural phospholipids. This leads to spontaneous membrane inter-digitation. Secondly, the presence of amide moieties increases the hydrogen bonding between the lipid head groups. This imposes higher lateral stability.

Here, we give an update on the Pad-PC-Pad vesicle research. In particular, we focus on the positive preclinical tests. We also report on microfluidic device testing showing localized cargo release at sites of high wall shear stresses only. Both results are an important step forward towards clinical applications of mechanosensitive drug delivery.

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ABSTRACTS OF THE POSTERS

TARGETED DELIVERY OF ANTI-HER2 AFFIBODY CONJUGATED STERICALLY STABILIZED CISPLATIN LIPOSOME IN HER2 RECEPTOR-EXPRESSING BREAST CANCER MODELS

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INTRODUCTION

Cisplatin, a widely used antineoplastic drug, is used in the treatment of epithelial associated malignancies. Though efficacious as the first-line treatment strategy, the dose limiting adverse reactions of cisplatin hampered dose increment and impeded its extensive clinical applications.
SPI-077 ™, the sterically stabilized liposomal formulation of cisplatin, is designed based upon liposomal formulation of doxorubicin, Doxil®. Though SPI077 ™ has shown superior therapeutic efficacy compared to free cisplatin, the formulation yet lacked efficacy gains in clinic.
Numerous studies reported use of targeting moieties to increase the therapeutic efficacy of encapsulated curative payload. We chose here anti-HER2 affibody molecule as an attractive tumor-specific ligand due to favorable structural and thermal stabilities as well as high binding affinity. Further, HER2 is highly overexpressed in a large proportion of cancers including breast cancer. Trastuzumab, MAb directed against HER2, represents one such clinical validation as a single agent; however, it is most efficacious when combined with chemotherapy.
To this end, we aimed at combining the tumor targeting features of specific anti-HER2 affibody with the drug delivery characteristics of long-circulating cisplatin liposome.

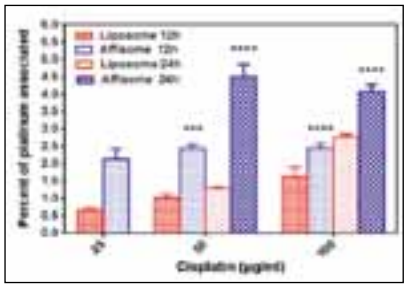
METHODS

For the first time, we developed a sterically stabilized cisplatin liposome composed of HSPC/ cholesterol/ mPEG2000-DSPE (56.5:38.5:5 molar ratio) by the ethanol injection method. Liposomes were then characterized by their size, zeta potential and cisplatin encapsulation efficiency.
Affibody conjugation to the linker was done by incubation of affibody with Mal-PEG2000-DSPE micelle and the covalent linkage of affibody to Mal-PEG2000-DSPE micelles was determined by SDS-PAGE analysis. Transfer of Affibody into preformed liposomes (affisome) was carried out by mixing aliquots of conjugated affibody-micelles with cisplatin-loaded liposomes to achieve an average number of 20 affibody molecules per liposomes.
Cellular platinum uptake and in vitro cytotoxicity assay were assessed on HER2-expressing human breast adenocarcinoma SK-BR-3 cells. Therapeutic efficacy was then investigated in BALB/c mice bearing HER2-overexpressing TUBO breast carcinoma tumor at 3 mg/kg cisplatin via a single tail vein injection every weeks for 3 consecutive weeks.

RESULTS

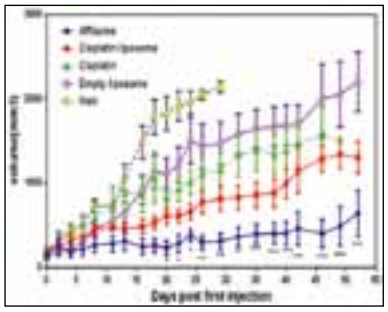
Negatively charged cisplatin liposomes and affisome had particle size of around 135 nm with poly dispersity index (PDI) of 0.04. Gel electrophoresis assay revealed approximately 70% conversion of the original affibody (M.w. 14 KDa) into slower-moving affibody-micelles conjugate (16.9 ~17 KDa) consistent with BCA results.
SK-BR-3 cells treated with liposome containing anti-HER2 affibody, demonstrated a significantly higher amount of platinum intracellularly compared to cisplatin. There was also a two- to three-fold increase in the association of affisome to SK-BR-3 cells compared to that of stealth liposome (no affibody) after 12 and 24 h incubation times at 50 and 100 µg/ml of cisplatin (Fig. 1)

Fig. 1: In vitro intracellular platinum uptake by SK-BR-3 cells at different time points. SK-BR-3 cells were treated with cisplatin containing liposome, affisome or free drug



Anti-HER2 liposome was 2-fold more cytotoxic than cisplatin-loaded stealth liposome which affected cell growth at higher concentrations.
Further, cisplatin-affisome had greater therapeutic efficiency than non-targeted liposome in HER2+ TUBO model (Fig. 2). Equally promising, the affisome-treated mice did extend the survival of animals by several days and even left a one tumor-free survivor.

Fig. 2: Inhibition of tumor growth in TUBO breast tumor model in BALB/c mice treated with free and cisplatin loaded liposome and affisome.



DISCUSSION

Clinical application of cancer-specific targeting vectors has long become a respectable therapeutic option in increasing the potency of the delivered drug. Up to date, 13 particulate nanomedicines equipped with targeting ligand have progressed into clinical evaluation. Indeed, active targeting via modification of nanocarrier surface with a targeting ligand provides benefit in terms of target cell internalization and retention. Upon binding, certain ligands release the nanoparticulate contents intracellularly by virtue of receptor-mediated endocytosis. Moreover, ligand-targeted nanoparticulate may circumvent the multi-drug resistance (MDR) through altered cellular fate after receptor-mediated endocytosis compared to passive diffusion of free drug over the cell membrane from the nanoparticulate devoid of targeting ligand.
We successfully decorated SPI077 ™ mimic with anti-HER2 affibody molecule. We found that anti-HER2 affisome not only efficiently bound to surface-exposed extracellular domain of HER2 of tumor cells such as SK-BR-3, but could be promptly internalized as well. Furthermore, the therapeutic efficacy of HER2 affibody-targeted cisplatin liposome was superior compared to unmodified SPI077 ™ mimic and free cisplatin against HER2-expressing TUBO cells.
Our data implied that the direct interaction of affibody-targeted liposome with HER2-overexpressing tumor cell could results in a more potent therapeutic efficiency than the gradual cisplatin release from stealth liposome in the tumor interstitium fluid.
We conclude here that targeted delivery using anti-HER2 affibody would improve the therapeutic index of cisplatin chemotherapy however; further studies with more specific approaches would be helpful for clinical translation of targeted liposomal vehicle in cancer therapy.

ACKNOWLEDGEMENTS

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A MULTIFUNCTIONAL HYBRID NANOPLATFORM FABRICATED BY ENCAPSULATION OF POROUS SILICON NANOPARTICLES INTO SELF-ASSEMBLING POLYMERIC NANOSSOMES FOR CANCER THERAPY

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INTRODUCTION

In recent years, several nanoparticle-based drug delivery systems have been developed in order to circumvent the deadlocks associated with the currently practiced modalities for cancer-specific targeting, imaging, and therapy.1

Polymeric nanossomes have recently focused attentions as promising drug delivery systems. The physicochemical properties of polymersomes allow them to overcome most of the challenges encountered by liposomes,2,3 exhibiting improved robustness and colloidal stability, higher membrane stability and lower membrane permeability, which can be controlled by varying the features of the constituent block copolymers.2 Additionally, the outer hydrophilic block prevents plasma protein adsorption, thereby evading the non-specific opsonization by the reticuloendothelial system (RES) and the versatile chemical surface of block copolymers enables the biofunctionalization with targeting moieties, resulting in the development of smart drug delivery systems.4,5

Porous silicon (PSi) has also recently emerged as a very promising platform for drug delivery, due to its remarkable physical properties, including fine-tunable nanoscale sizes, tailored shapes and pore sizes, large surface area, and large pore volume. These features permit the confinement of hydrophobic drugs in its amorphous state within the porous matrix, improving its aqueous solubility and, consequently, its bioavailability. Moreover, PSi is also characterized by suitable biocompatibility, biodegradation, and easy functionalization, rendering this material versatile for biomedical applications, particularly considering tumor drug delivery, imaging and targeting.6-13

OBJECTIVE

We aimed to fabricate a hybrid nanoplatform by nanoencapsulating of PSi nanoparticles loaded with a hydrophobic anticancer drug into polymersomes carrying a second hydrophilic cytostatic agent, resulting in a multistage and multifunctional nanodelivery system, which encompasses the attractive features of both components and is envisioned for combined cancer therapy.

EXPERIMENTAL METHODS

PSi nanoparticles and polymeric nanosomes were produced by electrochemical anodization and self-assembly co-solvation methods, respectively. Afterwards, the fabrication parameters and method of the hybrid nanoplatform comprising the aforementioned components were adjusted and optimized.

The produce nano-in-nano drug delivery system and its individual constituents were subsequently analyzed in terms of size, size distribution, zeta-potential, and morphology, using dynamic light scattering (DLS), electrophoretic light scattering (ELS), and transmission electron microscopy (TEM), respectively. Additionally, its stability in both aqueous media and human plasma was evaluated.

Furthermore, the ratiometric loading of two different hydrophobic and hydrophilic chemotherapeutic drug models into the designed nanosystem was tested by loading the hydrophobic agent into the porous matrix of the PSi nanoparticles and encapsulating its hydrophilic counterpart in the aqueous compartment of the polymeric nanossomes. The drug release profiles of the loaded antitumor agents were subsequently evaluated in different physiological relevant media.

RESULTS

In the preliminary development stage of the proposed nanocarrier (Figure 1(a)), we successfully produced polymeric vesicles by self-assembly of amphiphilic block copolymers in an aqueous medium (Figure 1(b)). Furthermore, the parameters for nanoencapsulation of PSi nanoparticles into the polymersomes were optimized and the nano-in-nano drug delivery platform was fabricated, as depicted in Figure 1(c), in which shows a PSi nanoparticle encapsulated in a polymeric nanossome.

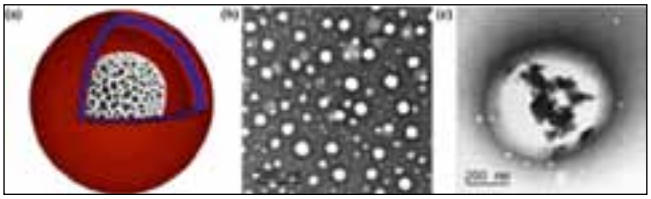

Figure 1. (a) Schematic representation of the hybrid nanocarrier. (b) TEM image of the bare polymersomes. (c) TEM image of the hybrid nanocarrier.

Aiming at the application of the developed nanosystem in combination cancer therapy, two chemotherapeutic agents with distinct physicochemical properties (i.e., one hydrophilic and one hydrophobic) were successfully loaded inside the nanocarrier. In addition, the loading of the combination therapy was precisely controlled in a mass ratiometric manner.

CONCLUSIONS

Herein, we demonstrated the fabrication of a hybrid nanocarrier by nanoencapsulation of PSi nanoparticles into polymeric vesicles, enabling the loading of combination cancer therapeutics. The drug nanodelivery system developed is envisioned to be further explored for cancer theranostic applications.

ACKNOWLEDGMENTS

Financial support from Academy of Finland (grant nos. 252215 and 281300), the ERC grant no. 310892 under the EU 7th Framework Programme (FP/2007-2013) and the Finnish Cultural Foundation (grant no. 00151092) are acknowledged.

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THE DEVELOPMENT OF MEDIUM – AND LARGE-SCALE SUSTAINABLE MANUFACTURING PROCESS PLATFORMS FOR CLINICALLY COMPLIANT SOLID CORE NANOPHARMACEUTICALS. 'NANOFACTURING'

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The Nanofacturing consortium will develop a new manufacturing platform process for solid core nanopharmaceutical products, capable of scale up to supply Phase III trials of a novel needle-free insulin formulation for the treatment of Diabetes, and gold nanoparticles (GNP) for the promising treatments for Central Nervous System, Antiviral and Oncology applications as both non-sterile and sterile formulations.
The aim of NANOFACTURING is to create an ambitious multiple-scale, manufacturing platform to support the wide range of nanopharmaceutical products being developed in Europe. It will address the small- and medium-scale needs of early phase clinical trials and applications, whilst also supporting later stage products with large potential markets, by developing clinically compliant, sustainable large scale manufacturing processes capable of taking these products through Phase III trials into commercial manufacture and supply. NANOFACTURING will specifically address cost and toxicity concerns, particularly over the use of gold, through state-of-the-art biological characterization.

THE SCIENTIFIC OBJECTIVES ARE:

1. Develop a new manufacturing platform process and accompanying Good Manufacturing Practice (GMP) plant design and concept brief for solid core nanopharmaceutical products, capable of scale up to supply Phase III trials and beyond which is cost effective, safe, efficient, robust and regulatory compliant.
2. Build a pilot scale GMP manufacturing line capable of supplying nanomedicines at scale.
3. Establish an open access pilot line in Europe as part of the existing UK innovation center for the process development and scale up of nanopharmaceutical manufacture.
4. Scale up the primary ligand component manufacture to meet the NP manufacturing requirements at all scales.
5. Establish a full spectrum of robust and practical chemical and biological characterization tests and procedures to meet stringent regulatory requirements for the manufacturing processes developed and guarantee the quality, safety and efficacy of the product(s) at all scales.
6. Bring an innovative healthcare solution closer to market for millions of sufferers of diabetes across the globe, and support the route to market for promising treatments in CNS, Antiviral and Oncology applications.
7. To create a European manufacturing ecosystem for nanopharma, which will support business and generate more high value manufacturing jobs in the EU.

The NANOFACTURING consortium will adopt a highly interdisciplinary approach between nanotechnologists, materials scientists, chemists, engineers, biomedical researchers, industrialists and regulatory specialists to realize the project objectives. The consortium includes:

MIDATECH PHARMA ESPAÑA S.L.U.

The SME Midatech Pharma España S.L.U. is the consortium leader and a global leader and centre of excellence in the emerging field of nanomedicine; they have developed a new broadly relevant class of glycan-coated gold NPs (GNPs). They have a fully licensed API cGMP NP manufacturing facility for solid core NPs.

CENTRE FOR PROCESS INNOVATION

The Centre for Process Innovation (UK), CPI bring broad interdisciplinary knowledge in biopharmaceutical manufacture, nanotechnology, green chemistry and industrial biotechnology. CPI will draw on their expertise in the development and scale up of sustainable and continuous manufacturing processes to create a new, sustainable, scalable, clinically compliant manufacturing process for nanopharmaceuticals.

FIRC INSTITUTE OF MOLECULAR ONCOLOGY

The Nanomedicine group of FIRC Insitute of Molecular Oncology (IFOM - Italy) is experts in the development and characterisation of functionalised NPs for diagnosis and therapy of different types of disease and have specific expertise in virology studies of NPs.

EPFL-ECOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

Supramolecular NanoMaterials and Interfaces Laboratory at Ecole Polytechnique Fédérale de Lausanne (EPFL – Switzerland, 3rd country entity) led by Prof. Francesco Stellacci are world leaders in gold NP synthesis, characterization, and application. The two groups have access to state-of-the-art services for NP characterisation and will provide an advanced suite of methods to prove product equivalence during scale up.

CENTRE FOR BIONANO INTERACTIONS

The Centre for BioNano Interactions (CBNI) at the National University of Ireland, Dublin, of prof. Kenneth Dawson, bring instrumenta-

tion and methodologies which allow fast and reliable multi-biological assay screening for in vitro nanomaterial toxicity, and specific expertise in quantitative bionanoscience of gold NPs.
SMEs in Spain and Poland with expertise in the development manufacture and scale up of the ligands to be used in the process (GalChimia, ProChimia and Applus). GalChimia bring the specific knowledge of GNP ligands. ProChimia bring interdisciplinary expertise of organic chemistry, physical chemistry, molecular biology and nanotechnology, with experience in the synthesis of thiolated compounds used in surface science. Applus has broad expertise in scale-up to pilot plant of chemical entities under GMP and in particular with the reactions involved in the synthesis of ligands used in the process.

MICROCAPILLARY LOCALIZATION OF CURCUMIN LOADED SPIONS: A PRECISE FOR TARGETED DRUG DELIVERY SYSTEM

MOHAMMED ANWAR[1], Mohammed Asfer[2], Neha Mallick[1], Asgar Ali[1], Farhan Jalees Ahmad[1]
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Curcumin loaded superparamagnetic iron oxide nanoparticles (CUR-SPIONs) were prepared by classical chemical co-precipitation method in an inert atmosphere (Figure 1 (a-d)). Characterization of SPIONs were done by dynamic light scattering (DLS), vibrating sample magnetometry (VSM), FT-IR, and transmission electron microscopy (TEM), which showed very small size (≈16 nm) of SPIONs(Figure 1 (e)) with a hydrodynamic size of ≈ 194.6±0.3 nm with PDI of 0.207±0.019. X-ray diffraction patterns of the native Fe_3O_4 nanoparticles shows a series of characteristic peaks at 2θ = 18.30°, 30.11°, 35.46°, 43.10°, 57.00°, 62.60° and 74.06° corresponded to the hkl values of {111}, {220}, {311}, {400}, {511}, {440} and {533}, respectively. From VSM studies, saturation magnetization of CUR-SPIONs was found to be 45 emu/g.

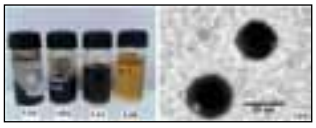

Fig. 1: Uncoated SPIONs (a), Aqueous ferrofluid (b), Curcumin loaded ferrofluid (c) CUR-SPIONs diluted with water (d), TEM image (e).

It's in vivo targeting ability was evaluated by simulating it's in vitro localization study and aggregation dynamics with a flow of blood inside a square glass capillary (500×500 µm2 cross section) in the presence of an externally applied magnetic field (Ms=1200 mT) (Figure 2).

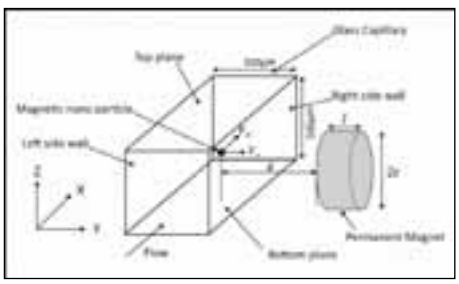

Fig. 2: Expression for the motion of SPIONs inside the glass capillary.

Visualization of CUR-SPIONs, mixed with blood (1:1 (v/v)) inside a glass micro capillary in the presence of magnetic field was done by fluorescence microscope. At a flow rate of Q = 5µL/min CUR-SPIONs were attracted towards the right side wall of the capillary adjacent to the permanent magnet and starts forming aggregate. The aggregate was observed over a time a period from t = 0+ to t = 800 s. After t = 500 s, no significant growth of aggregate was observed (Figure 3). This study simulates the insight behaviour of CUR-SPIONs in a blood vessel during targeted drug delivery system. The results reveal that aqueous phase transfer of optimized concentration of CUR-SPIONs can be used as a bio-safety carrier in application to targeted drug delivery systems.

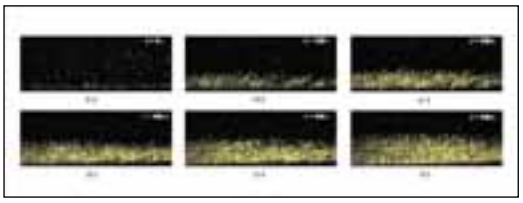

Fig. 3: In vitro localization study of CUR-SPIONs in blood after (a) t = 0 s, (b) t = 100 s, (c) t = 200 s, (d) t = 300 s, (e) t = 400 s, (f) t = 500 s.

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SIGNIFICANT IMPROVEMENT OF PHARMACOKINETIC AND THERAPEUTIC EFFICACY OF LIPOSOMAL DOXORUBICIN BY TARGETING THE CD44 MARKER IN COLON CARCINOMA

LEILA ARABI[1], Badiee A[1], Jaafari MR[1*]results indicate that the MSV can be used for safe and effective delivery of siRNA.
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OBJECTIVES:

In recent years, accumulating evidences support a role for cancer stem cells (CSCs) in resistance to conventional chemotherapy, metastasis and tumor recurrence (1-3). Putative CSC populations have been distinguished in several solid malignancies based on the expression of specific markers (4-7). Cells with high level expression of CD44 have been identified as candidate CSC in a variety of human tumors including leukemia, colon, breast, ovarian, prostate, pancreatic, and head and neck cancers. It has been shown that the high expression of CD44 play key roles in tumor maintenance, adhesion, cell survival, migration and invasion (8-9). The present study was designed to investigate CD44 expression in established C26 murine colon carcinoma cell line and targeted drug delivery to these cells via liposomal nanoparticles conjugated with CD44 monoclonal antibodies (mAbs).

MATERIALS AND METHODS:

The expression of CD44 marker was analyzed by flow cytometry (BD FACSCalibur). In order to target these cells, CD44 mAbs attached to the distal end of NHS functionalized mPEG3400-DSPE was post inserted into Doxil (10). Mean diameter, polydispersity index and zeta potential of liposomes were measured by Dynamic Light Scattering (Nano-ZS; Malvern, UK) in triplicate. In vitro cytotoxicity and uptake of liposomal preparations containing Dox was determined in C26 cell line. We evaluated the therapeutic efficacy and biodistribution of CD44-targeted Doxil (10-15 mg/kg) in comparison with Doxil (10-15 mg/kg) in in Balb/c mice carrying C26 murine carcinoma. We monitored tumor size, body weight and survival over time. Mouse survival was analyzed with GraphPad Prism version 5 and Mantel–Cox test. Regarding other comparisons, one-way ANOVA and Newman– Keuls multiple comparison tests were employed.

RESULTS AND DISCUSSION:

Flow cytometry results showed that CD44 was expressed at very high levels (>99%) of C26 cell line Compared to Doxil, CD44-targeted-Doxil showed a significantly higher binding affinity to C26 cells. In cytotoxicity assays CD44-targeted-Doxil showed lower inhibitory concentration at 50% (IC50) in-vitro compare to Doxil. (4.33 µg/mL versus 9.51 µg/mL, respectively). The higher interaction of

CD44-targeted Doxil could be translated in higher toxicity against these cells. CD44-targeted liposomes increased the survival of mice and inhibited tumor growth more significantly than the non-conjugated formulation. The uptake in liver and spleen, which are tissues rich in cells of the RES, was higher for CD44-Doxil, compared to Doxil. The tumor levels of CD44-Doxil were higher than Doxil. This increase was significant at 48 h ($p < 0.01$) but not significant at 24h. Since CD44-Doxil exhibited faster clearance rate compared to non-targeted liposomes, their high concentration in tumor was a great achievement.

CONCLUSION:

In this study we developed a nanocarrier capable to target CD44 positive cancer stem like cells within tumors. Here, we showed that the higher therapeutic efficacy in Balb/c mice carrying C26 murine carcinoma could be achieved as a result of coupling of anti-CD44 monoclonal antibody to liposomal doxorubicin. This study indicated that CD44-targeted therapy confer promising pharmacokinetic and antitumor activity and may be a successful approach for targeted delivery to cancer cells and CSCs.

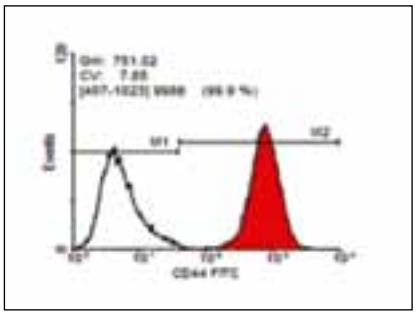

Fig. 1: Expression of CD44 receptor in C26 cells determined by FACS.

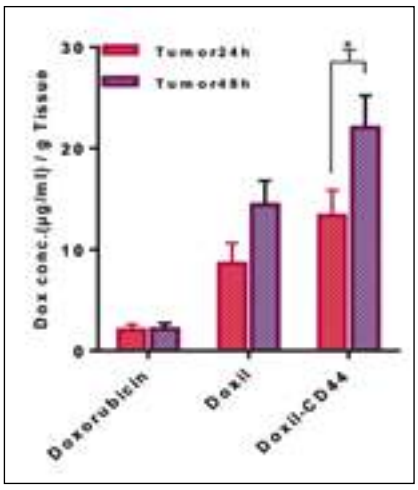

Fig. 2: Biodistribution of PLDs at different time points in organs including (A) Liver and Spleen; (B) Serum; and (C) Tumor in BALB/c mice bearing C-26 tumor after a single dose of 15 mg/kg liposomal Dox administered i.v. on day 12 after the tumor inoculation

KEYWORDS:

CD44, Cancer stem cells, Nanoimmunoliposome, Therapeutic efficacy

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NANOPARTICLES FOR DIAGNOSTIC IMAGING AND TREATMENT OF ATHEROTHROMBOSIS: STANDARDIZED PHYSICO-CHEMICAL CHARACTERIZATION AND IN VITRO CYTOTOXICITY ASSAYS.

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Background: Nanomedicine offers new avenues for an improved diagnosis of cardiovascular disorders, along with localized treatment of vulnerable plaque and stroke. However, for the application in humans, a substantial amount of preclinical studies is necessary to analyse the effects of nanoparticles on the vascular cells. Here, we report the establishment of standardized assays for physico-chemical characterization of nanosystems, and for the systematic toxicological examination of nanosystems' effects on endothelial cells, which are the first-contact cells of vascular wall for circulating nanoparticles.

Nanoparticle type	*Z-avg d (nm)*	*PDI*	*ζ (mV)*	*SD ζ (mV)*
LD-NP1	*53.3*	*0.156*	*- 7.0*	*14.5*
LD-NP2	*82.8*	*0.191*	*- 9.0*	*14.6*
LD-NP3	*120.1*	*0.151*	*- 8.8*	*8.4*
LP-NP1	*138.6*	*0.104*	*- 16.3*	*7.4*
LP-NP2	*108.8*	*0,034*	*-9.0*	*4.7*
PM-NP1	*145.1*	*0.072*	*- 51.0*	*5.6*
PM-NP2	*226.9*	*0.194*	*3.3*	*5.7*
IO-NP1	*78.7*	*0.145*	*- 37.3*	*12.9*
IO-NP2	*79.6*	*0.173*	*13.7*	*9.3*
IO-NP3	*57.5*	*0.217*	*- 24.9*	*8.4*

Table 1. Physicochemical characterization of nanoparticles Physicochemical characterizations were performed for various nanosystems 1 month after particle synthesis. Z-avg d, Z-averaged hydrodynamic diameter; PDI, polydispersity (PDI = SD2/d2); ζ, zeta-potential; SD ζ, standard deviation of zeta-potential; NP, nanoparticles; LD, lipidots; LP, liposomes; PM, polymeric NPs; IO, superparamagnetic iron oxide nanoparticles.

Methods: Lipid nanoparticles and liposomes, polymeric and iron oxide nanoparticle systems were produced. Z-averaged hydrodynamic diameter, dispersity (PDI) and ζ-potential were determined with a Zetasizer Nano ZS (Malvern). For long-term in vitro toxicity

testing, endothelial cell growth and vitality upon treatment with different nanoparticle systems (0-400 mg/mL) was monitored for up to 72h using two complementing methods: real-time cell analysis (impedance measurement, xCELLigence) and live-cell microscopy. Moreover, the effects of circulating nanoparticles on endothelium were assessed in an in vitro model of arterial bifurcations.

Results: We report here the establishment of a platform for systemic characterization and standardized toxicity testing of nanosystems intended for the intravascular applications. In total, 10 nanoparticle systems were tested (3 types of lipid nanoparticles, 2 types of polymeric nanoparticles, 3 types of iron oxide nanoparticles and 2 types of liposomes). The hydrodynamic diameter, depending on the nanosystems, ranged between 49 and 230 nm, and the ζ-potential between +9.4 and -53.4 mV (Table 1). All nanosystems were colloidally stable over the 6-month period of storage. In static conditions, the majority of nanosystems were well tolerated by endothelial cells up to the concentration of 100 µg/mL (Fig. 1 left panel), whereas liposomal nanoparticles showed no toxicity up to the highest tested concentration (400 µg/mL). In the dynamic in vitro assay, the majority of circulating nanoparticles were well tolerated up to a concentration of 400 µg/mL (Fig. 1, right panel).

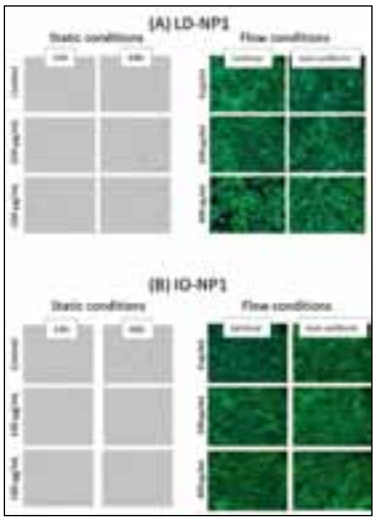

Figure 1. Effects on nanoparticles on endothelial cell viability and morphology under static and dynamic cell culture conditions. As an example, one type of (A) lipid nanoparticles, LD-NP1; and (B) iron oxide nanoparticles, IO-NP1 are shown. In static conditions, cells were treated with nanoparticles for up to 72 h. Phase-contrast images are shown. For flow experiments, ECs were grown in bifurcating slides until confluence and perfused for 18h with medium containing nanoparticles at 100 and 400 µg/mL. Fluorescent images of representative laminar and non-uniform regions are shown (green, F-actin; blue, nuclei).

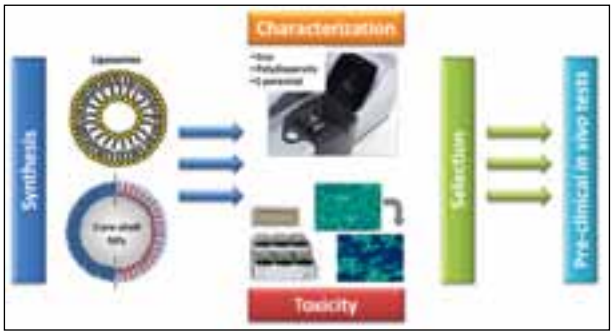

Figure 2. Standardized assays for the systematic physicochemical and toxicological characterization of nanoparticles are an essential part of the pre-clinical safety assessment of nanosystems intended for clinical use.

Conclusions: Characterization of nanosytems stability and biological compatibility is critical before their application in humans (Fig. 2). Our studies demonstrate that the tested nanosystems possess sufficient biocompatibility and are well-tolerated by primary human endothelial cells up to the concentration of 100 µg/mL or more. These findings constitute an essential part of nanotoxicology and safety assessment and are of importance for future clinical use of nanosystems intended for intravascular applications.

Funding: EU project FP7-NMP-2012-LARGE-6-309820 "NanoAthero".

A NON INVASIVE METHOD REGARDING QUALITATIVE AND QUANTITATIVE EVALUATION OF BONE FROM THE MANDIBULA AND THE MAXILLA. CORRELATING RESULTS OBTAINED BY RAMAN AND PYCNOMETER TECHNIQUES

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RESUME:

The bone density has primary influence on treatment planning, implant design, surgical approach, healing time and initial progressive bone loading during prosthetic reconstruction. [1] Depending on the location of the edentous ridge and the amount of time the area has been edentulous,the density of bone is variable. This survival is limited by bone quality, i.e. bone density. [1, 2] 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 not only a matter of mineral content, but also of structure.
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. [3]
Outcomes include mineral density and crystallinity, elemental composition, and collagen crosslink composition. Advantages include the detailed material characterization; disadvantages include the need for a biopsy for better results. Bone samples were obtained by drilling during implant surgery or just surgery. Regarding composition and crystallinity, investigation was performed by RAMAN technique. [4, 5, 6] 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 (v_1 mode) at 1107 cm^{-1}.

The full-width half-height of the $\dot{u}^1$ 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, apatite crystals develop with their long c-axes parallel to the collagen fibril axis. The collagen and associated proteins play an important role in determining nucleation, growth, and proliferation of these crystals – a nanoscale process.
Bone density was evaluated by pycnometer method, in order to complete the investigation. Values obtained, there were from 0.48 ÷ 2.28 g/cm3.

A correlation must be established between RAMAN spectra and density values in order to obtain a correlation function for one step investigation. Method easily can be adapted for "in vivo"

evaluation, being much less invasive method then the well known CT (computer tomography) or CBCT (con beam computer tomography) already used.

Fig. 1: Pycnometer technique – determination of bone density.

ACKNOWLEDGMENT:

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LIPOPOLYSACHARIDE – A MAJOR VIRULENCE FACTOR IN DISEASES

HADAR COHEN,

Lipopolysacharide (LPS endotoxin) is a glycolipid complex of Gram negative bacteria outer membrane which is considered to be a major virulence factor in many human and animal diseases. LPS is recognized as pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors such as Toll-Like Receptors (TLRs), here- TLR4. Once bound, it initiates an anti-inflammatory response which causes the secretion of pro- inflammatory cytokines and chemokines (TNFα, IL-6,MCP-1etc) and enhances phagocytic activity that at elevated concentrations might lead to sepsis and death. One of the potential neutralizers of endotoxin are antimicrobial peptides (AMPs). AMPs are central effector molecules of the innate immune system and produced as an initial response towards invasion.

MSI-78 peptide is a 22AA developed as antimicrobial peptide (megainine2 analog) which is being produced as a topical anti-infective and has a broad-spectrum of activity, covering Gram-positive and -negative bacteria, anaerobic bacteria and Candida albicans. Here we took the same amino acids composition as the parental peptide and formed segregated peptides which differ in properties. After analyzing we found out that the segregation forms less toxic peptides in comparison to the WT peptide, the peptide's hydrophobicity Is important for proper binding, replacement of L to D isomers increases the peptide's stability in solution thus makes it more active. It is also essential to mention that forming a peptide in the same chemical properties at the LPS molecule might enhances its binding capacity.

UPSCALING THE MANUFACTURING OF MICELLAR MPEGHEXPLA NANOCARRIERS LOADED WITH CYCLOSPORINE

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INTRODUCTION

The manufacturing of nanopharmaceuticals in quantity and quality (GMP) required to enter clinical trials is a critical step in the pharmaceutical development process. In order to ascertain the biological performance and therapeutic activity of nanomedicines, robust manufacturing processes have to be developed, with no or little impact on drug product specifications (e.g. particle size, polydispersity, stability or drug loading) at various upscale stages.

mPEGhexPLA is a co-polymer of methoxy poly-ethylene glycol (mPEG) and hexyl substituted poly-lactic acid (hexPLA) [1]. In an aqueous environment, mPEGhexPLA polymer spontaneously forms nanosized micelles in a self-assembly process.

Frequently, the manufacturing of drug loaded micellar nanocarriers involves a nanoprecipitation process: the polymer and the hydrophobic API are dissolved in a small amount of organic solvent and added drop-wise to the aqueous phase. In an energy requiring self-assembly process, the poorly water-soluble active pharmaceutical ingredient (API) is incorporated into the lipophilic core of the micelles. For small scale batches (10 mL) this energy is typically applied as ultrasonic waves using a sonication tip immersed into the aqueous formulation. At a larger production scale, the localized energy release, heat generation, foam formation and wear of the metal sonication probe are limiting factors. A promising alternative is the use of high pressure homogenization, a technique used in food industry and biotechnology; this technology allows high pressure application to the fluid flowing through a homogenizing valve. Potential advantages of this system include: no or very limited material wear, linear upscale, cGMP documentation and possibility for aseptic production. Here, we evaluated the impact of a set of process parameters (homogenization pressure and duration) and batch size (10mL – 1000 mL) on critical drug product characteristics.

METHODS

Micelle Preparation: At a typical labscale, Cyclosporine A (CsA) micellar formulations are prepared as 10 mL batches by drop-wise addition of acetone under sonication (S 450 D, Branson). Subsequently, acetone is removed under reduced pressure (Buchi Rotavapor R-210). Preparation of 10 x and 100 x higher batch volumes was tested using a high pressure homogenizer (Panda 1000, GEA Niro Soavi). Influence of pressure and homogenization time were studied with respect to encapsulation efficiency, micelle size and polydispersity index (PDI). In a first step 100 mL batches of 0.1% CsA mPEGhexPLA micelles were prepared testing 3 different homogenization pressures (200 bar, 400 bar and 800 bar); for each pressure condition aliquots were sampled after 2min, 4 min and 6 min of homogenization time. In a second step, batch size was progressively increased up to a 100 x.

Size measurement: Micelle size was characterized by dynamic light scattering with a Zetasizer Nano-ZS, Malvern Instruments using the DTS Nano software. Micelle size and distribution were obtained as size distribution by number, Z average diameter and PDI.

Molecular weight analysis: mPEGhexPLA molecular weight measurement was performed on a Viscotek GPCmax system equipped with a TDA 305 (Triple detection Array) detector. Separation was performed on a set of LT3000, T1000 and H100 columns using acetone as mobile phase. Molecular mass was calculated as a function of refractive index and light scattering signals.
Imaging: Micelle morphology was recorded on a transmission electron microscope (TEM) FEI Tecnai™ G2 Sphera. Micelles were stained with 0.2% uracyl acetate and deposited on a Carbon disc ultrathin grid.

RESULTS

mPEGhexPLA micelles are commonly prepared using the nanoprecipitation method (Figure 1A). At a 10 mL batch scale, this process results in transparent colloidal solutions (Figure 1B) containing micelles with spherical shape, small size (< 60 nm) and low polydispersity (Figure 1C).

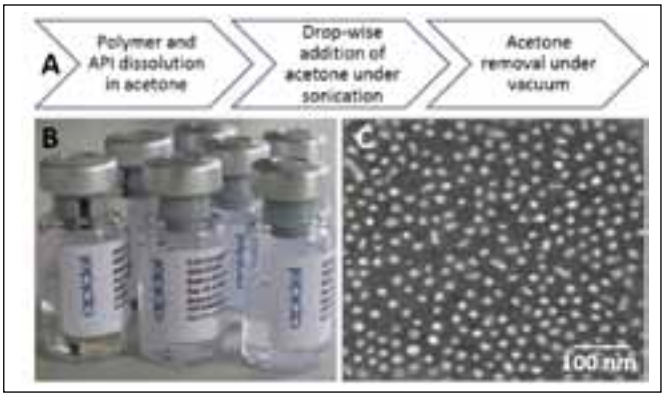
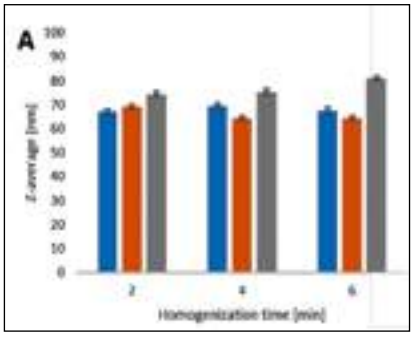


Figure 1: A) Flowchart of the manufacturing process of mPEGhexPLA micelles B) mPEGhexPLA micellar formulations result in transparent colloidal formulations C) TEM microscopy image of mPEGhexPLA micelles.

In initial experiments we increased the batch volume by a factor of 10 x (from 10 mL to 100 mL) and studied the impact of homogenization duration and pressure: independent of the homogenization duration or pressure applied, all prepared formulations were clear colloidal solutions and no drug precipitation was observed. Z-average values showed micellar population below 100 nm (Figure 2A). Very short homogenization times (e.g. 2-4 minutes) resulted in micellar formulations with sizes and size distribution similar to the conventional sonicator tip production method (Figure 2B). Formulations produced at a homogenization pressure of 200 bars for 4 minutes showed the lowest polydispersity index (PdI = 0.061) and were therefore selected for a further upscale.



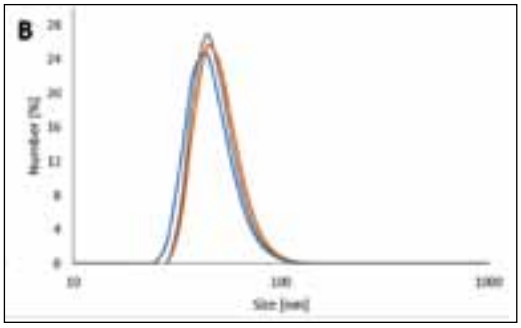


Figure 2: Optimization of the homogenization pressure and duration at a batch size of 100 mL. A) Micelle size (Z average) as a function of homogenization time, blue, orange and grey bars represent particles sizes (Z-average) of formulations manufactured at 200, 400 and 800 bar, respectively. B) Number weighed micellar distribution for homogenization at 200 bar. Blue, orange and grey curve represent 2 min, 4 min and 6 min homogenization time, respectively.

In a second step, the batch volume was further increased by a factor of 5 -10 x and CsA micellar formulation was prepared at a pressure of 200 bar. After only 10 min of homogenization a clear colloidal solution was obtained. Z-average value was in the same range as the smaller batch scale (i.e. 10 mL and 100 mL, (Figure 3) as well as the PDI.

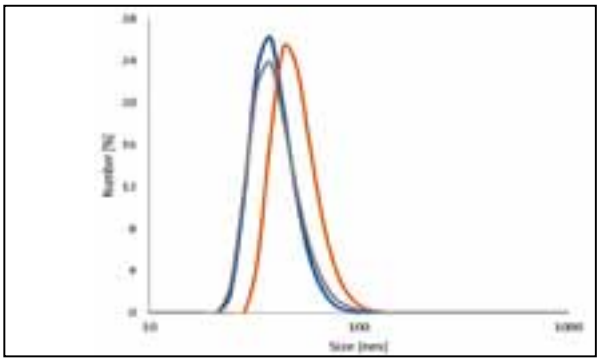


Figure 3: Number weighed micellar population distribution for 3 different batch scales. Blue, orange and grey represent 10 mL, 100 mL and 500 mL batches respectively.

To assess the stability of the polymer under different homogenization conditions, we analyzed the mPEGhexPLA molecular weight before and after the formulation process. GPC analysis showed no alteration of the polymer MW before and after homogenization, suggesting that even the highest pressure applied did not degrade the polymer (data not shown). The morphology of the mPEGhexPLA micelles produced by pressure homogenization was analyzed by TEM and did not show any significant change compared to the process using a sonicator tip at a small scale.

CONCLUSION

Formulation batch size of mPEGhexPLA micelles was successfully upscaled by a factor of 100 from 10 mL to 1000 mL using a high pressure homogenization method. Pressure homogenization was found to be a convenient and rapid process for the preparation of mPEGhexPLA micelles. Only a relatively low pressure and short homogenization time was needed to obtain a clear colloidal solution with a small micellar particle size and a low polydispersity index. mPEGhexPLA co-polymer remained intact after the formulation process, as well as the micelles morphology compared to the process used at a smaller scale. This new production process using a high pressure homogenizer shows great potential for the production of clinical batches of polymeric micellar formulation. A batch of 1 L, produced in an aseptic environment could result in 2000 mono-doses for a clinical trial in ophthalmology.

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PULMONARY INTRAVASCULAR MACROPHAGES: PRIME SUSPECTS AS CELLULAR TRIGGERS OF PORCINE CARPA

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Pigs provide a highly valueable in vivo model based of their sensitivity for complement (C) activation-related pseudoallergy (CARPA), a hypersensitivity reaction caused by some state-of-art nanomedicines. In an effort to understand the mechanism of the pigs' unique features for CARPA, our attention turned on pulmonary intravascular macrophages (PIMs), which are abundantly present in the lung of a wide range of certain mammal species like pigs. PIMs as a macrophage subpopulation have unique qualities explain some of the characteristic symptoms of CARPA in this species, most importantly rapidly (within minutes) releasing vasoactive substances and causing pulmonary vasoconstriction, leading to elevation of pulmonary arterial pressure. The unrivalled features of PIM cells include the following: 1) strong and constant adherence to the capillary wall via desmosome-like intercellular adhesion plaques, which secure stable and lasting direct exposition of the bulk of these cells to the blood stream; 2) their ruffled surfaced glycocalyx membrane engaged in intense phagocytic activity ensures efficient binding and phagocytosis of nanoparticles; 3) PIM cells express anaphylatoxin receptors, this way C activation can trigger these cells, 4) they also express pattern recognition molecules on their surface, whose engagement with certain coated nanoparticles may also activate these cells or act in synergy with anaphylatoxins; 5) their high metabolic activity and capability for immediate secretion of vasoactive mediators upon stimulation explain the circulatory disturbance and other robust pathophysiological effects that appears after their stimulation. Enzymatic digestion of the adhering structures seems to be an effective method to liberating PIMs and so making them available for further ex vivo examination (Fig.1.shows arrow marked PIMs in cell culture, 40x). These findings taken together with reports on liposome uptake by PIM cells during CARPA reaction and the possible presence of these cells in human lung suggests that PIM cells may be a potential therapeutic target preventing CARPA.

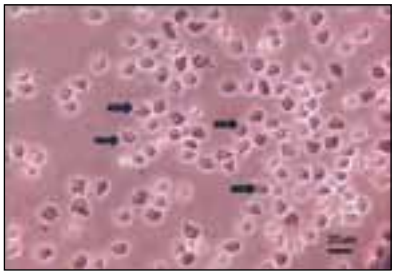

Figure 1. Porcine PIM cells adhered to plastic surface.

PHOSPHORUS DENDRIMERS: POTENTIAL NANOCARRIERS IN PHOTODYNAMIC THERAPY. FORMATION OF THE DENDRIMER-PHOTOSENSITIZER COMPLEXES AND THEIR IN VITRO ACTIVITY IN BASALL CELL CARCINOMA CELL LINES.

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Dendrimers are a very diverse group of polymers and this variety opens up many potential applications of these nanoparticles. The main advantage of dendrimers is in their three layered architecture. They consist of a dendrimer core, dendrons and many terminal groups. Thus, the strictly controlled chemical composition of a dendrimer determines the size, shape and the charge of the dendritic molecule. These features in turn have critical meaning for the potential application in the biomedical field. Dendrimers are being investigated, among others, as potential carriers of anticancer drugs, imaging agents, as antiviral therapeutic agents and as sensors. Hydrophilic and hydrophobic properties make them suitable agents for enhancing the solubility of different substances.
Limitations and side effects are connected to conventional chemotherapy and improved therapies are needed. However certain limitations are also connected to less invasive anticancer therapies such as photodynamic therapy. The efficiency of photodynamic therapy is limited mainly due to low selectivity, unfarorable biodistribution of photosensitizers, long-lasting skin sensitivity to light. Similarly, as in the case of chemotherapeutics, here, the use of nanocarriers may overcome the limitations mentioned above.
The general aim of our study is to check whether phosphorus dendrimers are suitable as carriers of photosensitizers. Firstly, we chose two phosphorus dendrimers for the study: a cationic phosphorus dendrimer of the third generation possessing 48 ammonium groups on the surface (1cat) and an anionic phosphorus dendrimer of the second generation bearing 24 carboxyl terminal groups (1an) (Figure 1). We chose two photosensitizers: rose bengal and methylene blue.

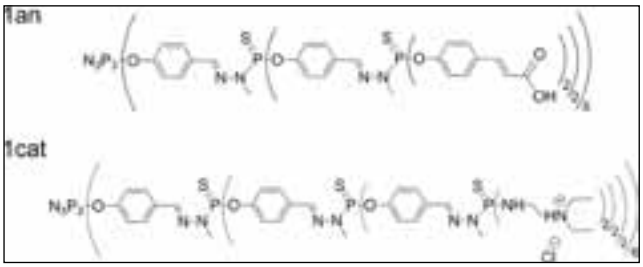

Fig. 1: Chemical structure of anionic phosphorus dendrimer of the second generation (1an) and cationic phosphorus dendrimer of the

third generation (1cat).
The first part of the research employed FTIR spectroscopy to study potential interaction between the dendrimers and photosensitizers. Obtained results showed that cationic dendrimer bound both photosensitizers and the anionic dendrimer interacted only with methylene blue. On the basis of the dissociation contants values, we chose only two complexes for further investigation. Spectroscopic analysis of the dendrimers' influence on the methylene blue absorption and spectrofluorimetric analysis of rose bengal allowed to determine the molar ratios of dendrimer-photosensitizers complexes. The stoichiometry of the rose bengal-cationic dendrimer complex was estimated to be 7:1 . In the case of methylene blue-anionic dendrimer complex the stiochiometry was 9:1. Figure 2 shows the changes in the absorption of the methylene blue and changes in fluorescence of rose bengal in the presence of anionic and cationic dendrimer, respectively.

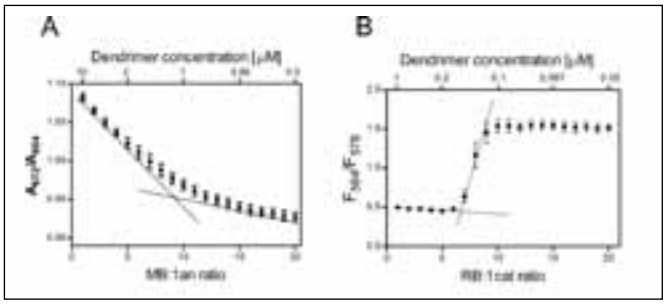

Fig.2: A - Changes in methylene blue (MB) absorption ratios A672/A664 as a function of MB:anionic dendrimer (1an) molar ratio; B - Changes in rose bengal (RB) fluorescence ratios F564/F578 as a function of RB:cationic dendrimer (1cat) molar ratio.

The determination of the stoichiometry was neccecary to find the optimal molar ratios for in vitro studies. In the case of methylene blue-anionic dendrimer complex we chose 5:1 molar ratio. Here, we report the comparison of the photodynamic action of methylene blue and methylene blue-anionic dendrimer complex. Both, free photosensitizer and the complex were studied after the photoirradiation and in the dark. The light source used for the experiments was the Q-light Pro Unit lamp equipped with a set of filters. For these experiments we chose murine basal cell carcinoma cell lines (ASZ001, BSZ and CSZ). Cytotoxicity was determined by MTT assay. Figure 3 depicts the viability of basal cell carcinoma cell lines after irradiation in the presence of free methylene blue and anionic dendrimer-methylene blue complexes.

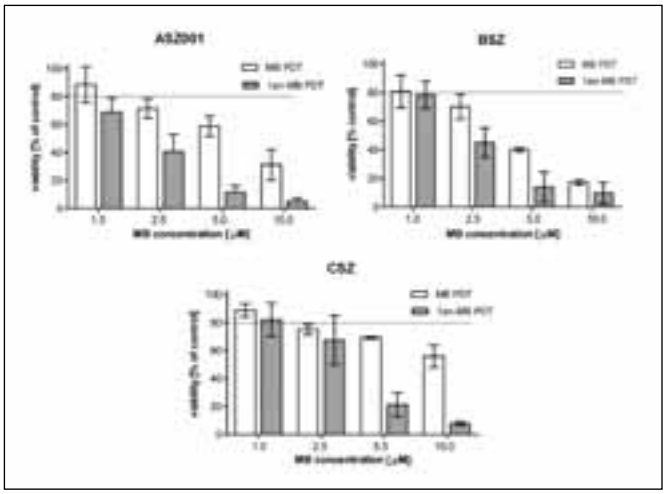

Fig. 3: The viability of murine basal cell carcinoma cell lines (ASZ001, BSZ and CSZ) after irradiation in presence of free photosensitizer (MB PDT) and the complex (1an-MB PDT). The ratio of dendrimer-methylene blue complex equals to 1:5.

The complex of methylene blue with anionic dendrimer showed higher cytotoxicity after irradiation than a free photosensitizer (Figure 3). In the case of ASZ001 and BSZ cell lines the complex was more toxic at the concentration of photosensitizer equal to 2.5 µM. There was no significant difference at this concentration in the case of CSZ cell line. In this case, the complex was significasntly more toxic at the concentration of 5 µM.

ACKNOWLEDGEMENTS:

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HEMOLYTIC AND PAN-C3 ASSAYS CAN DETECT LIPOSOMAL DRUG-INDUCED COMPLEMENT ACTIVATION WITH EQUAL EFFICACY IN THE RAT

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INTRODUCTION

The use of nanoparticles for diagnostic and therapeutic purposes greatly increases efficacy but also raises safety concerns, because the intravenous use of these agents activates the immune system. The response is a hypersensitivity reaction that is recently described as complement (C) activation-related pseudoallergy (CARPA)1. CARPA can mainly be observed following treatments with liposomes, nanoparticle-containing drugs, micellar solvents and biologicals and it is characterized by severe cardiopulmonary changes including arrhythmia, pulmonary edema, hypotension, airway occlusion, respiratory distress and cardiac arrest that in serious cases can cause cardiac (anaphylactic) shock and death2,3. Because of its potential fatal outcome the preclinical assessment the phenomenon has been recommended by the European Medicines Agency in the development of liposomal drugs4. However, the in vivo assays in which CARPAgenic properties of nanomolecules can be most reliably tested by i.v. application are not standardized. Currently the pig is the most suitable species for studying the mechanisms of CARPA and for performing safety tests, because the symptoms are quite similar to that of humans that can be elicited by low doses5. However, the model requires special skills. Therefore, the aim of the present study was to reveal the suitability of rats for testing CARPA6. In our experiments the use of the newly available Pan-C3 assay was also investigated.

MATERIALS AND METHODS

Experimental protocol: Male Wistar rats were anesthetized by thiobutabarbital (120 mg/kg i.p.), and the left femoral a. and v. and the right carotid a. were cannulated. Test substances were intravenously administered. Blood was collected before and 1-3-5-10-30 min after the treatments. Arterial blood pressure (SAP) was continuously recorded, and the heart rate (HR) was computed. The C activation was evaluated by a hemolysis assay based on antigen-sensitized sheep RBCs (CHA). Consumption of C3 was measured after conversion to SC5b-9 using a Pan-C3 assay, and thromboxane B2 (TXB2) was measured by ELISA. Blood cell counts (WBC, PLT) and other parameters were measured using an Abacus hematological analyzer (Diatron).
Blood sampling: Blood samples of 0.5 ml and 2 or 3 ml, each were collected from rats and pigs, respectively. Samples were collected into Eppendorf tubes containing 10 µl lepirudin (Refludan, 1mg/ml) before (time 0), and at different time points (1-3-5-10- 30 min) after the injection. Aliquots of 100 µl blood were drawn into tubes with

K2-EDTA for hematological analysis. Blood was centrifuged at 1500 rpm for 10 min at 4°C, and plasma was stored at -80°C until analysis. Complement hemolytic activity: The total complement hemolytic activity (CHA) was determined using a modified 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 540 nm. The amount of complement activity was determined by examining the capacity of test serum to lyse antibody coated SRBCs.

C3 levels: Consumption of C3 was measured after conversion to SC5b-9 using a Pan-C3 assay (TECO-Medical). For details see TECO-Medical booth at CLINAM.

Thromboxane B2 levels: Plasma TXB2 (the stable metabolite of plasma TXA2) levels were measured with an ELISA kit (Cayman Chemicals).

Test items: To induce CARPA, zymosan (Z, 10 mg/kg), and cobra venom factor (CVF, 12.5 IU/kg) was utilized for direct complement activation. AmBisome (A, 22 mg/kg phospholipid/kg), and the empty liposomes Ambisombo (same composition as AmBisome's vehicle), and 2K-PEG-Chol (PEG-Chol) served as liposomal complement activators.

RESULTS

The effects of liposomes on the cardiovascular (SAP, HR), hematological (WBC, PLT) and functional (CHA, TXB2) parameters are shown in Fig.1. The left panel shows the effect of high dose of AmBisome (22 mgPL/kg). Administration of this lipid vesicle leads to a gradual decrease in SAP by 40% after 5 min (A), while no change in HR (B) was found. However, significant initial leukopenia by 50% at 5 min, switching to leukocytosis by 10 min (C) could be observed. This change paralleled the thrombocytopenia by 60% after 3 to 5 min (D). This high dose gave us a reduction in CHA by 40% (E), however, plasma TXB2 rose only minimally (F). The effects of AmBisome were essentially identical to that of zymosan (10 mg/kg) communicated previously. The right panel shows the effect 10-fold higher dose (300 mgPL/kg) of PEG-Chol liposome. The reason of dose elevation is that an equivalent dose to AmBisome had no effect on any parameter. This higher dose exhibited similar, although much smaller effects than Ambisome in cases of all parameters, except that no leucocytosis could be observed. Ambisombo produced similar week effects (data not shown).

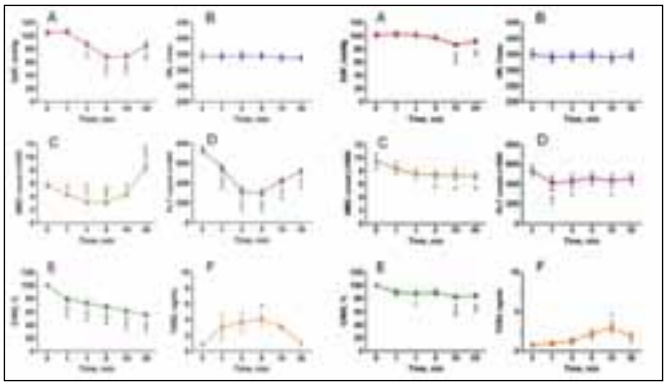

*Figure 1. Pathophysiological changes in rats injected i.v. with 22 mg PL/kg AmBisome (left panel) or 300 mg PL/kg PEG-Chol (right panel). Values shown are Mean ± SE for 8 or 3 animals, respectively. The curves were constructed from the 0, 1, 3, 5, 10 and 30 min readings of SAP and HR after injection, as well as of other parameters measured from blood samples taken at the same time points. *, **, ***: p<0.05, 0.01, 0.001 vs. the time 0 value, respectively. One-way ANOVA with repeated measures.*

Correlations between the SAP-lowering and C-activating effects of various CARPAgenic agents are shown in Fig. 2. The upper panel depicts correlations in the individual treatment groups using the SRBC (left) as well as the Pan-C3 (right) assay. These data demonstrate that agents with various physicochemical properties in a wide range of doses elicit very similar changes in both the cardiovascular and the immunological status of rats. The lower panel shows the overall correlation between the two methods. Group means of CHA and C3 consumption exhibit strong and significant correlation.

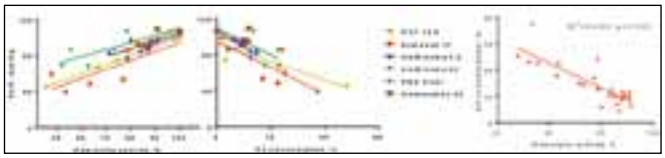

Figure 2. Correlations between the SAP-lowering and C-activating effects of CARPAgenic agents cobra venom factor (CVF; 12.5 IU/kg), zymosan (10 mg/kg), AmBisome (2.2 mg PL/kg and 22 mg PL/kg), and empty liposomes PEG-Chol (300 mgPL/kg) and Ambisombo (22 mgPL/kg) following i.v. injections (upper panel). Overall correlation between SRBC (hemolytic activity, %) and Pan-C3 (C3 consumption, %) assays (lower panel). Individual values are group means (n=6-8, each) of the respective agents at various time-points following the injection. For statistical analysis linear correlation and regression analysis was performed (Prism, Graphpad).

CONCLUSIONS

This study demonstrates the appearance of CARPA upon injections of various CARPAgenic agents, especially using liposomal nanoparticles in rats. The existence of CARPA was demonstrated by changes in cardiovascular, hematological as well as functional parameters, i.e. changes in C hemolitical activity, C3 consumption and TXB2 production. In comparison of loaded (AmBisome) and empty liposomes (PEG-Chol, Ambisombo) we found that AmBisome was stronger inducer of CARPA than its empty counterparts.

In addition, we compared two methods, determining the C hemolytical activity using the simple SRBC assay, and C3 consumption using the newly developed Pan-C3 assay to evaluate the effects of CARPAgenic agents on parameters of complement activation. Our data clearly proves the excellent applicability of the Pan-C3 assay in the rat CARPA model.

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NON BIOLOGICAL COMPLEX DRUGS: ARE THERE DIFFERENCES BETWEEN ORIGINATORS AND SIMILARS?

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INTRODUCTION

With approximately 1.6 billion of new cases annually, anemia represents the most important nutritional disease in the world [1]. Abso-

lute iron-deficiency anemia (IDA) may be caused either by a significant decrease in iron absorption or by severe blood loss [2]. In order to treat iron deficiency, either oral administration of iron salts or IV administration of iron carbohydrate drugs are applied [3]. Despite the easy access to oral drugs, limitations for this treatment due to unwanted gastrointestinal side effects in a high number of patients are reported [4]. An interesting alternative is proposed with IV iron carbohydrate drugs, which allow an efficient administration of large amounts of iron for a fast treatment of IDA [5].

IV iron carbohydrate drugs belong to a new pharmaceutical class of drugs, called Non-Biological Complex Drugs (NBCDs) [6]. NBCDs are synthetic polymeric complexes of high molecular weight where the entire complex represents the active pharmaceutical ingredient (API). Moreover, NBCDs are produced by a well-defined manufacturing process, which strongly influences the final properties of the drugs [7].

Iron sucrose (Venofer®), introduced in the market in the 1950's, represents the one of the products with the longest market experience for the IV treatment of iron deficiency. Chemically, it is a colloidal suspension composed of polynuclear iron(III)-hydroxide cores, which are surrounded by a sucrose shell [8]. During the last decades, different intended copies of Venofer® were introduced on various markets; however, clinical and non-clinical evidence suggests that the treatment with these similars may not lead to the same results as with the originator drug [9, 10]. Both FDA and EMA agree that there is an urgent need to define a regulatory approach for iron sucrose similars (ISSs) [11].

The aim of this work is to present a full physicochemical characterization of both the originator product and ISSs in order to lay the basis for an assessment of the safety and exchangeability of the different preparations.

MATERIALS AND METHODS

Prior to this comparative study, all protocols were validated by testing 13 different batches of Venofer®, obtaining always reproducible results (n=3). Successively, Venofer® was compared to seven different ISS (A-G). Different batches of the same ISS were analyzed, when samples were available (A and B). Dynamic light scattering (DLS) and transmission electron microscopy (TEM) were used to determine size and shape of the colloidal products. The molecular weight distribution of the complexes was investigated via GPC analysis, the charge determined by zeta potential measurements. The colorimetric method using chromazurol B [12] was used to quantify the amount of labile iron, and in vitro dissolution kinetics assays were performed [13].

RESULTS AND DISCUSSION

DLS analysis revealed that Venofer® is composed of particles with a size below 10 nm, with a monomodal distribution in Number. ISSs are nanoparticles of similar size, however, two ISSs showed a bimodal distribution in Number, as reported in Figure 1.

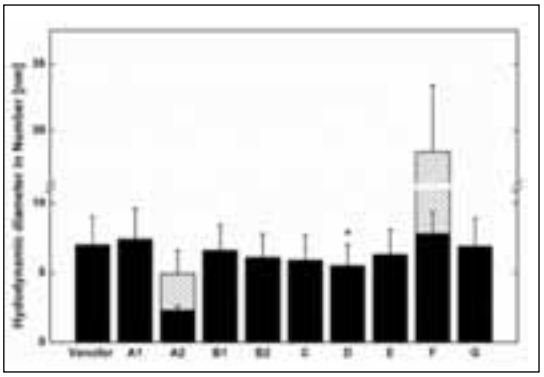

*Fig. 1: Hydrodynamic diameter in Number for different ISSs and Venofer®. All samples showed monomodal dispersion except for sample A2 and F. (n=3, * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001 and **** P ≤ 0.0001)*

Sample A2 showed a bimodal distribution in Number with two population of smaller size compared to Venofer®. Differences in the composition of the two batches for sample A were revealed. Sample F showed a bimodal distribution in Number, with bigger particles compared to the other preparations. Moreover, significant statistic differences were evaluated between Venofer® and sample D.

The shape of the different complexes was furthermore investigated by TEM. The images obtained confirmed the size and spherical shape of all iron sucrose complexes. The molecular weight distribution was investigated via GPC analysis, obtaining a weight average molecular weight (Mw) between 34 and 60 kDa for originator and ISSs, in agreement with the USP monograph for iron sucrose injection. The net charge was shown to be approximately −45mV for all complexes investigated.

The amount of labile iron was determined via a colorimetric method with chromazurol B, as reported in the Figure 2:

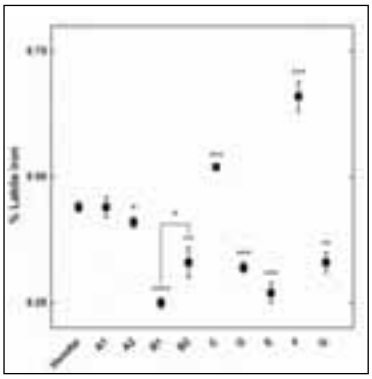

*Fig. 2: Relative percentage of labile iron for different ISSs compared to Venofer®. Significant differences were evaluated for all the samples, except sample A1.(n=3, * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001 and **** P ≤ 0.0001)*

Venofer® showed a relative amount of labile iron of 0.44 ± 0.01% whereas the ISSs showed a range of labile iron between 0.25 ± 0.01% and 0.66 ± 0.03%. Significant differences between originator and ISSs were evaluated for all the samples except for the sample A1. Moreover, a significant difference in the amount of labile iron for the two batches of samples B was identified.

Finally, the in vitro dissolution kinetics of both Venofer® and ISSs were examined, obtaining T75 values of 13 mins for the originator and 20 mins for the ISSs.

CONCLUSIONS

These first results may be helpful to clarify the reasons behind the discrepancy in clinical efficacy for Venofer® and ISSs. Variations in physicochemical properties for two different batches of the same product were found for samples A and B by different assays. Differences in the manufacturing procedure of iron sucrose drugs may explain the differences in resulting biodisposition and clinical activity.

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APOFERRITIN MODIFIED WITH ANTI-PSMA ANTIBODIES FOR TARGETED DELIVERY TO PROSTATE CANCER CELLS

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Apoferritin (APO) is a hollow protein cage of 450-475 kDa with internal diameter of about 7-8 nm that forms a basis for ferritin, protein responsible for a storage and transfer of ferric ions in many organisms. Due to its self-assembly activity, the formed cavities have uniform size, ensuring high reproducibility of cargo encapsulation [1].

The protocol for the encapsulation of drug molecules is very simple, based on APO responsiveness to the surrounding proton concentration [2, 3], in which it can reversibly dissociate and associate till pH bellow 1.0 when apoferritin denatures. The reversible disassociation begins in pH bellow 3.4 [4], therefore the encapsulated drug is not released until the apoferritin get to the late endosomes of cancer cells.

The passive targeting to the vicinity of tumour is enabled due to apoferritin size (12-13 nm) by the Enhanced Permeability and Retention (EPR) effect. Apoferritin can enter the cells through receptor-mediated endocytosis, as well as clathrin-mediated endocytosis by its specific receptors, transferrin receptor 1 and SCARA5. These, however, are found in the membrane of not only cancer, but also healthy cells [5]. Therefore, modification of apoferritin surface with targeting moieties should be employed. As a suitable targeting moiety can serve antibodies, which enable a wide variety of target cells. However, when using the native antibodies, they can be immunogenic by binding of their Fc domain to the Fc receptors on healthy cells. To eliminate this problem, it is possible to use antibody fragments, containing only the Fab region, or inactivate the Fc region by its binding to a peptide protein A derivative which can be attached to the apoferritin surface.

We used the HWRGWVC (HWR) heptapeptide, with N-terminus derived from protein A and able to bind the Fc region of antibodies and the C-terminus made of cysteine with high affinity to gold. The surface of apoferritin was modified with gold, to which HWR peptide and later anti-PSMA targeting antibody were bound. The employed antibody has high affinity towards PSMA antigen, found in the membranes of some prostate cancer cells, including LNCaP cell line [6]. Fig. 1A shows the design of suggested apoferritin nanocarrier. The anticancer drug doxorubicin (DOX) was encapsulated within APO to make use of its fluorescent properties for the detection of nanocarrier binding.

Next, we studied the influence of each component on nanocarrier ability to selectively target and bind to the target molecule. The experiment was performed using ELISA-like method with the surface of microtiter plate coated with antigen to the antibodies on the nanocarrier surface. After application of nanocarrier to the well and incubation for 2 h; the excess nanocarrier molecules were removed and the bound nanocarrier was acidified to release the DOX and 2× enhance its fluorescent signal (Fig. 1B-E). 5× higher amount of bound nanocarrier was achieved when using nanocarrier modified with all of the components in comparison with unmodified nanocarrier. The presence of each individual component was verified using gel electrophoresis separation with coomassie blue staining and the gold measurement in apoferritin bands was performed using the laser ablation with inductively coupled plasma mass spectrometry. The presence of all of the used components was found to significantly influence the nanocarrier geometry and thus its ability to bind to the target molecule.

The toxicity of targeted nanocarrier for healthy (HUVEC cell line) and prostate cancer cells (LNCaP cell line) was studied (Fig. F-G) and compared with free doxorubicin and non-targeted nanocarrier. According to the calculated values of IC_{50}, the targeted nanocarrier was significantly (20% at $p < 0.05$) more toxic for prostate cancer cells (IC_{50} 0.04) than both the free doxorubicin (IC_{50} 0.05) and non-targeted nanocarrier (IC_{50} 0.05). For healthy cells, the toxicity of both targeted and non-targeted nanocarriers was similar (IC_{50} 0.40 for targeted and 0.49 for non-targeted nanocarrier) to that of free doxorubicin (IC_{50} 0.39) and almost 9× lower than the toxicity for prostate cancer cells. However, after administration to the patient's body, the nanocarrier can make use of the EPR effect, thus its toxicity to healthy cells will be even more decreased.

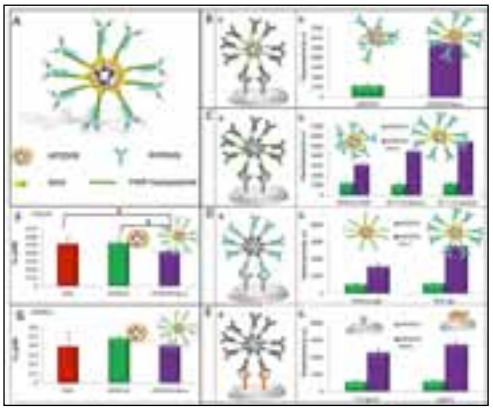

*Fig. 1: The influence of each component on nanocarrier geometry and toxicity of nanocarrier for cancer prostate and healthy cells. A) The design of apoferritin nanocarrier modified with antibodies via specific peptide linker derived from protein A. B-E) The influence of each component on nanocarrier geometry evaluated by ELISA-like method: a) The experimental design with highlighted respective part of nanocarrier and b) The influence of the respective component on the nanocarrier surface on the amount of bound nanocarrier detected by fluorescence (λ_{ex}=480 nm, λ_{em}=600 nm) with the respective part B) gold nanoparticles; C) HWR peptide; D) human IgG antibody; E)goat anti-human IgG antibody (antigen). F) The toxicity of suggested nanocarrier for cancer prostate cells (LNCaP cell line). * Significantly different at $p < 0.05$. G) The toxicity of suggested nanocarrier for healthy cells (HUVEC cell line).*

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INJECTABLE NANOCOMPOSITE FOR EMBOLIZATION AND MAGNETICALLY INDUCED LOCAL HYPERTHERMIA

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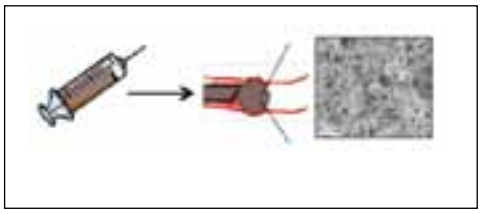
INTRODUCTION

Embolization of tumor-feeding blood vessels in combination with nanoparticle-mediated local hyperthermia could provide a successful combination for a therapeutic approach for solid tumors such as prostate cancer. Representing the most commonly diagnosed cancer in men (1), the significant side effects of radical prostatectomy or the possibility of active surveillance as common strategies for locally confined prostate carcinoma, require alternative treatments to fill this gap. For this purpose, injection of a liquid formulation based on superparamagnetic iron oxide nanoparticles (SPIONs), solidifying as a semisolid implant in-situ in the blood vessels as source for application of induced, local hyperthermia, offers a minimally invasive treatment option without major side effects.



We present a formulation ensuring appropriate injectability of the liquid suspension and safety of the solidifying implant material. Different compositions were evaluated in-vitro regarding their heating efficacy, in order to reach the threshold temperature of 42°C, known to induce cell apoptosis of the surrounding tumor cells (2).

A polymer consisting of water-insoluble mono-/ tri-iodo benzylether polyvinylalcohol (MTIB-PVA) was chosen (3). For adequate embolization performances, the polymer was solubilized in DMSO, an excipient commonly used in clinics for this purpose. The radiopaque iodine moieties allow real-time monitoring of implant distribution in-vivo using X-ray imaging. The incorporated SPIONs dissipate cytotoxic heat, when subjected to an external alternating magnetic field, allowing on-demand, repeatable hyperthermia sessions

EXPERIMENTAL METHODS

The liquid formulations were prepared by adding SPIONs embedded in mesoporous silica (silica-SPION-beads) to a solution of MTIB-PVA in DMSO, intensive vortexing and exposure to ultrasound.

Rheological measurements were performed using a cone-plate configuration at 25°C in order to determine the viscosity and the rheological behavior of the liquid formulations.

WST-1 cell proliferation assay was performed to evaluate the safety of the polymer MTIB-PVA on PC3 cells (human prostate cancer cells) and fibroblasts (healthy cells). Polymer dispersed in a fine suspension was tested to exclude a potential toxicity in the unlikely event of leakage of the polymer during the injection process. The MTIB-PVA were thoroughly washed to remove any DMSO and incubated for 48h, before WST-1 cell viability assay was performed following the manufacture's protocol. Cell viability was determined in comparison to untreated cells.

The heating efficacy was assessed by measuring the temperature increase of the implants' surface using optical fiber probes when exposed to an external alternating magnetic field applying magnetic field strengths ranging from 3 to 12 mT and a frequency of 300 kHz. During the measurements, the implant was placed in a PMMA chamber and surrounded by circulating, thermostated water mimicking blood flow cooling.

RESULTS AND DISCUSSION

In order to keep the viscosity as low as possible by maintaining homogeneous solidification, the MTIB-PVA concentration was set at 18% (data not shown). As expected and shown in Fig. 1, increasing the concentration of silica-SPION-beads resulted in an increase in dynamic viscosity. The formulations revealed Newtonian behavior for SPION concentrations up to 20%.

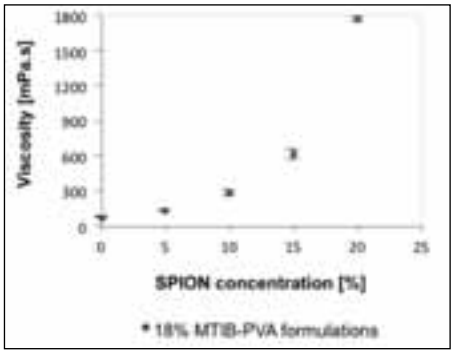


Fig. 1: Viscosity-concentration relationship of formulations containing 18% MTIB-PVA and varying concentrations of SPIONs (n=3).

No significant toxicity was detected for MTIB-PVA (Fig. 2). In addition, preliminary testing of pure polymer implants and silica-SPION-beads-loaded implants did not show any toxicity towards fibroblasts or PC3, suggesting safety of the polymer and the silica-SPION-beads.

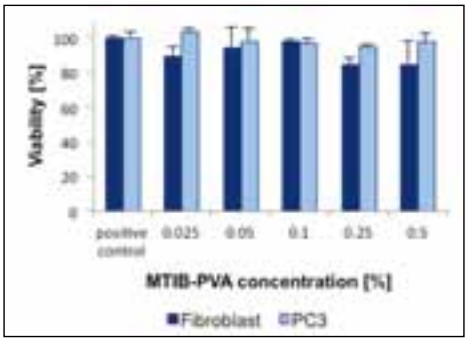


Fig. 2: Cell viability measured by WST-1 assay after an exposure time of 48h to MTIB-PVA suspensions in cell medium in comparison to untreated cells (n=3).

Figure 3 shows the temperature increase of the most promising implant composition with incorporated silica-SPION-beads, aiming a minimal temperature increase of 5°C to reach 42°C. Lower perfusion, such as found in a necrotic tumor core, might lead to higher temperature increase in-vivo.

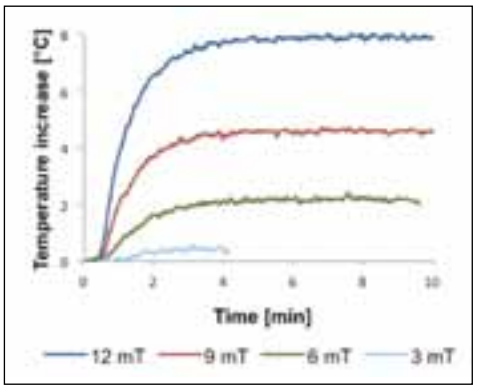


Fig. 3: Temperature elevation of an implant with concentrations of 18% MTIB-PVA and 20% SPIONs, when subjected to an alternating magnetic field (300 kHz, 3, 6, 9, 12 mT).

CONCLUSION

The proposed formulations showed an adequate injectability, forming an implant upon contact with aqueous solutions and the ability to block blood vessels in-vitro, revealing no polymer-related toxic effects in-vitro.
Mimicking blood flow-mediated tissue cooling and upon exposure to an alternating magnetic field (300kHz, 12 mT), the incorporation of 20% of SPIONs resulted in a temperature elevation of up to 8°C , which is aimed to induce apoptosis of surrounding cancerous cells. Consequently, the in-situ forming nanocomposite holds promise for local tumor thermotherapy.

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TOWARDS SAFE AND EFFECTIVE ANTIAGING SKIN TREATMENTS BASED ON BIODEGRADABLE NANOCAPSULES

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Potential use of nanocapsules for improved efficacy/toxicity profile of drugs has been widely proven. Indeed, numerous commercial and clinical formulations (in trials) contain liposomes, nanocapsules and micelles, to name a few. Especially in dermatological applications, relevant features from these nanoparticles are their higher skin penetration, slow drug release and improved biodistribution.1 Polyester nanocapsules are at the front line of attention because of their attractive safety profile. Since their degradation products are easily metabolized and eliminated, systemic toxicity associated with these nanocapsules for drug delivery is low.2
In this work, we present the development and comparative study of different nanoformulations for topical application with dermatological relevance, based on the use of polyester biodegradable nanocapsules (PCL, PLA and PLGA), containing active ingredients or drugs already used in formulations for topical treatments which present problems of solubility, stability, efficacy and/or secondary effects. The encapsulation of active ingredients has shown to improve their physicochemical properties and is expected to provide superior dermatological properties. An exhaustive characterization of the nanocapsules obtained by nanoprecipitation revealed mainly monodisperse samples with a small size (150-200 nm) and comparable between all the polymers used (Figure 1A). Besides, the encapsulation yield of the active ingredients reached values >80%, and depended essentially on the nature of those molecules. In addition, studies on the release kinetics of the payload indicated that, in all the cases studied, by one third of the active ingredient released in the first 3-4 hours and release was almost complete after 24 hours.
In vitro studies have also been performed in human dermal fibroblasts to demonstrate the biocompatibility of the mentioned nanocapsules, improved vehiculization and the detoxification of the active ingredient. It has been proven that, for active ingredients such as indomethacin, practically insoluble in water and widely used in antiinflammatory topical treatments, cytotoxicity is always lower when the drug is administered nanoencapsulated (see Figure 1B).
The presented methodology shows a great potential and feasibility for promising applications in skin treatments, such as antiaging or inflammatory diseases. In combination with the expertise of the Institute of Dermathology Umbert, specialists in personalized medicine, these formulations can be potentially applied in the near future in personalized topical treatments.

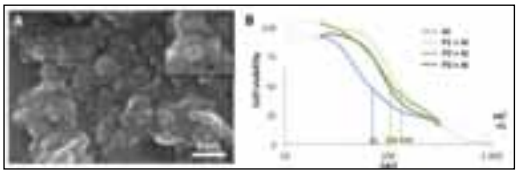

Figure 1. A) Representative scanning electron microscopy image from the synthesized nanocapsules entrapping an active ingredient; B) Toxicity profile from nanocapsules composed of different polymeric materials (P1, P2, P3) and containing an active ingredient.

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INTRACELLULAR SPION QUANTIFICATION BY FLOW CYTOMETRY IN COMPARISON WITH SPECTROSCOPIC METHODS

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BACKGROUND

Due to their special physicochemical properties, iron nanoparticles offer new promising possibilities for biomedical applications. For bench to bedside translation of SPIONs, safety issues have to be comprehensively clarified. To understand concentration-dependent nanoparticle-mediated toxicity, the exact quantification of intracellular SPIONs by reliable methods is of great importance.

RESULTS

In the present study, we compared three different SPION quantification methods (ultraviolet spectrophotometry (UVS), magnetic particle spectroscopy (MPS), atomic adsorption spectroscopy (AAS)) and discussed the shortcomings and advantages of each method. Moreover, we used those results to evaluate the possibility to use flow cytometric technique to determine the cellular SPION content. For this purpose, we correlated the side scatter (SSc) data received from flow cytometry with the actual cellular SPION amount.

We showed that flow cytometry provides a rapid and reliable method to assess the cellular SPION content (Table 1).

Table 1 Detection threshold for the UVS, MPS and ASS techniques with SPION-containing cell lysates indicated as µgFe/ml cell lysate. The detection threshold for SSc analysis is indicated in percentage compared to SSc data of untreated cells. The thresholds for UVS, MPS and SSc are dependent on the SPION nature, whereas ASS, a method quantifying elementary iron, is not. The determinations of the detection threshold were achieved using the 3σ criteria.

Our data also demonstrate that internalization of iron oxide nanoparticles in human umbilical vein endothelial cells is strongly dependent to the SPION type and results in a dose-dependent increase of toxicity (Fig. 1).

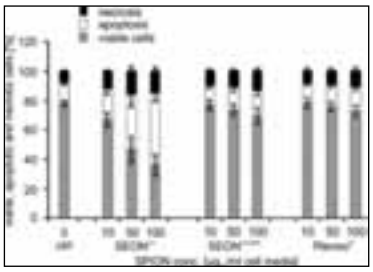

Figure 1 Association of cellular uptake with toxicity of SPIONs. HUVECs were incubated for 48 h with different amounts of SPIONs and cytotoxicity was analyzed by flow cytometry. Cell viability determined by annexin V/propidium iodide staining. Percentages of necrotic (PI+), apoptotic (Ax+, PI-) and viable cells (Ax-, PI-) are shown. Data are presented as mean standard error; n=3 with sample triplicates. The results were normalized untreated control cells, set to 100%

Thus, treatment with lauric acid (LA) coated SPIONs (SEONLA) resulted in a significant increase in the intensity of SSc and toxicity, whereas SEONLA with an additional protein corona formed by bovine serum albumin (SEONLA-BSA) and commercially available Rienso® particles showed only a minimal increase in both SSc intensity and cellular toxicity. The increase of the SSc was in accordance with the measurements of the SPION content by the AAS reference method.

SUMMARY

Our data revealed that flow cytometry analysis can be used for the estimation of uptake of SPIONs by mammalian cells and provides a fast tool for scientists to evaluate the safety of nanoparticle products.

ACKNOWLEDGMENTS

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INVESTIGATION REGARDING DENTAL ENAMEL ADULTERING BY BLACK SPOTS AND CARIOGENIC PROCESS. AN ADVANCED MECHANISM BASED ON DMSO CARRIER PROPERTIES.

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RESUME (PAPER AND POSTER ABSTRACT):

The environmental diversity of the oral cavity promotes different microbial communities, such as supragingival plaque, subgingival plaque and tongue coating. The properties of the environment determine which microorganisms can occupy a site, while the metabolic activities of those microbial communities subsequently modify the properties of the environment.

Enamel formation is structured by enamel prism which is formed by crystal apatite. Most apatite crystal founded is $Ca_{10}(PO_4)_6(OH)_2$ or known as hydroxyapatite (HA). HA arranged the enamel prism whose length is 120 to 160 nm and width is 25 nm on the narrow side and 40 nm on the wide side. Each HA crystal is arranged by apatite cell unit in lattice arrangement of P and Ca and also the lattice arrangement of O and H. Every cell unit of apatite suggested Ca ion position on hexagonal corner to form calcium column. Ca ion position is perpendicular to c-axis. Ca ion position is also on cell central canal which formed Ca triangles. Space between Ca columns were placed by two ions PO_4 on the hexagonal side. (OH) arrangement is vulnerable for special conditions.

Conversely, in subgingival sites, asaccharolytic microorganisms metabolize nitrogenous compounds derived from gingival crevicular fluid (GCF) and create a neutral pH and anaerobic environment abundant in short-chain fatty acids and ammonia. Other microorganisms are developing sulphur products. All those metabolic compounds combined with buffering saliva turned to an aggressive environment for dental enamel. This can be considered the first step, when mixed bacteria exposed to fermentable carbohydrates produce an acid environment, acting on dental enamel to dissolve hydroxyapatite and release free calcium and phosphates (vulnerability of OH arrangement). The second step of the process is the penetration at nano scale of the dental enamel crystal lattice, a model emphasized by us for „black stain" generation process (carious generation process as well). (Fig. 1)

We consider that for pointed places on dental enamel surface, occurs conditions for the following chemical reactions, in order of DMSO indirect obtaining process:

- $DMS + NO_2 \rightarrow DMSO + NO$;
- $(CH_3)_2S + H_2O_2 \rightarrow (CH_3)_2SO + H_2O$;
- $DMS + IO = DMSO + I$

For small quantities, the occurrence of DMSO and its metabolites, dimethyl sulfide and methyl sulfone ($DMSO_2$), have been widely reported in a variety of foods (corn, beans, tomatoes, cucumbers, onions, apples, milk, beer, coffee, tea).

We conclude that DMSO might be present in the oral biotop and conducting to a possible carrier process.

The model is based on DMSO carrier action and it will be proposed hereinafter as GCM (Gatin Ciobanu Mechanism). The nanoscale penetration of DMSO molecule is result of its small dimension (diameter about 30 ÷ 35 nm). The key role in the GCM mechanism is mainly due to the following DMSO properties:

1) DMSO, as solvent is miscible with reported aniline (other metabolic compounds as well) and is able to carry (to penetrate through the dental enamel because of its molecule nano dimensions) and to store those compounds into the organic matrix;

2 DMSO, due to calcium and phosphate stage of metastable equilibrium, is inducing HA formation. The mineralization is initiated

inside the matrix vesicles [Li et al., 2008].
The result is the adulterated dental enamel as "black stain" or discoloration spots (Fig. 1b)

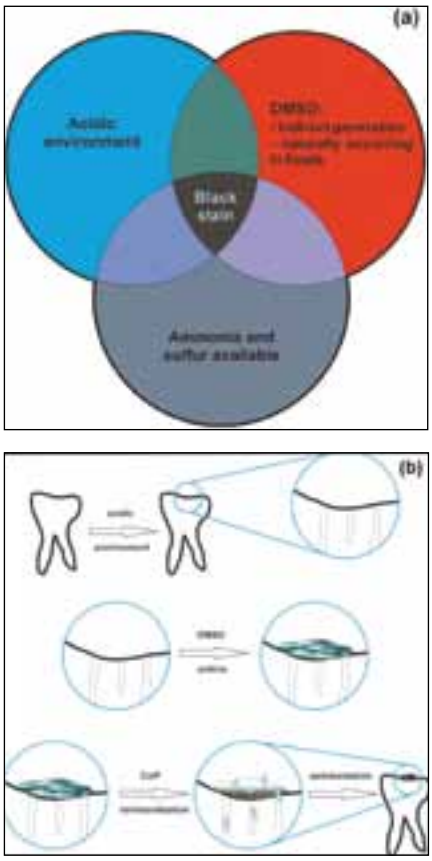

Fig. 1: Black stain, generation process (proposed mechanism GCM). Details: (a) oral environmental conditions, (b) generation steps process

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NANOMEDICINAL PRODUCTS: IMMUNOTOXIC EFFECTS AND CURRENT REGULATORY FRAMEWORK

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Nanomaterials (NM) are considered attractive biomedical and pharmaceutical tools due to their unique physical, chemical and biological properties. A major application area is drug delivery, including cancer treatment. Depending on the definition being applied, varying numbers of nanomedicinal products are on the market or in the premarket development phase, most often based on liposomal products, protein nanoparticles and polymer conjugates.
Nanomedicinal products can be designed to target specific tissues e.g. tumors. The toxicokinetic profile of nanoparticles is quite different from that of dissolved chemicals. In all cases, nanodrugs will probably reach the immune system. It has been shown that most NM end up in organs of the mononuclear phagocytic system, notably liver and spleen, after intravenous administration. Adverse immune effects, including allergy, hypersensitivity and immunosuppression, were reported after conventional drug as well as after nanodrug administration. Several examples are illustrated in Table 1 below. Interactions of nanomedicinal products with the immune system may therefore constitute important side effects.
Currently, there are no regulatory documents specifically dedicated to evaluate the immunotoxicity of NM or nanomedicinal products. Assessment of immunotoxicity of nanodrugs is performed based on existing guidelines for conventional drugs, including the ICH S8 guideline on immunotoxicity studies for human pharmaceuticals. Due to the differences between conventional drugs and nanoformulations, it is not clear whether the currently used set of testing methods provides sufficient information for an adequate evaluation of potential immunotoxic effects of nanomedicinal products. In the Table 1 is illustrated whether the testing methods included in the ICH S8 guideline as recommendation to detect immunotoxicity are expected to detect the immunotoxic effects reported after exposure to NM or nanomedicinal products.

Nanoparticles	ICH S8 guideline	Nanomedicines	ICH S8 guideline
Immunostimulation			
Inflammasome activation (e.g. CrO_3 and SiO_2 nanoparticles)	+ Included in vivo studies - Not included in vitro studies	Inflammation (e.g. gold nanoparticles)	+ Included in vivo studies - Not included in vitro studies
Inflammation after inhalation in vivo (e.g. TiO_2 nanoparticles)	+ Included in vivo studies		
Hypersensitivity			
T helper 1 cell-mediated immunity suppression (e.g. iron oxide nanoparticles)	+ Included in vivo studies - Not included in vitro studies	CARPA by liposomes, lipid-based nanoparticles, micelles	- Not included in vivo studies - Not included in vitro studies
		Anaphylactic reactions by liposomes	- Not included in vivo studies - Not included in vitro studies
Immunosuppression			
Reduction of IgG levels in vivo (e.g. Ag nanoparticles)	+ Included in vivo studies - Not included in vitro studies	Myelosuppression	- Not included in vivo studies - Not included in vitro studies
		Impairment of bacterial clearance by liposomes	+ Included in vivo studies - Not included in vitro studies

Table 1. Examples of immunotoxic effects of nanoparticles and immunotoxic effects induced specifically by nanomedicinal products. It is also presented whether the testing methods included in the ICH S8 guideline as recommendation to detect immunotoxicity are expected to detect the immunotoxic effects reported after exposure to NM or nanomedicinal products.

It is becoming increasingly clear that relying on only one parameter is not enough to assess immunotoxicity of nanodrugs. Immunotoxicity testing would be likely more accurately evaluated by a strategy using data generated by various assays. Certain immunotoxic effects, like complement activation, cytokine production and autophagy illustrate some of the adverse effects not readily detected by using current testing practices. One of the future actions in the context of nanomedicinal products should be directed to assessing the function of the immune system, qualify-

ing of tests already existing for different NM types, testing these approaches in an inter-laboratory manner and eventually standardize in vitro assays to be found sufficiently predictive of the in vivo situation.
The aim of our study is to compare the present status of the regulatory framework with regard to assessing the risk of adverse effects of medicinal products on the immune system with the accumulating knowledge and evolving state of the art on nanomedicinal products deriving from research and development, in order to identify potential gaps. Building on this, follow-up research will be performed aiming to fill these gaps.

SHEETS OF NANOSTRUCTURED BATIO$_3$ AS NEW MATERIAL FOR IMPLANTABLE DEVICES

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An important scientific goal in bioengineering research is overcoming or delaying the onset of the tissue response and consequent encapsulation of implanted biomaterial. Nanotechnology has the potential to address this problem by virtue of the ability of some nanomaterials to modulate interactions with cells, thereby inducing specific biological responses to implanted foreign materials. To this effect in the present study, we have characterised the growth of fibroblasts on nano-structured sheets constituted by BaTiO3, a material extensively used in biomedical applications. We found that sheets of vertically aligned BaTiO$_3$ nanotubes inhibit cell cycle progression - without impairing cell viability - of NIH-3T3 fibroblast cells. We postulate that the 3D organization of the material surface acts by increasing the availability of adhesion sites, promoting cell attachment and inhibition of cell proliferation. This finding could be of relevance for biomedical applications designed to prevent or minimize fibrous encasement by uncontrolled proliferation of fibroblastic cells with loss of material-tissue interface underpinning long-term function of implants.
The aim of this study is to explore the biological effects of sheets of BaTiO$_3$ nanotubes as a novel implantable material able to drive specific cellular responses and, more specifically, to gain control on processes that naturally occur when foreign materials are implanted in the human body. In particular this study targets fibroblasts, which are stimulated to proliferate and to deposit the connective tissue during a process of fibrosis [1], and explores potential mechanisms that could impair this phenomenon.
Here we demonstrate that AAO membranes filled with VANTs of BaTiO$_3$ clearly induce a specific biological response. Specifically, we observed in the embryonic fibroblast NIH-3T3 cell line that the nano-structured material influences the cell cycle by decreasing the rate of cell proliferation, without affecting cell viability.

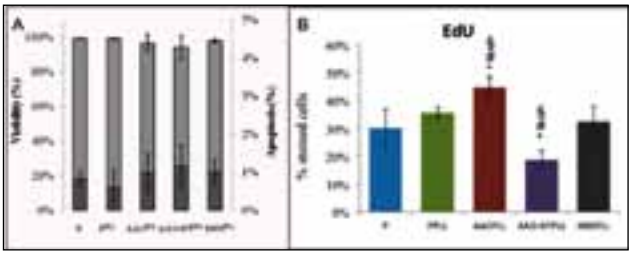

*Fig. 1: A) NIH-3T3 viability and apoptosis on different substrates. B) Cell proliferation on different substrates. P = plastic, PPLL = poly-L-lysin coated plastic, AAOPLL = poly-L-lysine coated AAO membranes, AAO-NTPLL = poly-L-lysine coated VANTs, NNSPLL = poly-L-lysine coated non-nanostructured BaTiO$_3$. * = different from P, # = different from PPLL, § = different from NNS.*

Furthermore, we observed that the attachment of cells to the VANTs substrate was stronger than in other groups. This effect cannot be attributed to any cytotoxic effect or impaired cell spreading, instead, it seems to depend on the strong improvement of mechanical adhesion of cells to the substrate, promoted exclusively by the VANTs. In fact, a strong cell attachment to the surface could be related to a decrease in cell proliferation [2].

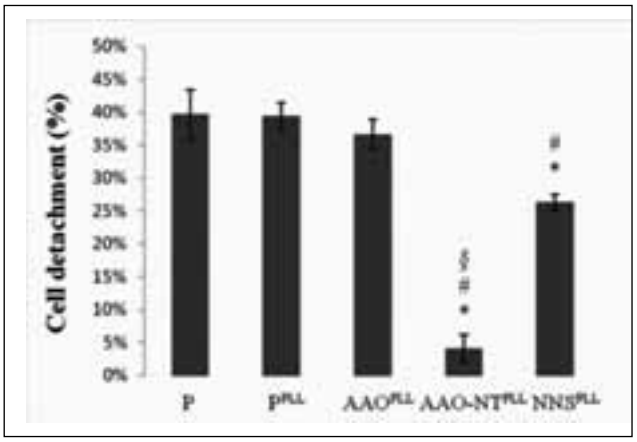

*Fig. 2: Percentage of cell detachment from different substrates after treatment with 50 mM EDTA and ultrasonication. P = plastic, PPLL = poly-L-lysin coated plastic, AAOPLL = poly-L-lysine coated AAO membranes, AAO-NTPLL = poly-L-lysine coated VANTs, NNSPLL = poly-L-lysine coated non-nanostructured BaTiO$_3$. * = different from P, # = different from PPLL, § = different from NNS.*

Currently we are studying the mechanisms responsible of such biological effects. In particular we are studying on the expression FAK protein, which is involved in molecular pathways related to substrate adhesion of cells and proliferation.
Because of the extensive use of BaTiO$_3$ in tissue engineering, our findings could represent a strategy to be explored for improvement in the overall performance of such implants by abrogation of the fibrous encapsulation. In particular, our work suggests that surface nano-structuration of BaTiO$_3$ could be investigated as a strategy to reduce the fibrosis which naturally occurs around implanted materials due to the uncontrolled proliferation of fibroblast cells around the implantation site.

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DESIGN OF NANOPARTICLES FOR THE TREATMENT OF BRAIN TUMORS

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Glioblastoma (GBM) is the most common primary brain neoplasm with an overall 5-year survival rate of less than 3,3% and a median survival time of approximately 14,6 months [1]. GBM is responsible of above 11000 patient deaths per year [2]. The present treatment for GBM consists of surgery, followed by radiotherapy and chemotherapy. This therapeutic strategy is associated with considerable toxicity and limited efficacy. Infiltrating cells that are not removed at the time of surgery disperse into unresectable brain regions, far beyond the margin of the surgery and radiation field, where they are securely protected from chemicals by the intact Blood Brain Barrier (BBB). Here they proliferate and recruit new vessels, leading to recurrence, that is the main cause of the poor prognosis associated with these tumors. Thus, to control GBM recurrence and realize long-term survival, it is essential to develop carrier able to penetrate the BBB and displaying cytotoxic anti-tumor drugs.
Considering that GBM cells express low density lipoprotein receptor (LDL-R) [3] and starting from previous data obtained by our group, showing that nanoliposomes (NLs) covalently coupled with a modified ApoE-derived peptide (mApoE) recognized by LDL-R can be successfully used to enhance the BBB penetration in the context of neurodegenerative diseases in vitro [4] and in vivo [5], the aims of the present work was to prepare liposomes multifunctionalized with doxorubicin (DOXO), an anticancer drug, and with mApoE (DOXO-mApoE NLs) (Fig.1), and to evaluate their in vitro tumor specific intracellular uptake and anti-tumor cytotoxic activity as a possible treatment of GBM tumor.

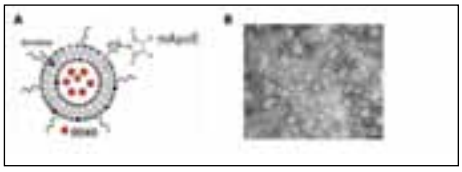
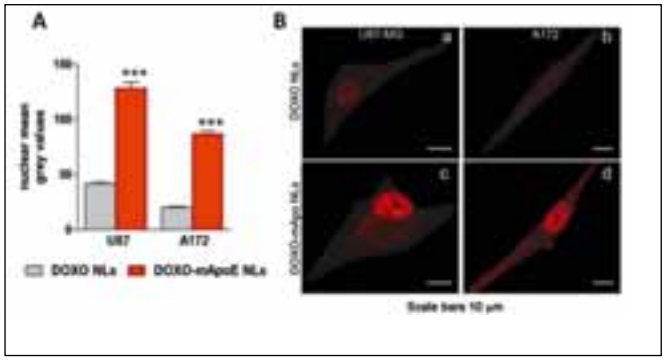


Fig.1: Schematic representation and TEM image of nanoliposomes double functionalized with doxorubicin and with a peptide derived from ApoE lipoprotein (DOXO-mApoE NLs).

Internalization of NLs in GMB-derived cell lines was analyzed by confocal microscopy. GBM-derived cell lines U87-MG and A172 were incubated for 4 hours with DOXO-mApoE NLs or DOXO NLs. DOXO intracellular uptake significantly increased in the presence of the mApoE functionalization (Fig. 2). These results suggest a DOXO-mApoE NLs internalization via receptor mediated endocytosis.
Inhibition of in vitro GBM cell growth was assayed by MTT test at 72 hours on U87-MG and A172 cells. DOXO-mApoE NLs inhibited GBM cell viability in a dose-dependent manner with IC_{50} values of DOXO comprised between 0,5 and 1,5 µg/ml. IC_{50} values of non-targeted DOXO NLs were noteworthy higher, comprised between 1,5 and 3µg/ml

Fig.2: Cellular uptake of nanoliposomes (NLs) by human GBM-derived U87-MG and A172 cell lines. Cells were incubated with DOXO NLs or mApoE-functionalized DOXO NLs (DOXO-mApoE NLs). DOXO concentration: 4 mg/ml; incubation time: 4 hours. DOXO intracellular distribution was determined by confocal microscopy (ex: 470 nm, em.: 600 nm). Nuclear fluorescence was quantified with ImageJ software..



The effect on BBB was evaluated in vitro by incubating NLs (containing DOXO up to 25 µg/mL) with a BBB in vitro cellular model (hCMEC/D3 cells). DOXO NLs with and without mApoE did not show cytotoxicity against BBB cells, while DOXO showed to be extremely cytotoxic, as assessed by MTT assay (Fig. 3). This result suggests a possible use of these liposomes in cancer therapy as DOXO carriers, to prevent side effects of free DOXO.

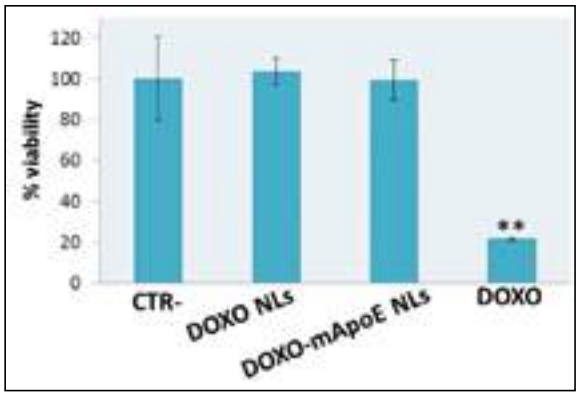


*Fig.3: Cellular viability of hCMEC/D3 cells used as BBB model after incubation with nanoliposomes embedded with DOXO and functionalized or not with mApoE. DOXO concentration: 25 µg/mL, 3 h of treatment, MTT assay after 72 h. ** = p<0,01*

The permeability of DOXO across the BBB model was also investigated by using a transwell system. Functionalization of NLs with mApoE enhanced DOXO passage at all concentrations tested (2.5 and 25 µg/mL of DOXO).
Considering the results on BBB cells, a co-colture has been set up with hCMEC cells and GBM cells (A172) to evaluate the ability of DOXO-mApoE NLs to kill GBM cells after passing through the BBB. Results showed that these liposomes retained a discrete ability to induce mortality on GBM cells (53.5% of viability), as assessed by MTT assay 72 h after liposome (25 µg/mL of DOXO) treatment.
Data obtained support the hypothesis that mApoE NLs are promising multi-task nanocarriers for the delivery of chemotherapeutics to GBM cells.

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NANOTECH DRUG DELIVERY FOR CANCER: FORMULATION STRATEGIES TO ACHIEVE OR AVOID SPECIFIC BIOLOGICAL ENDPOINTS

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INTRODUCTION

A major strategy in nanotech cancer drug delivery has been formulation of stable drug-platform complexes that take advantage of size-selective tumor distribution via the enhanced permeability and retention (EPR) effect. In this phenomenon, enhanced permeability of tumor vasculature provides entry to tumor interstitial space, and suppressed lymphatic filtration promotes accumulation. Though there has been recent progress in correlation of EPR activity to clinical response, and use of EPR activity for patient stratification for clinical trials [1], progress has been hampered by the heterogeneity of the EPR effect in different tumors and limited experimental data from patients [2]. In addition to exploiting the EPR effect and controlled drug release, there are several other nanotech formulation strategies that offer advantages for developing effective drugs. Such strategies are rarely researched in academic labs, but can provide significant incremental, and sometimes substantial improvements over standard-of-care cancer treatments. To be effective, these formulation strategies must be optimized to the physicochemical properties of the drug, its site and mechanism of action, potential off target toxicities and likely pathways of resistance. This poster provides examples of such strategies for delivery of small molecules, peptides, proteins, siRNAs and plasmids, using data from a new initiative in nanotech formulation by the the Nanotechnology Characterization Laboratory (NCL) at the U.S. Frederick National Lab for Cancer Research (FNLCR). We also highlight physicochemical, in vitro, and in vivo characterization methods that have proved useful in guiding formulation strategy.

MATERIALS AND METHODS

The Nanotechnology Characterization Laboratory (NCL) has developed an "Assay Cascade" for preclinical testing of nanoparticles intended as cancer therapeutics and diagnostics. The NCL has developed more than 40 protocols that rigorously characterize nanoparticle physicochemical properties, as well as in vitro immunological and cytotoxic characteristics and ADME/tox profiles in nonhuman animal models. These assays have undergone extensive in-house validation and are subject to regular revision to ensure applicability to a variety of nanomaterials. Selected protocols from the NCL Assay Cascade are available for download: http://ncl.cancer.gov/working_assay-cascade.asp. NCL has tested more than 300 nanomedicines, including liposomes, dendrimers and other polymers, quantum dots, gold colloids, metal oxides, and fullerenes. In addition to the testing service, NCL uses the Assay Cascade in support of industry-funded R&D to develop novel formulations and support discovery work (e.g. formulation, optimizing conjugation chemistries, targeting agents, particular surface coatings, methods development, etc.). Such work is conducted at the NCL and funded by biotech and pharma through collaborative research and development agreements (CRADAs).

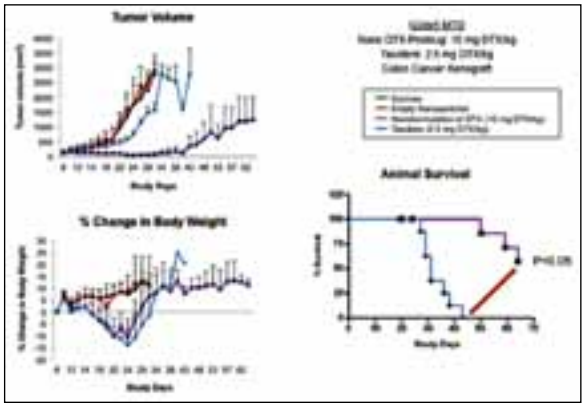
RESULTS AND DISCUSSION

The NCL formulation strategies discussed in this poster include:

- Reduce/avoid specific target organ toxicities (e.g., cardiotoxicity)
- Reduce/avoid specific routes of metabolism / degradation / elimination, both hepatic & extrahepatic (e.g., by enzymes in the blood, mucosa of the gastrointestinal tract, kidney, lung, brain or skin)
- Reduce immunotoxicity [3]
- Stimulate therapeutic immune response (e.g., act as adjuvants for cancer vaccines, immunostimulatory oligonucleotides) [4-5]
- Overcome pathophysiological barriers of tumors (e.g. the desmoplastic stroma in pancreatic ductal adenocarcinoma)
- Increase cellular uptake (e.g., by specific transporters or novel endocytic routes)
- Intracellular trafficking and transport (e.g. endosomal escape, nuclear targeting)

This poster will not provide specifics on proprietary chemistry, but will show data illustrating how formation strategies achieve specific biological endpoints, in vitro and in vivo.

Figure 1. Example of animal efficacy data [6-7] for a nanoformulation strategy optimized to the physicochemical properties of the drug, its site and mechanism of action, potential off-target toxicities and likely pathways of resistance. Such strategies can significantly increase efficacy and decrease toxicity, through mechanisms that may be independent of the enhanced permeability and retention (EPR) effect.



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"LARGE-SCALE MICRO-AND NANOFABRICATION TECHNOLOGIES FOR BIOANALYTICAL DEVICES BASED ON ROLL-2-ROLL IMPRINTING"

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Roll-to-roll (R2R) technologies are mature core processes in manufacturing lines for graphical printing industry. In several other areas (e.g. electronics or optics) R2R techniques are emerging, being expected to notably lower the unit prices of flexible devices. In particular, recently developed roller-based nanoimprinting methods enable unrivalled throughput and productivity for precise fabrication of micro- and nanoscale patterns. Areas that will benefit strongly from adopting such R2R nanoimprinting technologies are microfluidics and lab-on-chip products for diagnostics, drug discovery and food control.

The project R2R Biofluidics aims on the development of a complete process chain for first-time realization of production lines for two selected bioanalytical lab-on-chip devices based on high-throughput R2R nanoimprinting in combination with complementary printing and manufacturing technologies. Two types of demonstrators will be fabricated targeting application areas, which would clearly benefit from technology advancement in high volume manufacturing, show large potential for commercial exploitation and adopt current standard formats (microtiter plate and microscope slides).

Demonstrator 1 will represent an in-vitro diagnostic (IVD) chip suitable for point-of-care applications, showing improved sensitivity thanks to imprinted nanoscale optical structures and microfluidic channels. Demonstrator 2 will provide a device for improved neuron based high-throughput screening assays in drug development. It will consist of nano– to microstructured, interconnected channels in combination with dedicated biofunctionalized surfaces for alignment and controlled growth of neurons.

Nanotechnological applications development is closely related to safety concerns. Within this project, a so-called safe-by-design approach is chosen by addressing nanorelated environment, health and safety (EHS) issues connected to the design of the projects´ products. This will cover materials, processes and the products respectively their nano-related safety issues. Existing data on potential toxicity of materials/structures (e.g. biocompatibility tests according to ISO 10993, etc.) will be screened, evaluation of processes (with special focus on workers safety) including exposure measurements at the lab facilities will be conducted, and screening of potential safety concerns along the life-cycle (consumer and environmental issues) will lead to evidence of safety for the products of this project.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 646260

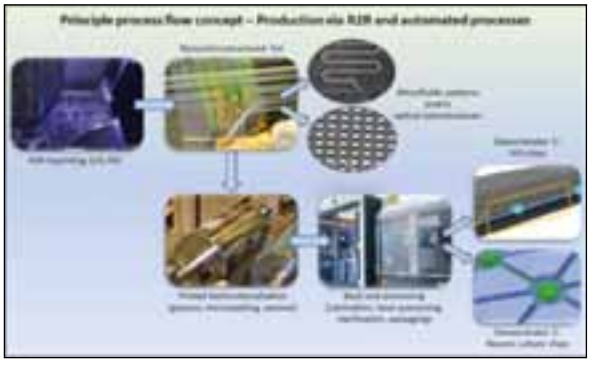

Figure 1: Overall concept for R2R production lines for bioanalytical devices

HER2-TARGETED HEPATITIS B VIRUS CORE PARTICLE AS NANOCARRIERS TO TREAT CANCER

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ABSTRACT SUMMARY

Hepatitis B Virus core (HBc) particles have been studied for their potential as drug delivery vehicles for cancer therapy. HBc particles are hollow nano-particles of 30-34 nm diameter and 7 nm thick envelopes, consisting of 180-240 units of 21 kDa core monomers. They have the capacity to non-specifically bind various cell types via the action of arginine-rich domain. This study focussed on the development of functional nano-assemblies for therapeutic applications. Herein we developed a genetically modified HBc particles to specifically recognise and target human epidermal growth factor receptor-related 2 (HER2)-expressing cancer cells. Our recombinant HBc particles are designed in such a way that non-specific binding property is reduced, via deleting C-terminal 150-183 aa part of the core protein that encodes arginine-rich domain (ΔHBc). HER2 target-cell-specific recognition was acquired genetically by inserting a Z_{HER2} affibody sequence into the 78-81 aa position of the core protein (Z_{HER2}-ΔHBc).

INTRODUCTION

HBc particles have the capacity to assemble/dis-assemble in a controlled manner allowing encapsulation of various drugs and other biomolecules [1, 2]. It is reported that the Major Immudominant Region (MIR) of HBc particles, located at the 78-83 aa, is able to express immunological epitopes by genetic modification to this region. Moreover, other functional motifs i.e., receptors, receptor binding sequences, proteins and elements recognising low molecular mass substrates can also be expressed [3]. The arginine-rich domain of the core protein is not critical for particles assembly but can induce, due to its positive charge, non-specific cellular uptake in cells [4]. We hypothesised that eliminating the arginine-rich domain can reduce non-specific cell binding, and expressing a targeting moiety into the HBc particles, at MIR region, can improve cellular targeting capacity.

EXPERIMENTAL METHODS

pET-22b(+)-HBc-His6, pET-22b(+)-ΔHBc-His6 and pET-22b(+)-Z_{HER2}-ΔHBc-His6 plasmids containing wild type HBc, recombinant ΔHBc and Z_{HER2}-ΔHBc protein sequence, respectively, were expressed in E.coli expression system. HBc particles were purified via affinity chromatography containing Ni^{2+}-charged resin and Urea-DTT dialysis method. To check the specificity of HBc particles, Western blot analysis was performed using anti-His6 antibody. Nanodrop UV spectroscopy was used to measure the protein concentration. Atomic Force Microscopy (AFM) and Dynamic Light Scattering (DLS) were used to characterise HBc particles morphology and size, respectively.

To evaluate the binding affinity to HER2-expressing cancer cells, HBc particles were labelled with Alexa Fluor 488, and incubated with either of the following HER2-expressing cell lines: HeLa (cervical cancer), IGROV-1 (ovarian cancer), MDA-MB-231 (breast cancer), SKOV-3 (ovarian cancer) and SKBR-3 (breast cancer) at concentrations of 10, 20 or 40 µg/ml. The median fluorescent intensity of cells was measured by flow cytometry after 4H and 24H of incubation.

RESULTS AND DISCUSSION

Schematic representations of constructed HBc proteins are shown in Figure 1.

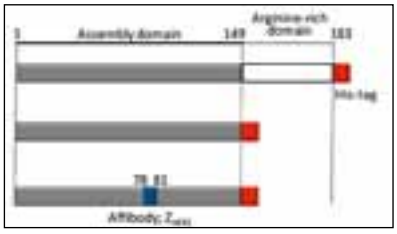


Figure 1. Wild-type HBc protein consisted of an assembly domain (grey body) and an arginine-rich domain (white body). For ΔHBc protein, the arginine-rich domain (150-183 aa) was deleted. For the Z_{HER2}- ΔHBc protein, Z_{HER2} affibody (blue body) was inserted between 78 and 81 aa. For all constructs, His-tag (red body) was fused to the C-termini.

Western blotting results confirmed the presence of specific protein bands at the desired positions (HBc, 21 kDA; ΔHBc, 17 kDa and ZHER2-ΔHBc, 24 kDa) (Figure 2).

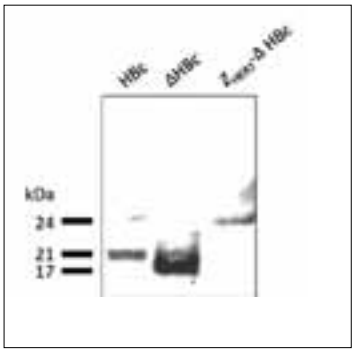


Figure 2. Western blotting analyses of purified core particles. Purified samples (HBc, ΔHBc and Z_{HER2}-ΔHBc) were subjected to SDS-PAGE followed by immune-blotting using anti-His6 antibody.

AFM confirmed the spherical structures of all assembled HBc particles (HBc, 33.77 ± 4.58 nm; ΔHBc, 30.24 ± 2.87 nm and Z_{HER2}-ΔHBc, 32.41 ± 2.33 nm) compared to the dis-assembled HBc which appeared as irregular structures (Figure 3).

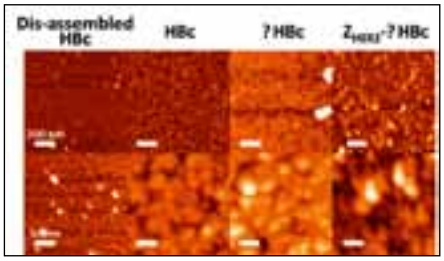
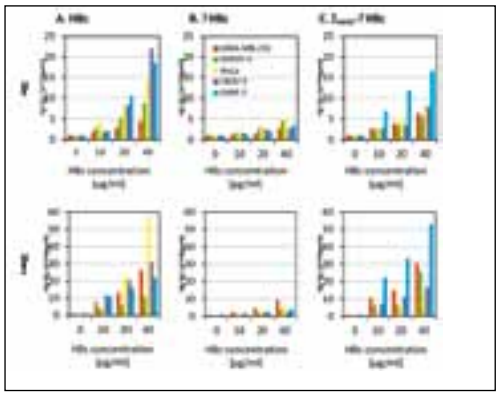


Figure 3. AFM images of dis-assembled and assembled HBc particles. All type of HBc particles (HBc, ΔHBc and Z_{HER2}-ΔHBc) were analysed using tapping mode AFM analysis deposited on the mica sheets.

The addition of HBc particles to cells caused a dose-dependent increase in median fluorescence intensity (MFI) of all cells (Figure 4A), indicating that wild-type HBc particles bind to cells in a non-specific manner. ΔHBc particles showed reduced MFI of all cells, supporting the hypothesis that arginine-rich domain deletion reduces the non-specific binding ability of the wild type HBc (Figure 4B). Z_{HER2}-ΔHBc particles showed enhanced uptake in all cells but the highest uptake was obtained in SKBR-3 cells (Figure 4C), as this cell type expressed the highest HER2 receptor among all cell types studied, determined by flow cytometry (data not shown). The increase in MFI in case of HBc and Z_{HER2}-ΔHBc was dose-dependent.

Figure 4. Fold increase in MFI of HER2-expressing cell lines treated with Alexa Fluor 488-labelled HBc particles. Fold increases were determined by flow cytometry. (A) HBc, (B) ΔHBc and (C) Z_{HER2}- ΔHBc. Time points: 4H and 24H.



CONCLUSION

Non-targeted and HER2-targeting HBc particles were engineered and self-assembled into spherical particles. Elimination of the arginine-rich domain reduced their non-specific binding to cells. HBc particles, expressing Z_{HER2} affibody, displayed specific binding to HER2-expressing cancer cells. Future experiments will focus on encapsulating siRNA inside the HBc particles for initial in vitro testing.

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NANOTOXICOLOGY – THE SEON-CONCEPT

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BACKGROUND

Nanoparticles offer promising new possibilities for a multiplicity of medical applications including therapy and diagnosis of various diseases. Especially nanoparticle systems with magnetic cores provide a broad application spectrum as contrast agents, magnetic transporters, or heat carriers in hyperthermia treatment. For bench to bedside translation of superparamagnetic iron oxide nanoparticles (SPIONs) as carriers for chemotherapeutic agents in Magnetic Drug Targeting (MDT) safety issues have to be clarified. For that reliable standards must established on the basis of comprehensively validated physicochemical and biological characterization methods.

Problematically, many recent studies investigating nanotoxicological topics are not qualified for safety assessment or regulatory purposes, because basic information as nanoparticle characterizations are not provided or tested concentrations are irrelevant. Additionally, many common toxicological assays like MTT of LDH based on colorimetric signals, fluorescence or luminescence might

be not suitable for the analysis of dark metal oxide particles due to optical interferences with the detection systems.

THE SEON-CONCEPT FOR NANOTOXICOLOGICAL INVESTIGATIONS

We are convinced that only the combination of proper physicochemical and biological characterization methods is able to draw a comprehensive picture of the nanoparticle-mediated effects. We are also persuaded that reproducible nanoparticle syntheses combined with physicochemical characterizations with repeated feedback cycles are fundamental requirements and of utmost importance for the successful attempt to transfer a nanoparticle system from laboratory to clinical use. Importantly, iron oxide and other nanoparticles have to be tested in their specific environment in which they are planned to be applied. That means, nanoparticle performance in complex media as human blood must be investigated, including testing on chemical stability, colloidal stability, drug release and activity properties.

Addressing this complex task, the establishment of a broad spectrum of expertise and reliable standardized tests is necessary. As depicted above, classical toxicological high throughput test systems are often not suitable for testing the biocompatibility of iron oxide and other nanoparticles and therefore, SEON is working on the establishment and validation of a combination of different in vitro assays that can be reliably used without nanoparticle interferences. Here, flow cytometry as well as real time cell analysis (RTCA) employing impedance measurement via the xCELLigence® -technique have been shown to enable interference-free analyses of nanotoxicity. Using multiparameter assays in flow cytometry allows us to analyze nanoparticle content per cell, apoptosis, necrosis, oxidative stress, mitochondrial membrane potential and DNA status in one single experiment. On the other hand, RTCA is a very sensitive method to noninvasively monitor the influence of substances on cell morphology and proliferation behavior of adherent cells over a time period of several days to weeks. The combination of these methods with fluorescence microscopy and immunological tests represents a powerful nanoparticle test battery providing valuable information for assessing biocompatibility and suitability of nanoparticles for medical applications.

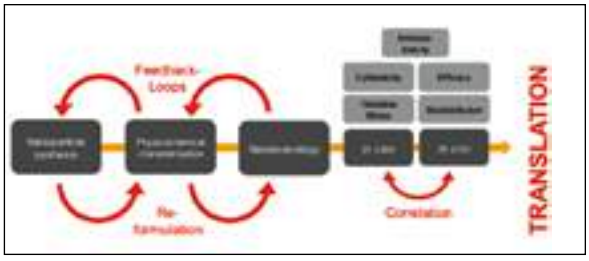

Fig. 1: Consecutive assay battery for the translation of SPION systems from bench to bedside.

SUMMARY

Nanotoxicology is a complex and interdisciplinary challenge, where physicochemical parameters, as well as in vitro and in vivo behavior of nanoparticles have to be considered. For a proper interpretation of the toxicological in vitro and in vivo data, reproducible nanoparticle synthesis procedures and full physicochemical characterizations of the nanoparticles are prerequisites. To address these basic requirements, SEON is working on a stringent and reliable, standardized road of characterization for (iron oxide) nanoparticles dedicated for medical use (Figure 1).

ACKNOWLEDGMENTS

Statements of funding: This work was supported by the Bavarian State Ministry of the Environment and Consumer Protection, and the DFG Cluster of Excellence Engineering of Advanced Materials (EAM).

SYSTEMIC GENE SILENCING IN PRIMARY T LYMPHOCYTES USING TARGETED LIPID NANOPARTICLES

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[1] These authors contributed equally to this work

Modulating T cells functions by down regulating specific genes using RNA interference (RNAi) holds tremendous potential in advancing targeted therapies in many immune related disorders including cancer, inflammation, autoimmunity and viral infections. Hematopoietic cells, in general, and primary T lymphocytes, in particular, are notoriously hard to transfect with small interfering RNAs (siRNAs). Herein, we describe a novel strategy to specifically deliver siRNAs to murine $CD4^+$ T cells using targeted lipid nanoparticles (tLNPs). To increase the efficacy of siRNA delivery, these tLNPs have been formulated with several lipids designed to improve the stability and efficacy of siRNA delivery. The tLNPs were surface functionalized with anti-CD4 monoclonal antibody (mAb) to permit delivery of the siRNAs specifically to $CD4^+$ T lymphocytes. Ex vivo, tLNPs demonstrated specificity by targeting only primary $CD4^+$ T lymphocytes and no other cell types. Systemic intravenous administration of these particles led to efficient binding and uptake into $CD4^+$ T lymphocytes in several anatomical sites including the spleen, inguinal lymph nodes, blood and the bone marrow. This resulted in the efficient silencing of the pan leukocyte surface marker CD45 in circulating and resting $CD4^+$ T lymphocytes. Taken together, these results suggest that tLNPs may open new avenues for the manipulation of T cell functionality and may help to establish RNAi as a therapeutic modality in leukocyte-associated diseases.

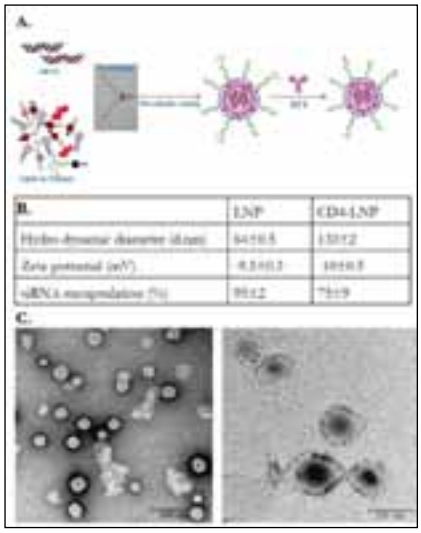

Fig. 1: Schematic illustration of the process which tLNPs are formed (A); Dynamic light scattering analysis of LNPs (B). (C) Transmission electron microscopy (TEM) images of unconjugated LNPs (left) or tLNPs (right).

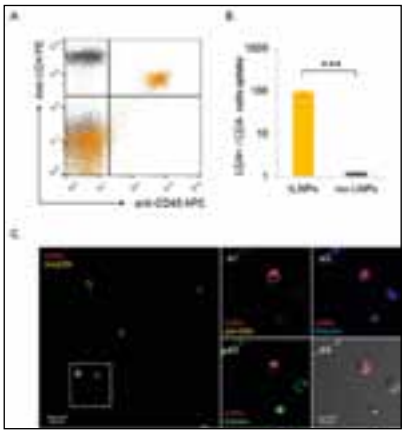

Fig. 2: tLNPs internalize into CD4⁺ T splenocytes ex-vivo. (A)Primary splenocytes were incubated with tLNPs (Cy5-siRNA) or isoLNPs (Cy5-siRNA) as a control for 30min. After labeling CD4⁺ cells with anti-CD4 PE, cells were analyzed for specific binding by flow cytometry. Representative dot blot is presented along with a column graph presents average of CD4⁺/CD4⁻ geometric mean (Cy5 florescence) (n=3,P = 0.0002) (B). (C) To test LNPs internalization, Primary splenocytes were incubated with tLNPs (Cy5-siRNA) or isoLNPs (Cy5-siRNA) as a control for 30min, followed by incubation in 37C for 30min to allow internalization. Cells were stain with Hoechst and calcein for nuclear and cytoplasm detection followed by staining of CD4+ membrane with anti-CD4 AF594. Cells were analyzed for internalization by confocal microscopy. Representative images are presented.

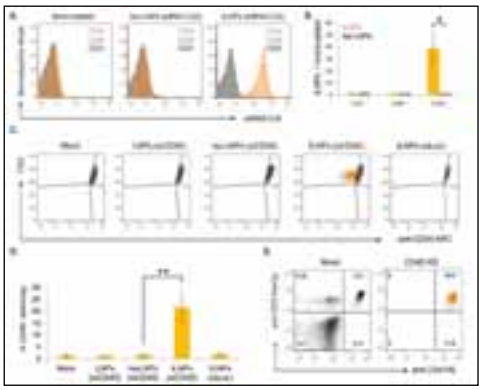

Fig. 3: tLNPs target CD4⁺ T cells in vivo and induce gene silencing. (A) tLNPs targets blood CD4⁺ cells in vivo. One hour after tLNPs administration, circulating lymphocytes were isolated and stained with a set of antibodies (anti-CD4 PE, anti-CD3 PerCp and anti-CD8 FITC). Representative histograms of gated cell populations are presented along with a column graph presents relative average of tLNPs or Iso LNPs geometric mean (Cy5 florescence) to mock cells of each gated population (n=3,P = 0.01)(B). (C) tLNPs silence CD45 in vivo. Saline (mock), tLNPs encapsulating Luc siRNA, Isotype IgG conjugated or uncoated LNPs encapsulating CD45 siRNA and targeted tLNPs encapsulating CD45 siRNA were injected into mice. After 5 days, circulating lymphocytes were isolated and stained for CD45. Representative dot blots analysis for live lymphocytes presented along with statistical analysis (n=3, p=0.0012) (D). (E) CD45 is silenced specifically in CD4+ circulating cells. Saline or tLNPs (siCD45) were administrated to mice. After 5 days, circulating lymphocytes were isolated and stained with a set of antibodies (anti-CD45 AF647, anti-CD4 PE, anti-CD3 PerCp and anti-CD8 FITC). Presented representative dot blot profile analysis of CD45 silenced cell population (CD45^{KD}) compare with saline treated lymphocytes (mock) (n=5).

THE EFFECT OF HOLLOW GOLD NANOPARTICLES AND GOLD NANORODS ON β-AMYLOID FIBER FORMATION IN PRESENCE OF NEAR-INFRARED IRRADIATION

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Amyloidogenesis has a devastating role in neurodegenerative diseases such as Alzheimer´s and Parkinson´s disease. Peptide aggregation into amyloid oligomers and fibrils is a multistep process that finally affects the brain depositing amyloid proteins and plaques the brain [1]. Therefore, the development of new methods to inhibit the aggregation process of peptides into β-amyloid fibrils and the deposition of β-amyloid fibrils as well as the dissolution of already formed β-amyloid aggregates reducing the toxicity would be highly desirable. One promising approach to inhibit the β-amyloid fibril formation is the use of β-amyloid specific peptides such as the CLPFFD-peptide. CLPFFD-peptide contains the LPFFD sequence that attaches selectively to the amyloidogenic Aβ1-42 structures and thereby affects their aggregation behaviour [2].
Recently, the inhibition of the β-amyloid fibril aggregation as well as the dissolution of already formed β-amyloid aggregates has been demonstrated by means CLPFFD-functionalized 15 nm solid gold nanoparticles (AuNP) upon weak microwave irradiation. However, with respect to in vivo application near infrared irradiation in the so-called optical window of biological tissues would be favourable. Therefore, peptide functionalized hollow gold nanospheres (HAuNS) and gold nanorods (AuNR) are chosen as a possibility to inhibit the β−amyloid fibrils formation with irradiation because they have, in comparison to solid nanospheres, the advantage of a tuneable absorption in the near-infrared (NIR) and optimal absorption coefficients for photothermal treatment [3], where the absorption of the constituents of the biological tissue is lowest [4].

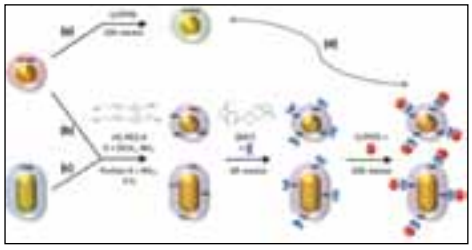
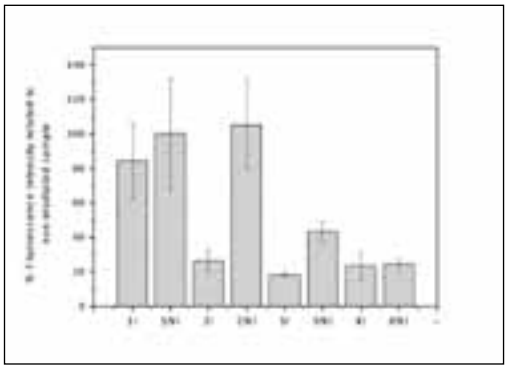


Figure 1: Schematic representation of the synthesis and functionalization of hollow gold nanoparticles and gold nanorods with PEGylated particles and CLPFFD-peptide.

In this work, we report the synthesis of HAuNS having a diameter of 36 nm, a shell thickness of 4.6 nm and an absorbance maximum at 855 nm. These particles were obtained by a galvanic replacement reaction of cobalt nanoparticles and HAuCl4 in the presence of sodium citrate as stabilizing ligand. HAuNS were functionalized with the CLPFFD peptide in two different ways: a direct functionalization (HAuNS-CLPFFD) and a functionalization on previously PEGylated particles (HAuNS-PEG-CLPFFD). In the case of AuNR, which were obtained by seed-mediated approach [5, 6] were functionalized with PEGligands, to which the CLPFFD peptide is coupled covalently (AuNR-PEG-CLPFFD) (Fig.1).

Figure 2: Fluorescence intensity signal from irradiated samples of β-amyloid fibrils in presence of HGNPs-CLPFFD, HGNPs—PEG-CLPFFD and AuNR-PEG-CLPFFD, irradiated with 808 nm, 450 mW continuous laser. The results are expressed as percentages with respect to the intensity from the non-irradiated sample and represent the standard deviation of n=3 (six each experiment). (1: Amyloid

fibrils, 2: Hollow gold nanoparticles with CLPFFD, 3: Hollow gold nanoparticles with PEG and CLPFFD and 4: Gold nanorods, I: irradiated samples, NI: Non irradiated sample).



Irradiation was applied for 2 h and after irradiation, the samples were placed in a thermomixer for 48 h at 37 °C and 300 rpm. The formation of fibrils was evaluated by using thioflavin-T fluorescence assay. In this assay, the amount of fibrils in suspension can be quantified by measuring the intensity of the fluorescence signal, which is proportional to the amount of formed fibrils. Figure 2 shows the percentage of fluorescence intensity related to non-irradiated samples. These results exhibit that the major effect with irradiation process is in presence of HAuNS-CLPFFD. In this case, the fluorescence of thioflavin-T decreased more than 60 % indicating that HAuNS-CLPFFD affects considerably the β−amyloid fibrils formation. Otherwise, for HAuNS-PEG-CLPFFD and AuNR-PEG-CLPFFD, independently of irradiation process, these nanoparticles affect the process of fibrils formation.
The inhibitory effect of HAuNS-CLPFFD in the amyloidogenesis process is potentiated by NIR irradiation, which is confirmed by transmission electron microscopy (TEM).
The aggregation process of β-amyloid to form amyloid fibrils is inhibited in the presence of peptide functionalized gold nanoparticles and after irradiation with NIR. HAuNS showed high potential for photothermal treatment of amyloidosis being more effective for the energy transfer after irradiation with respect to AuNR. These results are relevant for the development of a new strategy for the therapy of Alzheimer´s disease.

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COMPLEMENT ACTIVATION BY RITUXIMAB AND PACLITAXEL IN CANCER PATIENTS IN VIVO AND IN THEIR SCREENING SERUM IN VITRO: VARIABLE ASSOCIATION WITH HYPERSENSITIVITY REACTIONS

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To explore the role of complement (C) activation in the hypersensitivity reactions (HSRs) to some anticancer drugs, as well as the use of the C activation biomarkers (Cbiom) C3a, C5a and SC5b-9 C in the prediction of HSRs, we measured these Cbiom in plasma samples of cancer patients during infusion therapy, and in their pretreatment (screening) serum incubated with these drugs in vitro. Rituximab and paclitaxel caused mild to severe HSRs in 8/20 and 4/4 patients, respectively, which were associated with rises or falls of plasma and/or serum Cbioms. Among these changes, a rise of C3a in the plasma of 8/8 rituximab reactors and strong rises of Cbioms in the screening sera of all paclitaxel patents were most prominent. However, in the case of rituximab, significant Cbiom changes were also seen in nonreactors, while Cbiom changes were absent in the screening serum. Thus, C activation may be causally involved, but it is not rate limiting factor to HSRs to rituximab. Additional initial data in this study showed that a whole blood assay using hirudine is more sensitive to C activation by rituximab that the serum test; that trastuzumab and docetaxel also cause HSRs with changes of Cbioms, and that an anti-paclitaxel antibody (ADA) ELISA showed correlation with HSRs.
Keywords: anaphylaxis, anti-drug antibodies; cancer therapy; hypersensitivity reactions; immunogenicity; nanomedicines
Abbreviations: ADAs , anti-drug antibodies; C, complement; Cbiom, C activation biomarker(s); TCC, Terminal Complement Complex mAbs, monoclonal antibodies; HSRs hypersensitivity reactions; CARPA, complement activation-related pseudoallergy

TARGETED NANOGELS FOR DRUG DELIVERY TO BRAIN TUMORS

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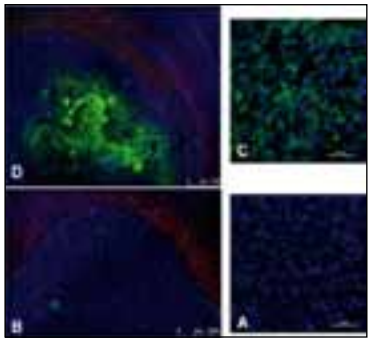
Conventional and combined chemotherapies are still low effective in case of brain tumors, especially glioblastoma multiform that is the most malignant and invasive among primary brain tumors. In spite of some therapeutics improvements from pharmacology, neurosurgery, radiology the main approach in treatment of brain tumors remains chemotherapy. Unfortunately, anti-tumor agents have extremely low specificity, which limits treatment efficiency and leads to adverse side effects. Nanocarrier-based therapeutics is widely investigated to overcome limitations of conventional chemotherapy. The enhanced permeability and retention effect leads to nanoparticles tumor accumulation. Passive targeting is the main powerful force for nanoparticles accumulation but in that case, the drug distribution could be heterogeneous. Consequently, active targeting may improve site-directed delivery in malignant tissue and make the drug distribution more specific. In terms of specific tumor-associated protein vascular endothelial growth factor receptor II (VEGFR2) is an attractive molecular target. VEGFR2 signaling plays an abundant role in tumor angiogenesis and cancer development. VEGFR2 expression was noted on endothelial tip cells that involved in formation of angiogenic sprout. Moreover, normal tissues have low expression levels of VEGFR2 comparing to cancer cells. In the present study, we demonstrate that conjugation of nanogels with monoclonal anti-VEGFR2 antibody enhances the uptake and accumulation of nanocarriers in an intracranial rat model of C6 glioma. Polyelectrolyte PEG-b-PMAA nanogels were chosen as a nanocar-

rier that are negatively charged, stable, spherical nanoparticles with narrow polydispersity. These nanogels can serve as a nanocarrier for different drugs with high loading capacity. As a targeting moiety, we selected VEGFR2 receptors that are known to be overexpressed in ovarian cancer, colorectal cancer, lymphomas, and gliomas. Therefore, nanogels were modified by anti-VEGFR2 monoclonal antibody that was obtained previously. To evaluate the cellular binding and uptake of anti-VEGFR2 nanogels we used glioma C6 and U-87 MG cells. It was shown that glioma C6 and U-87 MG cells treated with anti-VEGFR2 nanogels showed stronger fluorescence than non-modified and non-specific nanogels, suggesting that anti-VEGFR2 antibodies could facilitate effective cellular uptake of the nanogels.

To investigate the accumulation of anti-VEGFR2 nanogels in intracranial tumor model PEG-b-PMAA chains were modified with FITC-ED. Then samples were normalized by fluorescence of FITC and intravenously injected. Perfusion was performed 48 hours after injection and samples were analyzed. Additionally, anti-GFAP antibodies were used to visualize peritumoral regions. All samples (anti-VEGFR2 nanogels, non-modified nanogels and non-specific nanogels) were accumulated in tumor. Most of nanogels were accumulated in pathological vessels, but some areas of tumor remained free of nanogels. Targeting of VEGFR2 receptor lead to the most efficient accumulation of nanogels comparing with negative controls (Fig. 1 A, B). Consequently, targeting of nanogels improve distribution throughout tumor tissues comparing with controls.

Figure 1. Accumulation of FITC-labeled nanogels by glioma cells in intracranial tumor 48 hours after injection. Cellular nuclei are visualized DAPI (blue fluorescence). Nanogels shown as a green fluorescence, peritumoral regions shown as a red fluorescence. (A, B) Accumulation of anti-VEGFR2 nanogels in the glioma cells. (C, D) Accumulation non-specific nanogels in the glioma cells.



Our results indicate that anti-VEGFR2 nanogels can be efficient for site-directed delivery of therapeutic agents to the brain tumors that overexpress VEGFR2 receptor.

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POINT-OF-CARE MICROFLUIDIC DEVICE FOR QUANTIFICATION OF CHEMOTHERAPEUTIC DRUGS IN SMALL BODY FLUID SAMPLES BY HIGHLY SELECTIVE NANOPARTICLE EXTRACTION AND LIQUID CRYSTAL DETECTION

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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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EXPOSURE OF THE INTESTINAL BARRIER AS SECONDARY EXPOSURE ORGAN TO GRAPHENE-RELATED MATERIALS IN VITRO

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Graphene, graphene-related materials (GRM) and other 2D materials are the focus of outstanding expectations due to their unique combination of physicochemical properties and high technological potential (1, 2). But there is also broad discussion about the potential risks of graphene-related materials on human health as well as on the environment. Not only for envisioned biomedical application (3), GRM need to be safe showing no or negligible adverse effects. The discussion about possible risks is in part complicated by the deficit of information on human exposure as well as by the lack of a common nomenclature and classification system for GRM (4, 5). Graphene-related materials exhibit significant variability in their properties based on the applied synthesis route, the starting material, as well as the requirements for the targeted application. Since graphene oxide is one of the most important GRM due to its good dispersion ability in aqueous media as well simple manufacturing processes, we have focussed our work primarily on GO. Despite in vivo and in vitro studies addressing the possible influence of GRM on the air-blood barrier, information regarding other biological barriers such as the intestinal barrier is not available. The gastro-intestinal tract can be regarded as secondary exposure organ after inhalation as part of inhaled material is cleared from the lung by mucociliary transport and finally either coughed out or swallowed down. To close this knowledge gap, we investigated the interaction of GRM with the human intestinal barrier in vitro by application of the Caco-2 cell model. We will discuss the impact of pre-treatment of GRM in acidic environment on the material characteristics as well as the biological response. In addition, we will address the possible material uptake by Caco-2 cells in both differentiated and undifferentiated cells.

Figure 1: Scanning electron microscopy (SEM) image showing a folded commercial graphene oxide sheet (GO) on the surface of undifferentiated Caco-2 cells. Cells were exposed to 10 µg/ml GO for 24 h. The cellular surface shows typical microvilli arrangement. The GO flake was displayed in purple to enhance visibility.

The research leading to these results has received funding from the European Union Seventh Framework Programme under grant agreement n°604391 Graphene Flagship.

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LIPID NANOCAPSULES FOR DRUG DELIVERY IN RHEUMATIC CARTILAGE

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Rheumatoid arthritis is the most common inflammatory joint disease affecting 0.5-1% people worldwide (1). Loss of cartilage in the later stages of the disease leads to a significant reduction of life quality. Common antirheumatic drugs, such as analgesics, non-steroidal anti-inflammatory drugs, glucocorticoids and disease-modifying antirheumatic drugs suffer from low target specifity and fast elimination from the synovial fluid. With nanoparticles it would be possible to overcome these problems by modifying their surface with specific receptor ligands or extracellular matrix components. Antirheumatic drugs could be carried in the core or bound to the particle surface.
Nanoparticle transport into rheumatic cartilage depends on the stage of the disease. Early stage cartilage degradation is characterized by loss of glycosaminoglycans (GAG) and, therefore, loss of negative charge. With disease progression collagen is also removed from the "inflamed" tissue. To reduce side effects only pathological tissue should be reached by nanoparticles.
To the best of our knowledge diffusion experiments with nanoparticles were to date either done on healthy cartilage or after trypsinization, which removes the whole GAG. None of these settings reflects the complexity of the underlying pathological processes. Therefore, we used cytokines to induce inflammation in cartilage and get a pathological degradation. By adjusting cytokine concentration and incubation time different disease stages could be simulated.
Our goal was to investigate the penetration behavior of nanoparticles in intact and "rheumatic" cartilage. Important for the particle penetration are parameters like size and charge. Therefore, we designed different nanoparticles and tested their diffusion behavior on intact and "rheumatic" cartilage. With these experiments we wanted to figure out the physical properties that are necessary to have an efficient nanoparticle infiltration in each stage of disease.
For our experiments we used lipid nanocapsules (LNCs). These consist of a liquid lipid core, an aqueous phase and non-ionic surfactants. Big advantages of these nanoparticles are the possibility to tailor their surface charge by the use of modified surfactants and adjust their size by virtue of their composition. Lipophilic drugs or fluorescent dyes could be inserted in the core. LNCs were prepared as described earlier (2).
Bovine articular cartilage cylinders were treated with interleukin-1α (IL-1α) and oncostatin M (OSM) for 7 days. Control groups were treated identically but without addition of cytokines. Afterwards cartilage cylinders were incubated for 48 hours with either cell culture media, LNCs or free dye. From the harvested tissue samples 10 µm cryosections were prepared and stained for GAG with Fast Green/Safranin-O or investigated for the presence of nanoparticles.
Histological pictures showed that GAG was selectively removed at the superficial and calcified zone of cartilage after treatment with cytokines. The removal is due to the up regulation of matrix metalloproteinases (MMP) in chondrocytes. qRT-PCR data showed that the gene expression of MMP in chondrocytes is up to 2,800 times higher after adding IL-1α and OSM. In explants GAG was removed up to 700 µm in depth after one week incubation time. The released GAG could be found in the supernatant while it was free of collagen.
Cryosections of cartilage without nanoparticles showed no fluorescence and free dye did not penetrate into the tissue. 50 nm nanoparticles only diffuse in parts of cartilage where GAG was absent (Fig. 1). The depth of infiltration was about 700 µm which is consistent with the removal of GAG. Positively charged particles were found mainly in the cytoplasm of chondrocytes whereas neutral particles were in the extracellular matrix in a higher concentration.

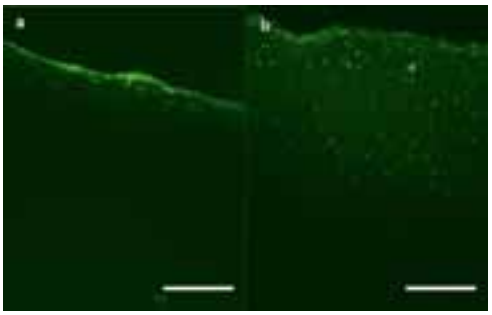

Fig. 1: Diffusion of non-charged lipid nanocapsules in cartilage. Nanoparticles were false color in green. Scale bar is 100 µm (a) untreated cartilage (b) cartilage treated with IL-1α and OSM

In summary we could show that 50 nm nanoparticles are able to diffuse into "inflamed" but not into intact cartilage. Positively charged nanoparticles preferentially target chondrocytes whereas non-charged particles stay in the extracellular matrix.

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IMMUNOLOGICAL ASPECTS OF MAGNETIC DRUG TARGETING (MDT)

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BACKGROUND

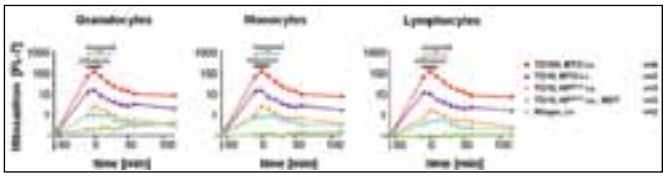
Surgery, radiotherapy and chemotherapy are standard therapies of cancer of today. The main disadvantages of chemotherapy are the severe systemic side effects of this therapy, although some progress has been achieved. The recent years have shown that new strategies like antibody based therapies are not only very cost intensive, but also could not or only in part fulfill the expectations scientists and oncologists have put in them.
Importantly, the immune system plays a key role in carcinogenesis

and cancer therapy since initiation, growth and metastasis formation require a modification or circumvention of the natural immune response. Additionally, conventional systemic chemotherapy lowers the ability of the body to induce immune reactions. Therefore, more and more attention is being paid to the immunological aspects of cancer therapy and the induction of anti-tumor immunity during radiotherapy and chemotherapy.
The goal of Magnetic Drug Targeting (MDT) is to multiply the effect of a cytotoxic drug on the tumor cells and to simultaneously reduce systemic side effects. Therefore, chemotherapeutics bound to superparamagnetic iron oxide nanoparticles (SPIONs) are accumulated in the tumor area by a strong external magnetic field after being applied intra-arterially in the vicinity of the tumor.
In the present study, mitoxantron (MTO) was used as cytostatic drug bound to SPION clusters with a hydrodynamic diameter of about 100nm. It is known that MTO impairs DNA replication, transcription and repair and is therefore used for the treatment of a variety of hematologic and solid tumors. As side effects MTO may cause nausea, fever, anemia, immunosuppression, and cardiotoxicity.

RESULTS

Aim of this pilot study was to investigate, if MDT with MTO as cytostatic drug is able to reduce the burden of circulating leukocytes with chemotherapeutic drugs in comparison to conventional systemic application of different doses.
For analyzing drug load of the leukocytes, we used flow cytometry (FC) and showed that nanoparticles can be easily distinguished from Jurkat cells in vitro by means of size and granularity. Forward and side scatter analysis (FSC/SSC blots) showed the SPIONs as a cloud of events two orders of magnitudes smaller and less granular than the cells. These morphological differences allowed us to gate exclusively on cells and to preclude SPION from the analysis. This finding also shows that FC is an excellent method for investigating nanoparticle mediated effects on cells without a disturbing background signal of the nanoparticles, like it is the case with other classical toxicological assays in vitro.
Next we investigated the uptake of MTO, SPIONs and SPIONs loaded with MTO by Jurkat cells and found a time and dose dependent uptake of MTO which was easily detectable by its inherent fluorescence using FC. The experiments also revealed that MTO uptake had dose and time dependent effects on cell proliferation and cell death and importantly there was no obvious difference whether MTO was bound to the nanoparticles or not.

Figure 1: Effect of Magnetic Drug Targeting on the burden of rabbit leukocytes with the chemotherapeutic drug mitoxantron (MTO). Compared to systemic application i.v. (red line), the intracellular MTO content of circulating leukocytes is clearly reduced in intra-arterial MDT (blue line).



Finally, we investigated the time dependence of the MTO-burden on circulating leukocytes accompanying to a MDT animal trial using New Zealand white rabbits. We compared conventional intravenous application of a 100% single MTO dose, as well as a 10% single MTO dose with the application of MDT using a 10% single dose of MTO bound to nanoparticles. As a control 2 animals were injected an equal volume of Ringer`s solution. Analysis of the intracellular MTO fluorescence revealed that MDT clearly reduced the chemotherapeutic burden on circulating leukocytes (Fig1).

SUMMARY

Major disadvantage of conventional chemotherapy is the occurrence of systemic side effects including effects on the immune system that not only have an impact on the quality of life of patients, but also on the therapy outcome as well as the treatment costs. The aim of MDT is to reduce these side effects and simultaneously improve the therapeutic outcome of cancer treatment.
This pilot study revealed that flow cytometry is an excellent tool for analyzing the effects of nanoparticles and MTO on human cancer cells in vitro. Uptake of free MTO as well as in its nanoparticle bound form was time and dose dependent and induced cytotoxicity. The animal experiments showed that the chemotherapeutic burden on circulating leukocytes was clearly decreased by MDT. Hence, one could assume that MDT not only represents a promising new cancer therapy but in fact reduces systemic side effects, at least on the immune system.

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FACTOR H INHIBITS LIPOSOMAL AND MICELLAR DRUG-INDUCED COMPLEMENT ACTIVATION

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Complement (C) activation can underlie acute, non-IgE-mediated allergic (infusion) reactions to particulate drugs, which reactions can occasionally be severe, or even lethal. Thus, the phenomenon represents a major immune barrier to the therapeutic use of many state-of-the-art "nanomedicines", including the antifungal drug, liposomal Amphotericin-B (AmBisome) and paclitaxel (Taxol), the most widely used anticancer drug that is solubilized by Cremophor EL (CrEL) micelles. Both AmBisome and CrEL were shown earlier to activate C (partly) via the alternative pathway (AP), and to induce CARPA (complement activation-related pseudoallergy) in pigs and dogs with symptoms mimicking the human anaphylactic reaction to these drugs. Thus, it was hypothesized that inhibition of AP C activation by AmBisome and CrEL might interfere with the CARPAgenic activity of these drugs. One of the well-known natural controlling factors of AP C activation is factor H, which we tested in the present study for inhibitory effect on Ambisome- and CrEL-induced C activation in vitro. Complement activation was measured in 3 normal human sera by measuring the formation of SC5b-9 with Quidel's TCC ELISA. As positive control we used Zymosan A. Both drugs caused significant, 4-8-fold rise of SC5b-9 over baseline, although the rise of SC5b-9 was less than that caused by Zymosan A. Exogenous factor H, added at 200 µg/mL concentration, led to >50% and >80% reduction of liposome- and CrEL-induced C activation, respectively. Addition of recombinant factor H had similar inhibitory effect on SC5b-9 generation. Our data suggest that factor H might play a key role in reducing CARPA and that its use could be a potentially useful approach to prevent this adverse immune effect of the studied and probably many other nanomedicines as well.

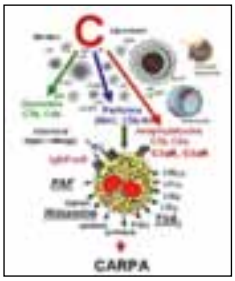

Figure 1. CARPA.1

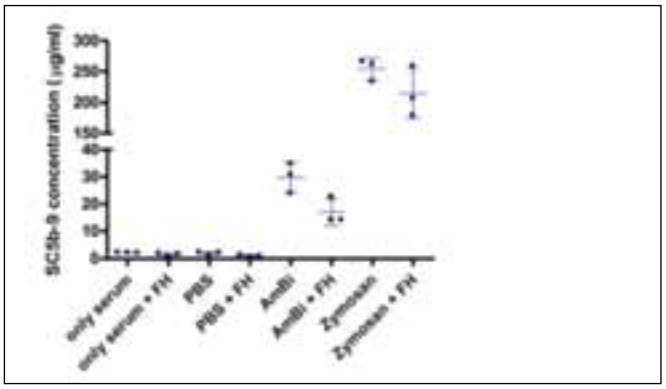

Figure 2. Factor H (FH) inhibits AmBisome-induced complement activation.

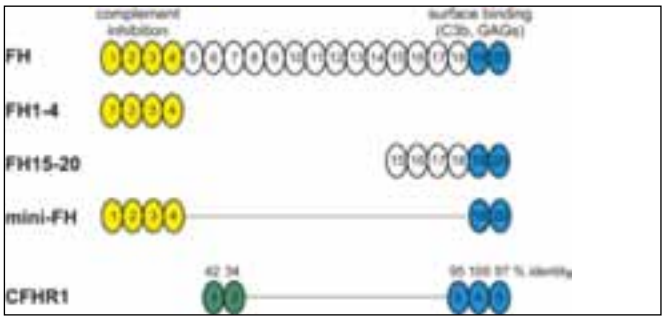

Figure 3. Factor H constructs used in the experiments.

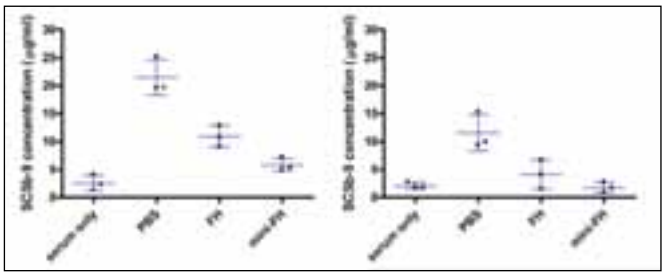

Figure 4. Mini-factor H (mini-FH) more strongly inhibits drug-induced complement activation compared with full-length factor H (FH).

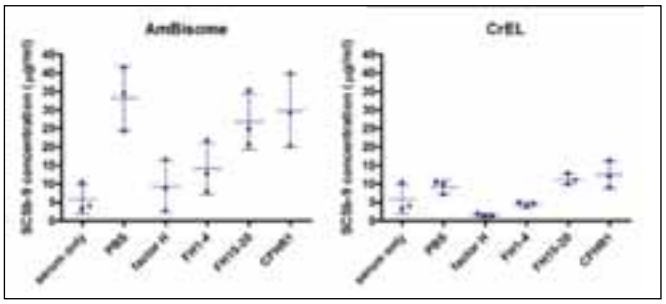

Figure 5. Evaluation of factor H fragments and the factor H-related protein CFHR1.

NANOMATERIAL BASED SUB-PROTEOME SELECTION FOR ANALYSIS OF THE ACTIVATION STATE OF MACROPHAGES

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INTRODUCTION

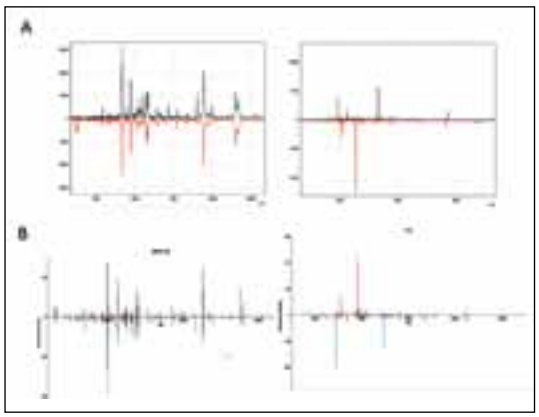
Macrophages are key cell players in the immune response associated with infection, cancer and tissues homeostasis. These cells present a high degree of phenotypic plasticity in response to their microenvironment. MALDI-TOF-MS analysis has been successfully used in the identification of microorganisms. Mass spectrum is used as a fingerprint without peak identification. It has been suggested that a whole cell analysis could be used also for macrophages. We decided in this work to explore this possibility and to explore sub-proteome analysis based on nanotechnologies (nanoporous silica chips and superparamagnetic beads) using a monocytic human cell line (THP-1) which could be differentiated into macrophage-like cell and stimulated by a TLR4 agonist.

METHODS

Human monocytic cell line THP-1 were differentiated in THP-1 macrophages by PMA (Phorbol 12-Myristate 13-Acetate) at 50nM during 48h. Both THP1 monocytes and THP-1 macrophages were also stimulated by a lipopolysaccharide (LPS O111B4 at 1µg/mL) during 24h. Cells were harvested and frozen at -80°C until use. Cells were secondly: 1. deposited directly with alpha-cyano-4-hydroxycinnamic acid (CHCA) or 2. cell lysis (urea 8M, 10minutes) were deposited on nanoporous silicon chip with CHCA or 3. cell lysis was incubated with superparamagnetic beads (chemically functionnalysed as hydrophobic, cationic or anionic) to further deposit the eluate with CHCA for MADI-TOF-MS analysis. Mass spectra were secondarily analysed with the ClinPro Tool software.

RESULTS

Mass spectra analysis (SNR>6) reveals detection of 90 peaks for whole cell method and 148 peaks for nanoporous silicon chip cell lysate method. The specific peaks analysis (normalized intensity > 1.5, ratio intensity >10) was able to identify 16 specific peaks for THP-1 monocytes and 12 specific peaks for THP-1 macrophages in the whole cell condition. The same analysis performed with the sub-proteome selection of the lysate of cell on nanoporous silicon chip reveals a significant higher number of usable peaks: 56 specific peaks for THP-1 monocytes and 39 specific peaks for THP-1 macrophages. (Figure A and B, monocytes in black, macrophages in red). To further evaluate the characterization of the activation state of cells we performed a peak intensity analysis (n=3 independent experiments, $p<0.05$ paired Student t-test) to evaluate the quantitative modification of mass spectra of macrophage under LPS stimulation. We found the following number of varying peaks: 14 for the whole cell method and 22 for the nanoporous silicon chip method. A correlation matrix analysis confirms these results. We also performed the same analysis for mass spectra obtained with superparamagnetic beads: we found 18 specific peaks for cationic beads, 16 for anionic beads and 12 for hydrophobic ones. These peaks associated with the LPS stimulation were all detected at different m/z demonstrating the complementary of these methods. It appears that combining different sub-proteome selection greatly increase the power of activation state characterization through MALDI-TOF-MS analysis.



CONCLUSION

MALDI-TOF-MS analysis on sub-proteome selection by nanomaterials opens new perspectives on the assessment of the immune response driven by macrophages.

EXOSOMES: COMPLEMENT-STEALTH NANOCARRIERS ENGAGED IN INTERCELLULAR MESSAGING

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Exosomes are natural nanocarriers that transport biological information. Their properties make them interesting objects for medical applications. The interaction of exosomes with the immune system has been one of the focal points of interest, but the mechanisms leading to exosomes averting adverse immune reactions are still not fully understood. In this review, after giving an overview of recent findings on the role of exosomes in disease pathogenesis and physiological functions, we focused on their interaction with the immune system and explored the possibility of creating a complement-stealth nanoparticle model based on exosomes that are better tolerated by the immune system than the presently available synthetic drug delivery systems, which may represent a promising new approach in nanomedicine.

NANONIZATION AND ORAL BIOAVAILABILITY ENHANCEMENT OF CURCUMIN THROUGH SUPERCRITICAL ANTISOLVENT (SAS) PROCESS

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Curcumin (CUR) was nanonized by combining the supercritical antisolvent (SAS) process with Tween 80 as a solubilizing agent and permeation enhancer. Different processing parameters were well investigated by manipulating the types of solvents (S) , mixing vessel pressure (P) , mixing vessel temperature (T) , CO_2 flow rate (CFR) , solution flow rate (SFR) and solution concentration (SC) to optimize average particle size with narrow size distribution. Curcumin nanoparticles were characterized by Fourier transform infrared spectroscopy, differential scanning calorimetry, dynamic light scattering, scanning electron microscopy, and powder X-ray diffraction study.

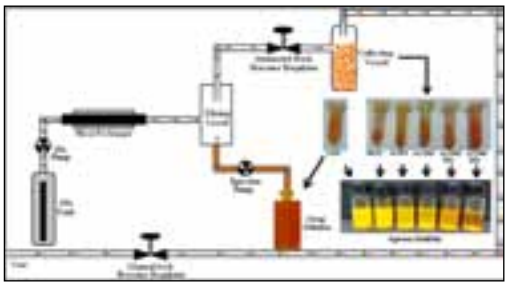

Figure 1. Schematic representation of curcumin nanonization through SAS process.

The optimum operating process parameters were set at P = 14 MPa, T = 40°C, CFR = 40 g/min, SFR = 0.5 mL/min, SC = 0.5 % (w/v) and metal frit diameter (D) = 100 µm. By using this optimum condition, CUR was nanonized 430.6 ± 4.1 nm by employing acetone as solvent. Mean particle size was increased to 635.5 ± 5.3 nm and 750.4 ± 6.8 nm at a Tween 80 concentration of 1% and 2% (v/v), respectively. On increasing Tween 80 concentration above 2% (v/v), mean particle size of SAS-processed CUR was found to be in the micron range and particles were tended to stick to the wall and metal frit of the reacting vessel. A significant increase in the solubility of SAS-processed CUR was observed due to its smaller particle size and hydrophilic coating of Tween 80 surfactant, where Tween 80 might have formed a soluble complex and acted as a solubilizing agent SAS-processed CUR exhibited approximately 11.6-fold ($P < 0.001$) enhancement in oral bioavailability as compared to unprocessed CUR in male Wistar-strain rats.

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GOLD NANORODS FOR THE TREATMENT OF ALZHEIMER'S DISEASE

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The Alzheimer Disease (AD) is a neurodegenerative disease which his main characteristic is the loss of the cognitive capacity and memory, impeding the normal development of the daily activities. The causes of AD is multifactorial being very relevant for the development of this disease the accumulation of toxics aggregates of β amyloid (ATAβ) in the brain [1].
Gold nanorods (GNR) are interesting nanomaterials for use them in photothermal therapy in different pathologies, like AD and cancer, because have the capacity to absorb large amounts of energy and then dissipate it as local heat, when are irradiated with a near infrared laser (NIR) where the tissues have a minimal absorption, called "biological window" [2].
GNR can be used for disaggregating ATAβ, reducing their toxicity, however, thinking in a possible therapy as was described in our group by Adura et al. it is necessary that GNR reach the brain in adequate levels crossing the blood brain barrier.2
For such purpose in this work we multifunctionalized GNR with the peptide Angiopep 2 (Ang2), used as a shuttle to the central nervous system [3], and with other peptide consisting of amino acids of the D series, D1, which has a high affinity for ATAβ [4]. Also, the surface was modified using polyethylene glycol to avoid unspecific interactions with the biological media. We tested the cytotoxicity of multifunctionalized GNR in SH-SY5Y cell line and in primary culture of hippocampal neurons. Finally we probe in vitro adhesion/internalisation of GNR in SH-SY5Y cell line and in primary culture of hippocampal neurons
We characterize the multifunctionalized GNR by UV-Vis-NIR, DLS and TEM observing a longitudinal plasmon in the NIR region, a zeta potential negative with a hydrodynamic diameter nearly to 90 nm, and in the pictures of TEM we can see out nanoparticle with rod shape (Figure 1)

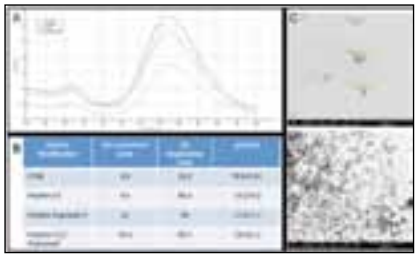

Figure 1. GNR characterization by UV-Vis-NIR (A), DLS (B) and TEM (C)

On the other hand we observed that GNR does not affect the viability of cells at the times and concentrations tested (figure 2).

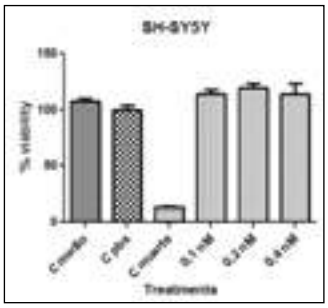

Figure 2. Effects of GNR on the cell viability of SH-SY5Y neroblastome cell line, measured by the MTS assay.

Finally, we evaluated adhesion/internalization of GNR and found a time -dependent increase, at the times and concentrations tested. The obtained functionalized GNRs are good candidates for in vivo experiments to improve their delivery to the central nervous system.

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TOPICAL AMPHOTERICIN B IN ULTRADEFORMABLE VESICLES: FORMULATION, SKIN PENETRATION STUDIES, ANTIFUNGAL AND ANTILEISHMANIAL ACTIVITY.

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Introduction: Amphotericin B (AmB) is a polyene antibiotic with potent antifungal and leishmanicidal activities. AmB is a high molecular weight (924 Da) molecule with amphoteric and amphiphilic behavior, poorly soluble in aqueous media and in most organic solvents. Micellar formulation of AmB with deoxycholate (Fungizone) and the unilamellar liposomal formulation of AmB (AmBisome) are used for parenteral treatment of invasive fungal infections such as Candida albicans or Aspergillus fumigatus (mostly in inmmunocompromised patients) and for treatment of visceral and mucocutaneous leishmaniasis.

In the last 30 year the number of cases of invasive and superficial mycosis of Candida spp. has increased, mainly in inmmunocompromised patients. Almost 90% of AIDS patients develop at least one fungal infection over the course of disease, out of which 10–20% of infections prove fatal. Treating these patients with systemic AmB it is quite difficult because of the ongoing treatments with antiretroviral and other immunosuppressant drugs. On the other hand, Candida spp. infection in the context of burn wounds leads to invasive disease with a 14-70% mortality rate. AmB is the first line treatment for burn-related fungemia attributed to Candida species as well as many moulds. Topical AmB is the election treatment for fungal occular infections caused by Candida spp., which can cause blindness, and for cutaneous leishmaniasis (major tropical skin diseases caused by a variety of parasites of the Lesihmania genus). Counting on a topical formulation of AmB would be a goal to safely treat locally invasive fungal disease and leishmaniasis. Topical route enables a painless self administration, avoiding the use of injectablea and of systemic cytotoxicity caused by parenteral AmB. However the large molecular weight and amphoteric nature of AmB in Fungizone (the micellar formulation) hinders its adequate cutaneous penetration. Besides, the high cost and low skin penetration of AmBisome (the liposomal formulation), limits its clinical use in topical applications.

Several attempts have been made to develop a topical formulation for AmB, namely liposomes, microemulsions, nanoemulsions, chitosan nanoparticles, lipid-based microtubes and ethosomes. Excepting the ethosomes however, the reminder AmB formulations act as skin surface depots, which do not enhance the AmB penetration within the epithelia.

In this work we show that a deep access of AmB towards deep epithelial layers, with no aid of permeation enhancers, was achieved by loading AmB within ultradeformable nanoliposomes. Ultradeformable nanoliposomes (UDL) are highly deformable (elastic¬/flexible) nanoliposomes made of phospholipids plus edge acti¬vators (EA, surfactants of high radius of curvature and mobility), capable of penetrating the intact skin across the stratum corneum and reach the viable epidermis.

The challenge of this work was tuning the UDL bilayer composition so as to effectively achieve the loading of a quantitative amount of AmB. To that aim, we prepared and characterized diferent UDL lipid matrices containing AmB, soya phosphatidylcholine (SPC) and sodium cholate or Tween 80 as EA. The toxicity on keratinocytes and machophages by MTT and LDH leakage was determined for those formulations with the highest elasticty and AmB content. After that, the in vitro antifungal activity of UDL-AmB against albicans and non-albicans Candida ATCC strains, and against clinical isolates of C.albicans albicans were tested. The in vitro antileishmanial activity of UDL-AmB on Leishmania braziliensis promastigotes and intracellular amastigotes were also determined. Finally the in vitro skin penetration of UDL-AmB on human skin and storage stability were determined.

Results: In first place, the influence of type of EA, SPC concentration, AmB concentration and SPC:EA ratio on deformability and AmB incorporation was tested. Nanoliposomal formulations contaning Tween 80 showed the highest deformability and AmB incorporation. The size of the resultant UDL-AmB was 107 ± 8 nm with polidispersity index of 0.078 and Z-potential of -3 ± 0.2 mV, encapsulation eficiency of 75%, being unilamellar vesicles as shown by transmission electron microscopy. UV-Vis spectral characterization of UDL-AmB showed that AmB fully associated to nanoliposomal bilayers in the monomeric form.

Overall, both AmBisome and UDL-AmB caused more damage on plasma membrane than on mitochondrial activity, meaning that no cytotoxicity, as measured by MTT, was registered. Nonetheless, the lowest and highest toxicities of UDL-AmB ocurred on cells exhibiting lower (HaCaT) and higher (J774) endocytic uptake capability, respectively. The macrophage toxicity of UDL-AmB was higher than that of AmBisome: the UDL-AmB IC50 was < 1.25 µg/ml, vs 6 µg/ml for AmBisome. On the contrary, no differences were found on UDL-AmB and AmBisome IC50 on HaCaT cells cells, which resulted > 6 µg/ml.

We also found that fungal strains were more sensitive that mammal cells to AmB liposomal formulations. UDL-AmB MICs (total inhibition of visible growth, equivalent to 99% inhibition) were equal or lower than AmBisome and free AmB MICs for the 5 Candida ATCC strains, and the clinical isolates of C.albicans albicans. UDL-AmB MICs were between 0.06 and 0.25 µg/ml and between 0.12 and 1 µg/ml for AmBisome. These values were 5-24 and 24-50 folds lower that IC50 on J774 for UDL-AmB and AmBisome, respectively.

Besides, confocal fluorescence microscopies of rhodamine-labeled UDL-AmB incubated with C. albicans at concentration > MIC showed a fast transfer of the fluorescent dye within the fungus body.
UDL-AmB showed comparable anti-leishmania activity than AmBisome. UDL-AmB showed 100% of anti-promastigote activity at 0.6 µg/ml and 25 and 75 % anti-amastigote activity at 0.6 and 1.25 µg/ml, respectively (figure 1).

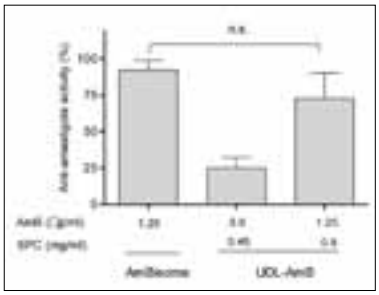

Figure 1. Anti-amastigote activity of AmBisome and UDL-AmB on intracellular L. braziliensis. Anti-amastigote activity was expressed as: % AA=[1–(no. of amastigotes/100 cells) treated/ (no.of amastigotes/100 cells) untreated infected macrophages]×100. n.s.: not significat difference by one-way ANOVA followed by Tukey's test

Finally, upon 1 h of incubation on human skin, 33 ± 2 % and 7 ± 2 % of the administered dose of UDL-AmB was found in the stratum corneum and viable epidermis, respectively (figure 2). In contrast, AmB was not found in the stratum corneum and less than 2 % of the administered dose was found viable epidermis when AmBisome was applied. The total accumulation of AmB in skin was 40 folds higher when applied as UDL-AmB than as AmBisome.

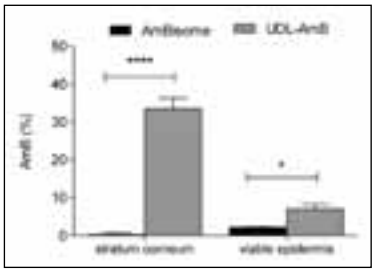

*Figure 2. Percentage of AmB accumulation upon 1 h incubation of AmB liposomal formulation on human skin. * p < 0.05; *** p < 0.001*

Conclusions: the most remarkable finding of this work can be summarized as follows: altough UDL-AmB formulation showed comparable anti-fungal and anti-leishmanial activities with AmBisome, only UDL-AmB provided a considerable increased AmB skin deposition. Therefore the UDL-AmB formulation would be advantageous over other liposomal AmB formulations for topical fungicidal and leshminicidal treatments that require a targeted delivery of AmB to the viable epidermis and dermis.

ALBUMIN NANOPARTICLES INCREASE THE ANTI-CANCER EFFICACY OF ALBENDAZOLE IN OVARIAN CANCER XENOGRAFT MODEL

NOORANI LUBNA

Epithelial ovarian cancer is the leading cause of death among gynaecologic malignancies. The majority of the patients present with an advanced-stage disease (Stage III and IV) and experience recurrence of the disease. This cancer tends to be relatively aggressive and there are no proven early detection tests [1]. Standard treatment of ovarian cancer currently includes the surgical staging and debulking followed by chemotherapy. Chemotherapy such as paclitaxel (PTX) coupled with a platinum salt such as cisplatin/oxaliplatin is considered as a standard treatment of ovarian cancer [2]. However, due to poor solubility in water PTX was first formulated in a mixture of cremophor EL/absolute ethanol (1:1 in volume) as 'Taxol'. Due to the large amount of solvent used and the nonspecific bio-distribution of the drug in both the tumor and normal tissues, Taxol has been associated with serious side effects such as severe hypersensitivity reactions, myelosuppression and neurotoxicity [3]. In addition, PTX resistance is also very common due to the poor availability of systematically administered drugs and phenotypic alteration in the cancer cells [4]. Therefore, there is a great necessity to develop a new treatment strategy such as different drug or an alternative delivery system to increase the drug concentration at tumor sites and to maximize the therapeutic efficacy while minimizing the side effects of ovarian cancer.
Albendazole (ABZ) [1 methyl (5-proylthio) 1H-benzylBZ-2yl carbamate], a well-established anti parasitic drug, has already been effective against preclinical models of ovarian cancer. Albendazole inhibits microtubule polymerization by binding and interference with β tubulin (isotype 2) dynamics [5] leading to the disruption of microtubules during cell division. Interestingly, microtubule disrupting agents (MDAs) are a highly effective group of drugs widely used in the treatment of cancer [6, 7]. ABZ has been shown to be a potential inhibitor of VEGF [8], hypoxia inducible factor (HIF1-α) [9] and angiogenesis [10] that are responsible for the development and progression of ovarian cancer. Inhibition of VEGF suppresses tumour growth and malignant ascites formation [8]. Substantial evidence suggests that VEGF promotes the formation of ascites and is present in very high levels in the ascites of patients with advanced ovarian cancer [11].Treatment with a suspension of albendazole in hydroxypropyl methylcellulose (0.5% HPMC) in nude mice showed the inhibition of tumor growth and metastasis [12]. It also showed superior effects over paclitaxel in suppression of VEGF and ascites in ovarian cancer bearing nude mice [13]. Therefore, it has the potential to be repositioned as an anticancer agent and preferably for the treatment of chemo resistant ovarian cancer with malignant ascites. With these perspectives, in mind, albendazole may be repurposed as a new treatment for ovarian cancer due to its anticancer properties.
Due to the extreme low aqueous solubility (0.55 µg/ml) and poor bioavailability [14], the potential of ABZ for the treatment of cancer is limited. We perceived that nanotechnology and in particular nab-technology as a potential drug delivery system for albendazole to increase solubility and selective delivery to tumors. Nab-technology is basically non-covalent association of hydrophobic drugs with albumin and the formation of nanoparticles that are readily water dispersible without any solvent and surfactant. It achieved targeted and improved drug delivery to tumors by exploiting the biological pathways of albumin [15]. One of the most important features of a nab-technology based drug delivery system is the enhanced accumulation of nab drug conjugates in tumor tissue due to the leaky vasculature found in the angiogenic vessels in solid tumors [16]. Drug delivery via nanoparticles presents novel therapeutic opportunities for the drugs which were previously unsuitable for traditional, oral or injectable therapeutic formulations, hence allowing active agents to be delivered effectively while minimizing side effects.

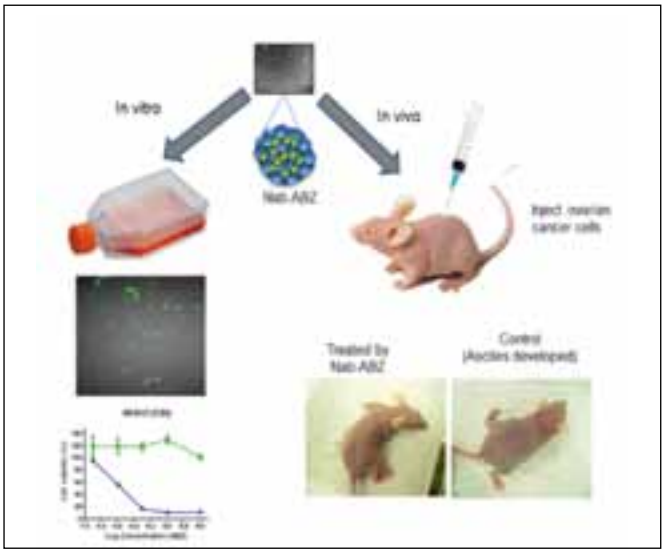

Figure 1: In vitro and in vivo effects of nanoparticle formulation.

In this study, we have assembled ABZ with bovine serum albumin into nanoparticles with a size range of 7-10 nm (BSA-ABZ) and 200-250 nm (Nab-ABZ). We further examined the anticancer effects of ABZ carrying nanoparticles in ovarian cancer cells, in both in vitro and in vivo models. Drug release studies demonstrated that about 93% of ABZ was released from BSA-ABZ 10 nm in comparison to 83% from Nab-ABZ 200 nm at pH 7.4 in 8 days. In vitro cell proliferation studies showed that the BSA-ABZ 10 nm exhibited the highest killing efficacy of ovarian cancer cells with surprisingly least toxicity to healthy ovarian epithelial cells. Confocal microscopy and fluorescence activated cell sorting analysis (FACS) revealed more efficient internalization of the BSA-ABZ 10 nm by cancer cells. For in vivo studies, we examined the tumor growth, ascites formation and the expression of VEGF and secreted protein acidic and rich in cysteine (SPARC) in tumor samples and only VEGF in plasma samples. The BSA-ABZ 10 nm reduced the tumor burden significantly ($p<0.02$) at a much lower drug dose (10 µg/ml) compare to free drug. Both formulations were capable of suppressing the ascites volume significantly ($p<0.05$) and reducing the number of ascites cells. The expression of VEGF and SPARC was also reduced, which indicates the underlying therapeutic mechanism of the ABZ.

Our data suggest that the BSA-ABZ may hold promise for the treatment and control of progression of ovarian cancer with ascites. However further studies are required to examine the efficacy of both the formulations in aggressive models of recurrent ovarian cancer with a more comprehensive efficacy comparison with respect to particle size and dosing parameters.

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EX VIVO MRI CHARACTERIZATION OF THE PERMEABILITY TO NANOPARTICLES OF ATHEROSCLEROTIC PLAQUES IN APOE (-/-) MICE

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Atherosclerosis is an artery degenerative disease resulting in plaques leading to stenosis, embolization and thrombosis. In order to develop and validate novel nanosystems for targeted imaging and therapy of advanced atherosclerotic diseases, the FP7 project "NanoAthero" has been funded and is now in progress with the active contribution of several European research labs. A specific task of NanoAthero is the definition of an MRI method to evaluate the efficacy of nanosystem-based therapies; efficacy which is known to be dependent on the plaque endothelium permeability. Such MRI method would be a powerful tool in the hands of clinicians to address tailored therapeutic treatments for every single patient.

Different animal models have been so far used to study pathogenesis and potential treatment of atherosclerotic lesions. In particular it has been shown that apolipoprotein E-deficient mice (ApoE-/-), created by homologous recombination in embryonic stem cells, develop severe hypercholesterolemia and atherosclerosis with characteristics and distribution similar to those observed in humans.

In this study, plaque permeability was evaluated in ApoE (-/-) mice using ex vivo MRI and the albumin binding contrast agent B22956/1. The histological analysis of plaques is in progress to explore the possible occurrence of a correlation between the plaque composition/vulnerability with MRI signals.

ApoE (-/-) mice were fed with a high-cholesterol diet from 8 weeks of age. MRI experiments were performed at three time points to evaluate innominate artery plaque development: group 1 - mice at early stage (from 8 to 11 weeks of high fat diet); group 2 - mice at intermediate stage (from 14 to 19 weeks of high fat diet); group 3 - mice at very advanced stage (from 29 to 32 weeks of high fat diet). B22956/1 was intravenously repeatedly administered (5 times, with an interval of one hour between each treatment) at a dose of 0.03 mmol/kg. Then, mice were submitted to myocardial perfusion to recover the innominate artery and the arch; vessels samples were placed on a layer of agar (2%), embedded in OCT (Optimal Cutting Temperature compound) into an embedding mold and imaged on a 3T MRI system. Coronal and transverse T2-weighted Spin-Echo images (SE) were acquired in order to identify the best geometry to visualize the innominate artery and T1-weighted gradient echo (GE) images to evaluate the contrast agent effect.

MRI plaque differential signal was obtained by subtracting background from the raw signal intensity into the plaque (Figure 1).

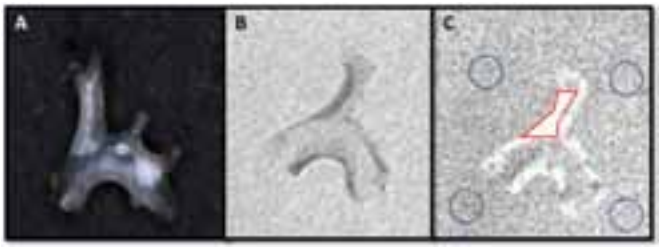

Figure 1. Region of Interest (ROIs) definition: bright field (A), T_2-

weighted Spin-Echo (B) and MRI T_1-weighted gradient echo (C). Blue ring → background; red zone → plaque in the innominate artery
The following evidences were found in the analysis of differential MRI signals at three different staging time points of innominate artery plaques.

- In untreated animals the innominate artery staging is reflected by the decrease of the mean differential signal;
- The differential signal intensity in the plaque shows a large variability within all groups of B22956/1 treated mice;
- After B22956/1 injection, plaque signal is strong in mice at early (group 1) and very advanced (group 3) plaque stage with respect to untreated animals, differently from what occurs in animal belonging to the intermediate (group 2).

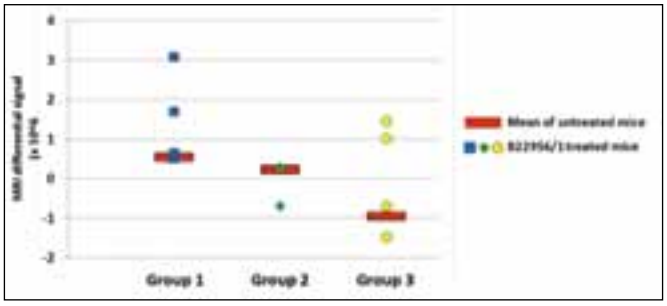

Figure 2. MRI signal analysis.

The MRI analysis demonstrates that without a clear assessment of the plaque composition by means of a suitable histological analysis, it is not possible to understand the diagnostic value of a contrast enhanced MRI procedure. Moreover the inherent differences among the MRI signal of plaques at increased fat diet times suggests that the composition of plaques is changed among the groups possibly with a reduction of the amount of interstitial water which could affect the estimation of the amount of extravasated contrast agent into the plaques.

PRODUCTION OF SCR1-3 COMPLEMENT-ACTIVATING PROTEINS

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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.Figure 1

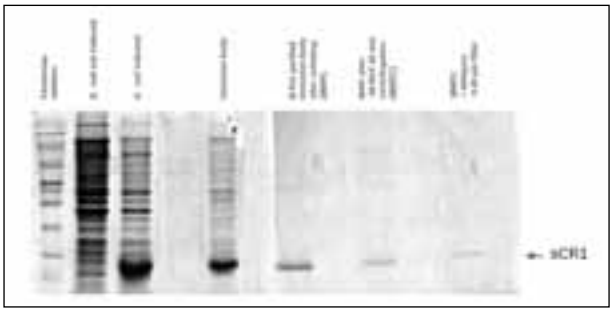

Figure1: sCR1-3 expression and refolding sCR1-3 was expressed in E. coli Rosetta DE3 strain under autoinduction conditions (Studier W. 2005), inclusion body was purified using B-PER reagent (Lane4), resolved in 8M UREA, 1M NaCl, 50 mM bME and refolded in 20 mM ethanolamine buffer pH10 with the presence of GSH/GSSG redox pair. (lane 5). Subsequently the unfolded protein aggregates were pelleted by centrifugation (30000 g/30 min) Lane 6, and filtered (lane 7)

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LIPOSOME-ENTRAPPED MITOMYCIN C PRODRUG (PROMITIL®) IS STABLE IN PLASMA BUT RAPIDLY ACTIVATED IN TUMOR AND TISSUES

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INTRODUCTION

A preclinical formulation of lipid based mitomycin-C prodrug (MLP) formulated in pegylated liposomes (PL-MLP or Promitil®) was previously studied for its pharmacokinetics and therapeutic efficacy in various tumor models. All these studies consistently showed plasma stability, long circulation time, superior efficacy and lower toxicity of PL-MLP compared to free mitomycin C (1-3). The present study examines the biodistribution of MLP in tumor-free and tumor-bearing mice in-vivo and the cleavage and activation of MLP by mouse tissues in an ex-vivo assay. MLP is cleaved by thiolysis, generating mitomycin C, in the presence of thiol donors, with or without involvement of the thioredoxin-thioreductase system (1,2). Prodrug-generated mitomycin C alkylates and cross-links DNA or undergoes rapid inactivation by further reductive processes (4-6).

EXPERIMENTAL METHODS

The pharmacokinetics of the prodrug MLP and of the liposomal carrier was measured in plasma and in various tissues after i.v. administration of a radiolabeled H3-cholesterol (H3-chol) PL-MLP formulation in M109R and KB tumor bearing mice and in normal mice. Additional ex-vivo tests were performed to study prodrug activation in various tissues excised from tumor bearing mice, disrupted by homogenization and sonication, and incubated with PL-MLP in PBS at 37°C for 2 hr with shaking. Tissue samples from in vivo or ex vivo studies were extracted in isopropyl alcohol and then analyzed by HPLC for quantification of MLP. A ratio of 100 mg tissue for 250 µg/ml MLP was used in the ex-vivo assay.

RESULTS AND DISCUSSION

The plasma levels of MLP and H3-chol of radiolabeled PL-MLP liposomes were high and comparable in relative terms during the

first 24hr after injection as shown in Figure 1, indicating that no apparent leakage or cleavage of MLP occurs in plasma.

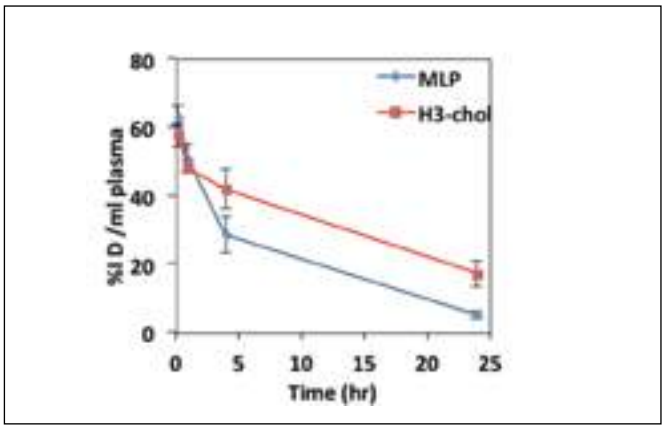

Figure 1: The plasma levels of MLP and H3-chol at different time points after the injection of radiolabeled PL-MLP in normal mice.

In biodistribution studies, the highest levels of MLP were found in spleen and in tumor tissue as shown in Table 1. The levels of MLP in liver and lung were not detectable or very minimal, suggesting that these tissues can activate and metabolize MLP at a much faster rate than other tissues. H3-chol levels, indicative of the presence of the liposome carrier, were relatively much higher than those of MLP in all tissues indicating that the prodrug MLP is cleaved and free MMC released shortly after liposomes deliver it to tissues. Ex-vivo results revealed that liver tissue is a potent activator of prodrug MLP, with ~70% of the prodrug degraded after a 2 hr incubation, while other tissues appear to have much less or much slower metabolizing activity.

Table 1: Plasma and tissue distribution of MLP and H3-chol in KB tumor bearing mice after 24 hr injection of radiolabeled PL-MLP.

	KB tumor bearing mice			
	% ID/ml plasma or gm of tissue			
	MLP	SEM	H3-chol	SEM
Plasma	9.3	1.39	7.0	0.64
Liver	n.d.	n.d.	14.0	1.11
Spleen	1.0	0.23	16.4	2.68
Kidney	0.7	0.06	6.6	1.00
Lung	n.d.	n.d.	13.9	2.03
Heart	0.1	0.01	4.7	0.21
Tumor	1.0	0.12	4.8	0.35
Skin	0.7	0.06	2.6	0.07

CONCLUSION

PL-MLP liposomes demonstrate long circulation and very high stability in plasma with relatively equivalent levels of the prodrug MLP and H3-chol indicating prodrug leakage, or cleavage occur in circulation at very low levels. In contrast to plasma, the prodrug MLP is rapidly metabolized in tissues with liver and lung, being the most effective metabolizers. Ex-vivo results confirm the in-vivo data signifying the high potential of liver above other tissues to metabolize the prodrug. High levels of prodrug were detectable in tumor tissue where the prodrug is slowly cleaved releasing active free drug. These results have important implications on the pharmacology and toxicology of the PL-MLP formulation which is currently undergoing clinical testing (7).a

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NOVEL CHITOSAN DERIVATIVES FORMULATIONS FOR WOUND HEALING

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Background: Dermal wound healing is a complex process and includes four overlapping steps, which are: inflammatory, migratory, proliferatory and maturation phase [1]. In most cases, tissue repair can occur spontaneously, but this process depends on the size and depth of the wound. In the case of non-healing wounds, regeneration needs to be guided by biological cues.
Aim of the study: The CMTMC synthesis optimization and assessment of two different formulations as hydrogels and as lyophilized wound patches.
Materials and methods: In this view, we synthesized O-carboxymethyl-N,N,N-trimethyl-chitosan (CMTMC) from chitosan via trimethylation and carboxymethylation steps [2]. CMTMC derivative is known to promote cell adhesion and differentiation. However, previous described CMTMC in the literature lead to low carboxymethylation efficiency. Hence, the first aim of this study was to optimize CMTMC synthesis protocol towards high carboxymethyl grafting (Fig. 1). A high degree of carboxymethylation is needed in order to achieve efficient peptide grafting. Peptide moieties like Arg-Gly-Asp (RGD) were used, which are known to induce cell adhesion and migration [3].

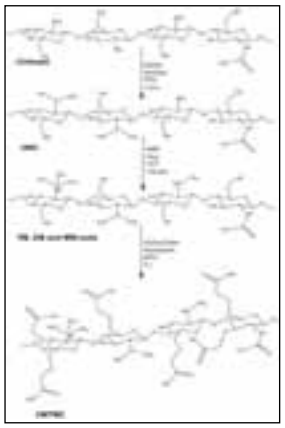

Fig. 1. Improved CMTMC synthesis via trimethylation and carboxymethylation.

Wound dressings comprising healing-promoting peptides and polymers to be applied as hydrogels for topical application were studied. Here, the second aim of the study was to investigate two formulations: hydrogels and lyophilized wound patches. This was performed using different concentrations of both CTMC and hyaluronic acid (HA).
Results: CMTMC was synthesized from TMC through an optimized protocol, and its structure was characterized by 13C-NMR and 1H-NMR. The optimized protocol yielded a very high substitution degree of carboxymethyl grafted on the TMC (>85%). In this view, the newly developed carboxymethylated chitosan can be used as starting material for different approaches like gene and drug or protein delivery systems. The cytotoxicity tests in vitro proved that

our CMTMC had no toxic effects on human dermal fibroblasts at concentration up to 5 mg/ml.
We investigated composite hydrogels based on CMTMC blended with HA in NaCl 0.9% and 1.2%. Combining CMTMC and HA led to polyelectrolyte formation in a viscous gel which could be used for a topical application.
As for a wound patch application, all formulations prepared in NaCl 1.2%, after lyophilization were brittle, while those prepared in NaCl 0.9% were soft foams. Hydrogels became more viscous with both increasing shear rate and HA concentration (Fig. 3). The CMTMC amount was maximized to increase peptide content, and thus bioactivity. Concentrations s of 0.5% of HA and 3.0% CMTMC led to a clear phase separation in both 0.9% and 1.2% of NaCl. The samples were further defined based on stability and viscosity criteria. The selected formulation for topical application as hydrogels was CMTMC 3.0% and HA 2.0% in NaCl 0.9% (Fig.2) and as lyophilized formulation is CMTMC 3.0% and HA 1.5% in NaCl 0.9% (Fig. 3).

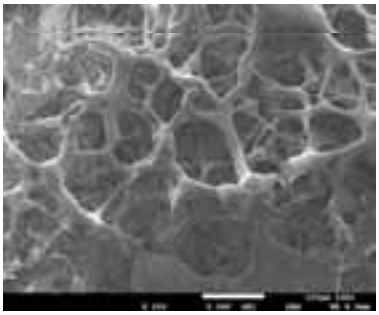

Fig. 2. SEM photo for lyophilized hydrogel : CMTMC 3.0 and HA 1.5% in NaCl 0.9%

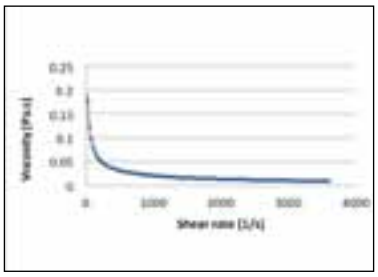

Fig. 3. Effect of shear rate on CMTMC formulations viscosity

Conclusions: The obtained results showed a successful carboxymethylation with a high degree of substitution (>85%). Such carboxymethylated chitosan could further serve as a starting material for numerous applications such as vaccine, protein or drug delivery, in order to enhance cell uptake. Composite hyaluronic/derivatized chitosan formulations were designed for topical application, either under a gel or a dry patch form, that included peptide-displaying nanocomplexes. Bioactivity of these formulations and wound healing promotion need to be evaluated in vitro and in vivo.

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SHELLFISH POISONING INTOXICATION PREVENTION: NOVEL APPROACH BASED ON PLASMONICS SERS OF AG NANOPARTICLES-BIOTOXINS INTERACTION AND DETECTION

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Keywords: DSP, ASP, PSP toxins, shellfish poisoning, molecular plasmonics, SERS.

This paper provides our recent developments in the optical biosensing nanotechnology in answer to the current need for seafood control and intoxication prevention. Shellfish poisoning diseases produced by consuming oyster, mussels, clams and other seawater products enriched in harmful compounds as a consequence of unpredictable seasonal algal bloom. Such events could be fatal for consumers when prompt methods to freshly evaluate the toxins content status are lack in the field. Here we describe the novel approach based on surface enhanced Raman scattering of various paralytic shellfish poisoning (PSP), amnesic shellfish poisoning (ASP), diharretic shellfish poisoning (DSP) biotoxins that could the accumulated in the edible tissue of the seafood products.
Although considered a delicacy and largely appreciated for unique taste and health benefits, seafood is one of the most perishable product that could reach consumers is the shortest time. Increasing number of intoxication cases are reported worldwide. Patients seeking treatment for seafood-borne illnesses may present a diagnostic challenge to the care provider as they may have variable presentations of signs and symptoms, different degrees of severity, as well as unclear timelines in relation to ingestion. The scope of seafood-borne illness is vast, and, with increased travel and expanding seafood trade, the likelihood of a care provider seeing a seafood-borne illness is increasing.
Conventional agreed techniques of monitoring cannot provide prompt answer on the product status and chemical composition. For example, the first symptoms in the case of PSP intoxications appear after the toxin adsorption through the buccal mucosa further resulting in tingling sensation or numbness around lips and spread gradually to the face and neck in several minutes after shellfish ingestion. Many seafood detoxification methods were proposed [1] most of them with poor success rate, since some species could remain in edible tissue for months, depending on the shellfish species an local environmental conditions.
Aiming to translate SERS technique to the specific monitoring programs for fast and prompt screening in the aquaculture sector [2], we investigated here the SERS response of the first group of DSP toxins, namely okadaic acid (OA), dinophysistoxin-1 (DTX-1), dinophysistoxin-2 (DTX-2) in the presence of silver nanoparticles (AgNPs). These species were accessible at extremely low concentrations (13.7, 15.1 and 7.8 µg/ml respectively) in methanol with 0.1 mM acetic acid and were determined by HPLC.
Because of their extremely weak concentrations of the commercially available solutions, to record Raman spectrum of the solid species for reference database purpose was a challenge. Additionally, the conventional SERS sampling used for various molecular species requiring about 50:1 (v/v) AgNPs/ dissolved sample ratio failed in the case of biotoxins solutions. Exploiting the flexibility of the Renishaw InVia Raman instrument with a modified SERS sampling protocol we succeeded in recording excellent SERS signal of toxins (Fig.2). SERS spectra revealed a series of similarities consistent with the related molecular structures. For instance, OA was clearly differentiated by DTX-1 and DTX-2 through the missing band at 1017 cm^{-1} assigned to $-CH_3$ vibrational mode. All of these three toxins have a variety of 7-O-acyl ester derivatives that are produced by shellfish. DSP toxins are produced by select dinoflagellates belonging to the genus Dinophysis and Prorocentrum.
The observed SERS bands were assigned with the accomplishment of the DFT calculations. For example, in the case of okadaic acid, the common vibrational modes of the C=C, C=O, C-C, C-O, C-OH, C-O-C, -OH, -COOH, $C-CH_3$ groups were dominantly observed in SERS at 1649, 1619, 1585, 1441, 1370, 1291, 1175, 1017, 909, 806, 581, 478, 441, 422, (Fig. 2) and the similar Ag-toxin SERS band at 237 cm-1 suggests similar chemisorption on the AgNPs.
The ultimate challenge was the similarity of the SERS signal with that of natural seawater. In this respect, systematic assessment of the seasonal variance of the seawater was achieved. The long term run of the study was focused on the seawater and seafood in the aquaculture area in South-Eastern Adriatic coast of Croatia with particular emphasis on the aquaculture area in Mali Ston Bay. Seawater and seafood samples were

collected from the aquaculture area in Mali Ston Bay in early spring, when the dinoflagellates dominates the plankton community [3] as well as in summer, autumn and winter, to assess their spectral variability link with the food web. A complete analysis of the toxin profile in shellfish farmed on the Adriatic Sea Coast of Croatia has been achieved using the LC-MS/MS technique [4]. For reliable SERS spectral database a complete theoretical characterization of the toxins structure was achieved using DFT calculations. Optimized detection protocols were set up to overcome the limited amount of sample commercially available. As such, microcrystals of OA were assessed in special measurements conditions to provide the pure and highest concentration available for spectral reference. Okadaic acid DSP toxin was detected using LC-MS method at 7 µg/kg shellfish meat (SM). The EU regulation limit value is 0.16 mg/kg tissue while SERS achieved detection at ng/mL seawater level. The promising results are attractive for translating knowledge to the seafood control in real time. Presentation will resume the specificity in SERS detection for each toxin group and will highlight the advantage of technique for low cost, fast and sensitive alternative for intoxication prevention.

Fig. 1. Graphical sketch of the SERS –based plasmonics detection of biotoxins in shellfish edible tissue. The approach is strongly dependent on the prior development of adequate SERS database.

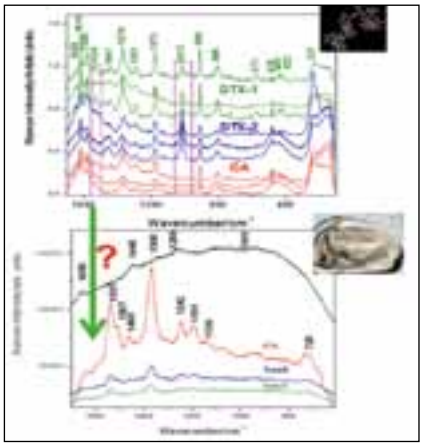

Fig. 2. Characteristic SERS signal of DSP toxins, okadaic acid (OA) and its structural analogs dinophysistoxin-2 (DTX-2), the methylated derivative and dinophysistoxin-1 (DTX-1), used for tissue analysis. The specific differences are highlighted. Laser line 532 nm. Molecular structure of OA toxin is inserted.

Bottom: Typical SERS signal collected from edible tissue after NPs incubation. Better enhancement is achieved upon sample drying. Noteworthy the strong fluorescence of the tissue when (black spectrum) conventional Raman techniques are applied to characterize its chemical composition. The spectral range in topic focus is around 1616-1648 cm^{-1} where the toxin SERS fingerprint revealed highest intensity for all the three groups (PSP, ASP, DSP toxins).

ACKNOWLEDGMENTS

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A NANO-DISC FORMULATION OF A NOVEL SMALL MOLECULE TLR4 ANTAGONIST (IAXO-102) INHIBITS AORTIC ANEURYSMS DEVELOPMENT

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Objectives: Toll-like receptors (TLRs), including TLR4, have been shown to play a crucial role in vascular inflammatory diseases, such as atherosclerosis and aneurysm. The main goal of this study was to determine the potential of IAXO-102 a novel small molecule TLR4 antagonist, to modulate non-hematopoietic TLR4 proinflammatory signalling and inhibit experimental abdominal aortic aneurysm (AAA) development using LipodisqTM as a novel drug formulation.
Methods: Angiotensin II-induced experimental AAA development was used as a murine in vivo model. TLR4 antagonist IAXO-102 formulated in Lipodisq™, a lipid based discoidal nano-particle preparation (Malvern Cosmeceutics, Malvern, UK) was provided by Innaxon, UK.

IAXO-102 DOWN-REGULATES TLR4 SIGNALLING IN MOUSE AORTA

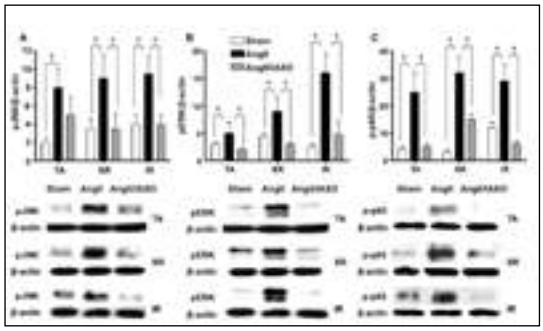

Figure 1. IAXO-102 inhibits Angiotensin II-induced JNK, ERK and p65 NF-kB phosphorylation in the initiation stage of experimental aneurysm. Mice were divided into three groups: sham control negative group, Angiotensin II group, Angiotensin II/IAXO-102 co-treated group. IAXO-102 (3 mg/kg/day in 50 µl Lipodisq™) was administered s.c. up to 72 h. Tissue samples from thoracic aorta (TA); supra-renal aorta (SR) and infra-renal aorta (IR) were prepared at 72 h, and soluble proteins were analysed for JNK phosphorylation (A), ERK phosphorylation (B) and p-65 NF-kB phosphorylation (C) using immunoblotting analysis. Actin was used as a loading control. Data are mean ± SD, n = 4 at each data point. p<0.05 [Anova].*

IAXO-102 MODULATES EXPRESSION OF ANGIOTENSIN II DRIVEN PROTEINS IN MOUSE AORTA

Pro/inflammatory proteins	Sham	AngII	AngII IAXO	Pro/inflammatory proteins	Sham	AngII	AngII IAXO
1. RLC	1.0	1.12	1.31	21. IL-17	1.0	2.32	1.26*
2. CD40L	1.0	0.90	0.95	22. I-TAC	1.0	2.33	1.91
3. Eotaxin 1	1.0	1.29	1.28	23. KC	1.0	1.74	0.99*
4. Eotaxin 2	1.0	0.84	0.84	24. Leptin	1.0	2.11	1.09*
5. FasL	1.0	1.03	1.12	25. GDF	1.0	1.37	1.11
6. Fractalkine	1.0	1.37	0.93	26. Lymphotactin	1.0	1.11	0.88
7. GCSF	1.0	2.45	0.99*	27. MCP-1	1.0	1.00	0.90
8. GM-CSF	1.0	2.51	1.42*	28. MCSF	1.0	1.11	0.98
9. IFNg	1.0	1.50	1.09*	29. MG	1.0	1.33	1.25
10. IL-1a	1.0	1.36	1.18	30. MIP-1a	1.0	1.72	0.92*
11. IL-1b	1.0	1.61	1.40	31. MIP-1c	1.0	9.50	3.00*
12. IL-2	1.0	0.90	0.55	32. RANTES	1.0	1.81	0.84*
13. IL-3	1.0	2.41	1.79*	33. SDF-1	1.0	1.72	1.11*
14. IL-4	1.0	1.11	1.09	34. TCA-3	1.0	1.83	0.88*
15. IL-6	1.0	1.40	1.40	35. TECK	1.0	1.88	0.90*
16. IL-9	1.0	1.31	1.21	36. TIMP-1	1.0	0.77	1.12*
17. IL-10	1.0	3.33	2.00*	37. TIMP-2	1.0	2.11	0.81*
18. IL-12p40	1.0	4.03	2.80*	38. TNFa	1.0	1.66	0.90*
19. IL-12p70	1.0	1.67	0.97*	39. TNFRI	1.0	2.10	1.68
20. IL-13	1.0	1.42	1.08*	40. TNFRII	1.0	1.10	0.88

Table 1 Mice were divided into three groups: sham control negative group, Angiotensin II group, Angiotensin II/IAXO-102 co-treated group. IAXO-102 (3 mg/kg/day in LipodisqTM) was administered s.c. up to 72 h. Tissue samples from supra-renal aorta were prepared at 72 h, and soluble proteins were semi-quantitatively analysed on mouse Inflammation antibody array (Ray-BioTech) following manufacturer's instructions. Data are normalized to array controls and expressed as a fold change in comparison to untreated controls. IAXO-102 down regulates TLR4-dependent pro-inflammatory proteins (*).

IAXO-102 INHIBITS THE RUPTURE, INCIDENCE AND DEVELOPMENT OF EXPERIMENTAL AAA

Groups	Mice number	Early rupture (%)	AAA Incidence (%)	Aortic diameter (mm)
Sham	6	0	0	0.86+/-0.08
Ang II	10	30	86	1.86+/-0.56
Ang II IAXO-102	10	0	30	1.05+/-0.18

*Table 2. Twenty six 6 months-old male Apo-E deficient C57BL/6 mice were divided into three groups: sham control, negative group, Angiotensin II group, Angiotensin II/IAXO-102 co-treated group. All animals had osmotic pumps implanted at time 0. The sham group (n=6) had pumps delivering saline whereas the other two groups (n=10) had pumps delivering Ang II (1 mg/min/kg). The Angiotensin II/IAXO-102 co-treated group received s.c. injections of IAXO-102 (3 mg/kg/day in 50 µl Lipodisq™) whereas the other two groups received daily s.c. injections of vehicle (Lipodisq™). Animals were euthanized at day 28 after pump implementation and the maximal aortic diameter was measured by computer micrometry. Data are mean ± SD, n = as indicated. p***<0.001 [Anova].*

Conclusion: IAXO-102, is as a glycolipid, poorly soluble in physiological solutions, which limits its bioavailability and distribution in vivo, By using Lipodisq™ technology we demonstrate the ability of IAXO-102 to modulate TLR4 signalling and to efficiently inhibit experimental AAA development, suggesting the potential therapeutic use of this TLR4 antagonist nano-carrier system for pharmacological intervention of AAA.

ACKNOWLEDGEMENTS

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COMPARISON OF POLYMERIC NANOPARTICLES AND OIL-IN-WATER NANOEMULSIONS FOR THEIR POTENTIAL TO INDUCE IMMUNE RESPONSES AGAINST M. TUBERCULOSIS IN VITRO AND IN VIVO

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INTRODUCTION

Nanotechnology in vaccines is of great scientific interest as nanoparticles may serve as delivery systems for the antigen and, depending on the composition, offer intrinsic adjuvant properties. In the present study N-trimethyl chitosan (TMC) nanoparticles and the oil in water emulsions Cationorm, a commercially available cationic nanoemulsion, as well as the cationic squalene in water emulsion SWE, were investigated as adjuvants and delivery systems. Chitosan derived polymers have been shown to activate innate immune receptors and influence dendritic cell maturation [1]. Oil in water emulsions activate immune responses upon cellular uptake, due to changes in lipid metabolism that is closely linked to innate immunity [2].

Furthermore, the cellular immune system can specifically be targeted by immunostimulatory adjuvants resembling certain antigenic parts of the pathogen, such as CpG motifs in bacterial DNA and muramyl dipeptide (MDP), being the minimal structures of bacterial cell walls.

In this study three different nanocarrier systems were evaluated in vitro and in vivo as delivery systems for genomic pDNA encoding Mtb antigen 85A. This plasmid also contains unmethylated CpG sequences that are known to activate TLR-9. Subsequently muramyl dipeptide (MDP) as a second immunostimulatory, targeting NLR-2, was added to the DNA nanoparticle conjugates to investigate immuno-potentiating effects in vitro.

EXPERIMENTAL METHODS

Trimethyl chitosan (TMC) nanoparticle formation was the result of polymer-polymer interaction of the oppositely charged polyelectrolytes TMC and chondroitin sulfate. Furthermore the two oil-in-water (O/W) nanoemulsions, squalene-in-water (SWE) with DOTAP and the commercially available Cationorm® formulation were investigated. Size and zeta potential of the nanoparticles was measured by means of dynamic light scattering and laser Doppler anemometry. The plasmid was adsorbed to the surface by electrostatic interaction and MDP either incorporated into the TMC nanoparticles or added to the aqueous phase of the O/W nanoemulsions.

RAW 264.7 murine macrophages were stimulated with pDNA loaded nanoparticles and the release of proinflammatory cytokine TNF-α determined by ELISA. A cell proliferation assay (XTT) was performed to confirm cell viability of these cells after exposure to the formulations for 24h. The potential to stimulate systemic immune responses by DNA nanoparticle conjugates and the adjuvant effect of these vaccine formulations was evaluated in C57BL/6 mice following intramuscular administration. IgG antibodies against antigen 85A were detected in the serum via endpoint ELISA.

RESULTS & DISCUSSION

Synergistic effect in vitro

No significant toxicity could be detected for all nanoparticle formulations compared to the untreated control. Levels of the proinflammatory cytokine TNF-α in the cell culture supernatant were determined by ELISA, as shown in Fig. 1. Marked increase of cytokine release with pDNA decorated nanoparticles compared to pDNA in solution could be observed with TMC nanoparticles. This also accounts for the delivery of MDP, showing only an adjuvant effect with TMC nanoparticles, but not with the nanoemulsions. However, the adjuvant function of TMC nanoparticles and Cationorm seemed to be important for the co-delivery of pDNA and MDP in one formulation. Herein a synergistic effect could be observed, as cytokine production of the combination of immune receptor ligands was higher than the calculation of the sum of the two applied as single ligands.

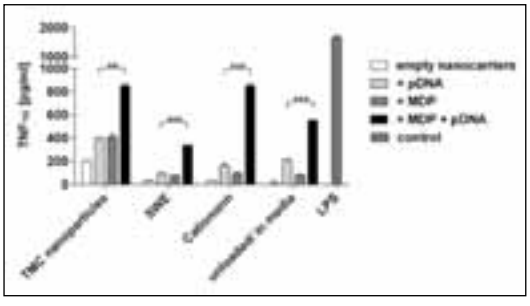

Figure 1: TNF-α release from RAW264.7 murine macrophages on exposure to different stimulating solutions. Ag85A-pDNA and MDP were applied either alone or in combination in solution, with TMC nanoparticles, SWE, and Cationorm®, respectively. Significant differences between the DNA formulations and the DNA+MDP

*formulations are indicated with **, *** (p <0.01, 0.001, respectively, Student's t-test).*

Adjuvant effect in vivo

In vivo a marked increase in IgG titers to Ag85A-pDNA loaded nanoformulations could be shown in vaccinated mice. IgG in sera of SWE and Cationorm® adjuvanted mice was enhanced, however, only in TMC vaccinated mice significant increased titers could be observed in comparison with pDNA alone. Ongoing in vivo studies are continued now with TMC nanoparticles as the most successful candidate for DNA delivery and will reveal if MDP also shows an immunopotentiating effect in mice as proven in vitro.

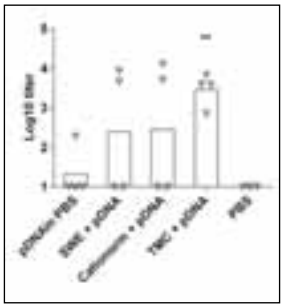

*Figure 1: Total IgG titers against Ag85A in serum from vaccinated C57BL/6 mice on exposure to different stimulating solutions one week after the last injection. pDNA in PBS, pDNA adsorbed to TMC nanoparticles, SWE and Cationorm®, respectively, and PBS where injected three times into mice in three week intervals. Significant differences between the DNA alone and adjuvanted DNA formulations are indicated with ** (p <0.01, Student's t-test).*

CONCLUSION

This study demonstrated that plasmid DNA adsorbed to TMC nanoparticles markedly increased pro-inflammatory cytokine release from murine macrophages in vitro. In all adjuvanted nanoformulations a synergistic effect could be observed for DNA in combination with MDP. According to the in vivo observations TMC nanoparticles in DNA vaccine formulations have shown to influence activation of the immune system, leading to significantly enhanced antigen specific immune responses.

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PACLITAXEL NANOCRYSTALS FOR TARGETED TUMOR DELIVERY

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Preparation of nanosuspensions of poorly soluble drugs is particularly attractive due to high drug loading and minimal use of excipients.[1],[2] In this work, we aim to obtain long circulating nanoparticles of hydrophobic anticancer drug paclitaxel, capable to accumulate passively in the tumor tissue via the enhanced permeability and retention effect after intravenous administration. Nanocrystals of paclitaxel were coated with multiple layers of oppositely-charged polyelectrolytes (layer-by-layer assembly) to control the dissolution rate of the drug. Poly(ethylene glycol) (PEG) was introduced in the last coating layer to reduce opsonisation and provide steric stabilization. To further improve the tumor targeting, Designed Ankyrin Repeat Protein (DARPin) binding to EpCAM molecule, overexpressed by many tumor types was selected (Figure 1).

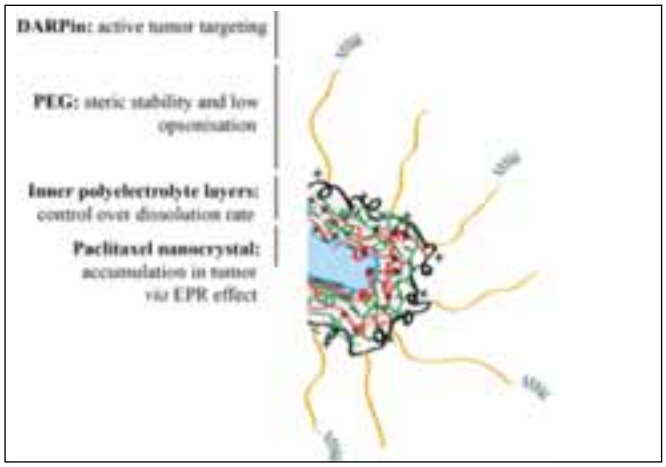

Figure 1. Graphical representation of the nanoparticulate system.

Paclitaxel nanocrystals were obtained by wet milling of the drug powder with a solution of sodium poly(styrene sulfonate). These template negatively charged nanocores were coated layer-by-layer with poly(L-arginine) (PLR) as polycation and poly(styrene sulfonate) as polyanion. It was found that the amount of the drug released from the nanoparticles was reduced after deposition of 5 layers of polyelectrolytes by ca. 30% after 8 h for PLR terminated particles. The last PEGylated layer consisted of the graft copolymer poly(L-lysine)-g-PEG/alkyne-PEG (PLL-g-PEG/alkyne-PEG) with ~40% of lysine residues functionalized with methoxy-PEG and ~10 % with alkyne-PEG. The alkyne groups will allow the further conjugation of a "clickable" azide-bearing DARPin.[3] The PEGylated nanoparticles had a diameter of 250±33 nm, zeta potential of around -7 mV, and were stable for at least 24 h when stored in cell culture medium at 37°C. In vitro cytotoxicity was assessed on human colon carcinoma cells (HT-29) with the MTS assay. It was found that the "non-targeted" coated nanocrystals exhibited an activity similar to that of the commercial nanoparticulate paclitaxel formulation Abraxane®. In subsequent experiments, the PLL-g-PEG/alkyne-PEG polymer will be conjugated to a "clickable" DARPin and used to decorate the nanoparticles. The cytoxicity and the cellular uptake of the "targeted" formulation will be compared with the "non-targeted" version.

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CYTO- AND GENOTOXICITY OF SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES IN GRANULOSA CELLS

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Nanoparticles, with the purpose to target cancer cells but spare healthy tissue, provide an attractive platform e.g. for hypothermia or as carriers of chemotherapeutics. According to literature, there are diverse effects of nanoparticles described on mammalian cells refered to reproductive tissue. To address nanoparticle impact on cytotoxicity and genotoxicity, we examine the effect of superparamagnetic iron oxide nanoparticles on granulosa cells, which play a

key role in ovarian function and female fertility. Human granulosa cells (HLG-5) are treated with nanoparticles either coated with dextran, lauric acid only or additionally with BSA (Fig. 1). Different concentrations are established for experiments and in a time dependent manner. Live/dead staining via flow cytometry (Fig.1) as well as impedance measurements give insights about cytotoxic effects on cells when treated with nanoparticles. Furthermore, detection of strand breaks are evaluated via gamma H2A.X and ATM antibody detection as well as via miconuclei scoring using flow cytometry and fluorescence microscopy. Since there are enormous advantages in using nanoparticles for medical applications we want to outline the importance of cyto- and genotoxic effects of nanomaterial on reproductive cells.

KEYWORDS

Superparamagnetic iron oxide nanoparticles; protein corona; cancer therapy and diagnosis; nano-toxicology, reproductive health; granulosa cells

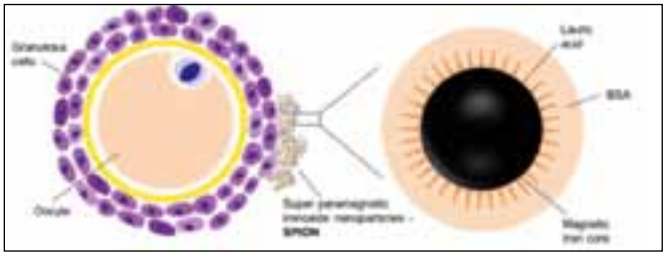

Fig. 1: a) Granulosa cells treated with super paramagnetic iron oxide nanoparticles – SPION. b) enlarged SPION, coated with lauric acid and a protein layer – bovine serum albumine to provide biocompatibility.

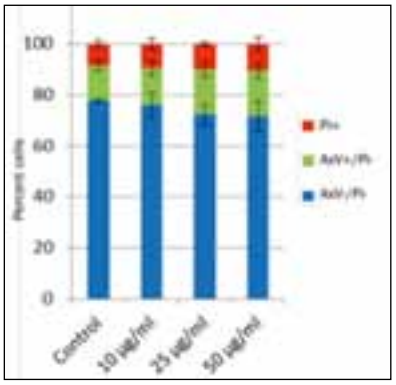

Fig. 2: Flow cytometry analysis of HLG-5 cell viability after treatment with superparamagentic iron oxide nanoparticles coated with lauric acid and bovine serum albumin. Annexin V/ propidium iodide staining, showing viable (AxV–/PI–), apoptotic (AxV+/PI–) and necrotic cells (PI+). Data indicating a vibrant trend that protein coated nanoparticles are highly biocompatible and therefore no maltreatment on reproductive tissue takes place.

LOCALIZED RNAI THERAPEUTICS OF CHEMORESISTANT GRADE IV GLIOMA USING HYALURONAN-GRAFTED LIPID-BASED NANOPARTICLES: LOCALIZED RNAI THERAPEUTICS OF CHEMORESISTANT GRADE IV GLIOMA USING HYALURONAN-GRAFTED LIPID-BASED NANOPARTICLES:

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Glioblastoma multiforme (GBM) is one of the most infiltrating, aggressive, and poorly treated brain tumors. Progress in genomics and proteomics has paved the way for identifying potential therapeutic targets for treating GBM, yet the vast majority of these leading drug candidates for the treatment of GBM are ineffective, mainly due to restricted passages across the bloodbrain barrier. Nanoparticles have been emerged as a promising platform to treat different types of tumors due to their ability to transport drugs to target sites while minimizing adverse effects. Herein, we devised a localized strategy to deliver RNA interference (RNAi) directly to the GBM site using hyaluronan (HA)-grafted lipid-based nanoparticles (LNPs). These LNPs having an ionized lipid were previously shown to be highly effective in delivering small interfering RNAs (siRNAs) into various cell types. LNP's surface was functionalized with hyaluronan (HA), a naturally occurring glycosaminoglycan that specifically binds the CD44 receptor expressed on GBM cells. We found that HA-LNPs can successfully bind to GBM cell lines and primary neurospheres of GBM patients. HA-LNPs loaded with Polo-Like Kinase 1 (PLK1) siRNAs (siPLK1) dramatically reduced the expression of PLK1 mRNA and cumulated in cell death even under shear flow that simulate the flow of the cerebrospinal fluid compared with control groups. Next, a human GBM U87MG orthotopic xenograft model was established by intracranial injection of U87MG cells into nude mice. Convection of Cy3-siRNA entrapped in HA-LNPs was performed, and specific Cy3 uptake was observed in U87MG cells. Moreover, convection of siPLK1 entrapped in HA-LNPs reduced mRNA levels by more than 80% and significantly prolonged survival of treated mice in the orthotopic model. Taken together, our results suggest that RNAi therapeutics could effectively be delivered in a localized manner with HA-coated LNPs and ultimately may become a therapeutic modality for GBM.

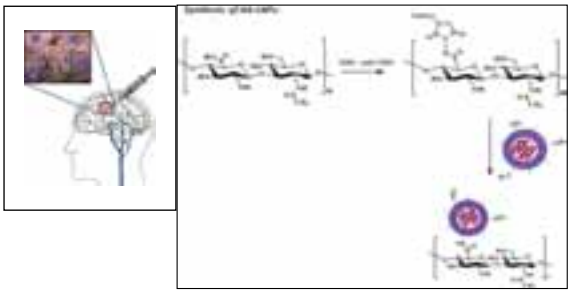

Figure 1: Schematic illustration of HA conjugation to LNPs-PEG-NH2. HA (5KDa) is activated by classical amine coupling strategy (EDC and Sulfo-NHS) as detailed in the Methods. LNPs-PEG-NH2 entrapping siRNAs using the microfluidic nanoassmblr is then mixed and incubated with the activated HA. Purification of unbound HA is performed using extensive dialysis as detailed in the experimental section.

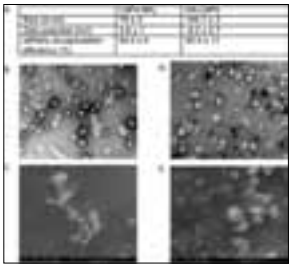

Figure 2: Physicochemical and structural characterization of HA-LNPs and LNPs-NH2. (A) Size distribution and ζ potential are performed on a Malvern ζsizer as detailed in the Methods. Entrapment of siRNAs is measured using Ribogreen assay as detailed in the Methods. (B and D) TEM analysis of LNPs-NH2 and HA-LNPs, respectively. Bar scale: 200 nm. (C and E) SEM analysis of LNPs-NH2 and HA-LNPs, respectively. Bar scale: 1 µm. Methods and instrumentation are detailed in the Methods. LNPs-NH2 were found to have globular shapes and round surfaces whereas HA-LNPs exhibit a flower-like shape on the particles.

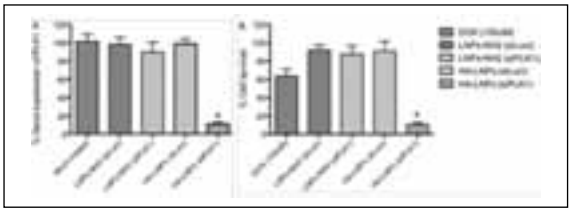

Figure 3: PLK1 induce specific cell death in glioma cells. (A) PLK1 gene expression was quantifying using QPCR as detailed in the

*Methods. U87MG cells were incubated with HA-LNPs or LNPs-NH2 with either siLuci or siPLK1 under shear flow conditions to simulate CSF flow. A robust knockdown was observed in the siPLK1 treatment when delivered via HA-LNPs. (B) XTT cell survival assay was performed on cells treated with the same types of treatments as listed in (A). Doxorubicin (DOX), a known chemotherapy, was used as a positive control. *p < 0.001.*

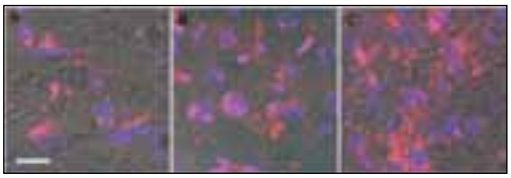

Figure 4: Cy3-siRNAs are taken up by U87MG cells. Representative confocal microscopy images are presented. HA-LNPs were injected into tumor site as detailed in the Methods. Three hours (A), 6 h (B), and 24 h (C) after LNPs administration, animals were sacrificed and the Cy3-siRNA (red) location was detected using confocal microscopy analysis. DAPI (blue) was used for nuclear staining. Bar scale, 50 µm.

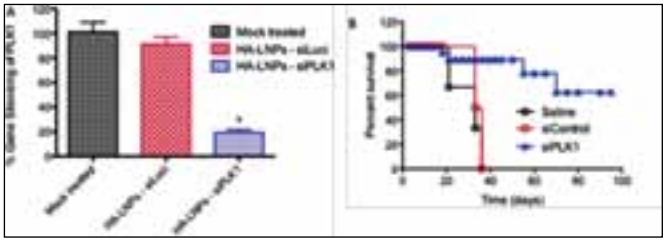

*Figure 5: Therapeutic gene silencing prolongs the survival of GBM-bearing mice. (A) Robust in vivo gene silencing in siPLK1 treated mice is shown (n = 10 mice/group). Animals were treated twice as detailed in the Methods. Tumor cells were FACS sorted via a surface marker, and PLK1 mRNA level was quantified using QPCR. *p < 0.001. (B) Kaplan–Meier survival analysis of GBM-bearing orthotropic U87MG cells (n = 10/group) treated with siControl (siLuciferase), siPLK1, or saline. Overall, four administrations were given at days 7 and 9 post tumor inoculation and then at days 20 and 22 post tumor inoculation*

ENCAPSULATION OF GOLD NANOPARTICLES INTO GELATIN FOR MULTISTAGE DELIVERY OF CHEMOTHERAPEUTICS

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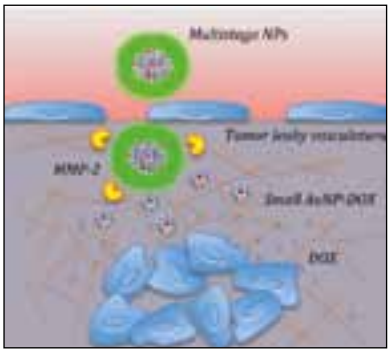
Most effective anticancer drugs have a restricted clinical use due to their dose-dependent toxicity and their low specificity against cancer cells. Multifunctional nanoparticles (NPs) offer advantages in ameliorate physicochemical properties of drugs, change the pharmacokinetics and reduce their side effects. Many of these nanotherapeutics can passively accumulate around the leaky regions of the tumor vasculature due to the enhance permeation and retention effect (EPR), but their large size (> 150 nm) hinders the penetration into the dense collagen matrix. Herein, we design a multistage system in which bigger polymeric NPs can be degraded to 5-nm drug-loaded-NPs after they extravasate from the tumor vasculature, and these small NPs can more readily diffuse throughout the tumor environment. The degradation process is triggered by the matrix metalloproteinases (MMPs) that are highly expressed by the tumor microenvironment.



By following the concept of supramolecular receptors developed by Stellacci and his group [1], small AuNPs having two bi-functional ligands suitable for entrapping the anticancer drug doxorubicin (Dox) were synthesized and encapsulated into Gelatin-NPs (Gel-AuNPs). The ligand-coated gold NPs were obtained via a modified one-phase synthesis [2] and they were included into the gelatin during the two-step desolvation process adopted for the Gelatin-NPs preparation [3].
The particles were uniform in size and well dispersed as confirmed by DLS (Gel-Dox-AuNPs 138 ± 2 nm, PI 0.08; ζ-potential + 27,4 mV), TEM (AuNPs 3.6 ± 0.8 nm) and SEM. Confocal microscopy revealed that Gel-Dox-AuNPs penetrate tumor cells, cultured as monolayer or 3D spheroids. Gel-Dox-AuNPs were stable in PBS pH 7 after 24 h at 37°C, while they released Dox after 24 h in PBS pH 5. MTT proliferation assay demonstrated that Gel-Dox-AuNPs were toxic for tumor cells after 24 h and 48 h, while no toxicity was observed for the control NPs. Moreover, for a better understanding of the mechanism underlying Dox release, we added glutathione (GSH), a molecule highly concentrated inside the cellular cytosol, but not outside. We found that GSH triggers the drug release from the small AuNPs to 50 % within 24 h. The assumed mechanism is that the drug is released from the AuNPs by ligand exchange of the surface thiols with GSH.
Thanks to its dimension this drug delivery system is expected to reach the tumor via the EPR effect and to deeply penetrate into the tumor microenvironment. The controlled drug release in combination with a biodegradable gelatin carrier offer potential therapeutic advantages of enhanced tumor cell localization and reduced systemic toxicities of the drug.

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DEVELOPMENT OF IRON OXIDE NANOPARTICLES AS MAGNETIC LABELLING AND DRUG CARRIERS FOR MICROBUBBLE DRIVEN MULTIMODAL IMAGING AND THERANOSTICS FOR GLIOMAS

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Malignant gliomas (MG) constitute at least 35% of all primary brain tumors and are the third leading cause of death from cancer in people from 15 to 34 years of age. Surgery plays a major role in the treatment of MG, as the surgical extent of resection is clearly associated with improved patient survival[1]. Unfortunately, a complete surgical removal of these tumors is achieved in less than 20% of cases [2,3] also because of technical difficulties in identifying the tumor borders during surgery[4]. Intra-operative fluorescence and ultrasound (US) guidance are functional to improve tumor resection, however both techniques have several limitations . Therefore, improving intra-operative US image quality with an appropriate contrast agent enhancement (CE), and combining it with intraoperative fluorescence and pre-operative Magnetic Resonance Imaging

(MRI) is highly desirable in order to achieve a better intraoperative discrimination of the tumour margins and safely maximize resection[5]. Microbubbles (MBs) are a well-established contrast agent for US imaging. Moreover poly (vinyl alcohol)-based MBs already allow to support three different imaging modalities - US, MRI and single-photon emission computer tomography[6-9].

The idea of TheraGlio project is to develop a multimodal imaging system for Theranostics (therapy+diagnosis) of patients bearing malignant glioma, the most common primary brain tumor. This technology will avail of new generation Microbubbles (MBs) that can simultaneously act as drug delivery system and contrast agent for Magnetic Resonance Imaging, intra-operative Contrast-Enhanced UltraSound and intra-operative fluorescence microscopy (Figure 1). This novel imaging system will provide multimodal image guidance during tumor resection with the final goal of prolonging patients' survival, as a result of a safer and larger tumor resection and tailored delivery of specific chemotherapeutic molecules.

In this framework we are developing functionalized iron oxide nanoparticles suitable for MicroBubble multi-labeling for MRI imaging and targeted drug delivery [10,11]. We focused our work in preparing suitable superparamagnetic iron oxide nanoparticle (SPION) core and tested a panel of functionalizations to allow conjugation onto polymeric microbubbles (MB) and coupling with targeting biomolecules and drugs. We selected a co-precipitation synthesis route for SPION which is fast, cheap, scalable and easily adaptable to green chemistry, compared to other approaches reported in the literature[12-14]. Size, shape distribution and magnetic susceptibility depends on the ratio of precursor in the SPION synthesis, and we selected protocols that allow 8-12 nm spherical SPION with narrow distribution. Although catecholamines, carbohydrates and phosphonates were investigated, highly dispersable SPION were obtained with aminosilane functionalization. This coating gave also improved MBs loading. As density of amino groups on nanoparticles is linked to cytotoxicity, we are investigating different mixed coatings. We also started introducing temozolomide (an anticancer drug) onto the surface with both cleavable and non-cleavable strategy.

ACKNOWLEDGMENTS.

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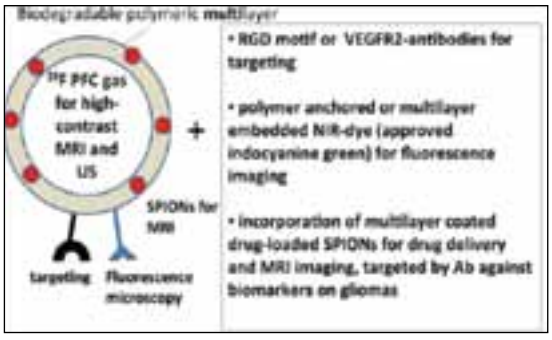

Figure 1. The surface of polymeric biodegradable MBs can be functionalized for multimodal imaging and loaded with NPs as drug carriers; the degradation of the polymer shell will allow the drug to be released.

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PATCHY GOLD/IRON NANOPARTICLES AS NOVEL APPROACHES TO TREATMENT AND IMAGING OF EPILEPSY.

BARBARA SANAVIO[1] (PRESENTING AUTHOR), Paolo Pennacchio[2], Laura Librizzi[2], Fernanda Sousa[1], Carolina Frassoni[2], Marco de Curtis[2], Silke Krol[1], Francesco Stellacci[3]

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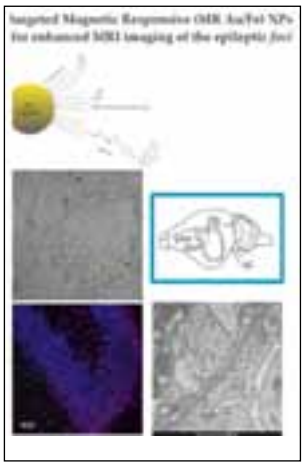
Epilepsies comprise a family of neurological disorders affecting about 1% of the world population and six million European citizens currently suffer from active epilepsy Epilepsy is characterised by an enduring predisposition to generate epileptic seizures, and by the neurobiologic, cognitive, psychological, and social consequences of this condition. An epileptic seizure is a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain. While many patient achieve control of the epileptic syndrome with pharmacological treatment, about one third of patients have or develop treatment-resistant epilepsy that impairs their neurological, cognitive and social condition and

for which surgery is the only options. Unfortunately, a considerable subset of patients fails to undergo surgery because of the poor localization of the epileptic foci.
We aim at developing targeted Magnetic Responsive (MR Au/Fe) NPs for enhanced MRI imaging of the epileptic foci1.
We investigated the penetration of MR-NPs under a facilitated experimental condition in which the interface between the plasma and the cerebral tissue is functionally preserved2.



The isolated guinea pig brain maintained in vitro by arterial perfusion has been demonstrated to retain the structural and functional preservation of neuronal and vascular compartments as well as the blood-brain barrierfor several hours after the in vitro conditions establishment 2,3,4. The effect of MR-NPS perfusion into the isolated brain preparation was evaluated in control condition and after the induction of seizure-like events (SLE). Preliminary results from confocal laser scanning fluorescence microscopy, silver staining and TEM analysis suggest that NPs not only decorate the lumen endothelium, but are transcytosed and penetrate through endothelial contacts towards astrocytes, glial cells and neurons even without apparent damage to the BBB.

ACKNOWLEDGMENTS

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DISTRIBUTION OF VEGF-TARGETED IMMUNOLIPOSOMES INTO BRAIN TUMORS

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The main purpose of active targeting is enhancing site-directed delivery and tumor-targeted particularly. However active targeting integrated with passive delivery that is why contribution of both should be investigated separately. Therefore the aim of present study was thorough researching of VEGF-targeted immunoliposomes internalization, uptake, accumulation and distribution into brain tumor. VEGF is widely expressed by many tumors, including gliomas [1], and can be considered as a target for nanocarriers delivery [2,3]. VEGF is secretory but there are cell- and matrix-associated heparin-binding isoforms which are necessary to tumor targeting.
We synthesized PEGylated liposomes conjugated with monoclonal anti-VEGF antibody [4], bevasizumab and non-specific IgG. Immunoliposomes had a narrow particle size distribution and high dispersion stability. Affinity of conjugated anti-VEGF was about 70% of initial. All samples of liposomes were taken up by rat C6 and human U-87 MG glioma cells. While VEGF-targeted immunoliposomes accumulated on membrane and into lysosomes up to 50 times greater than IgG control. However study of immunoliposomes accumulation in C6-bearing rats did not show benefits of anti-VEGF liposomes. Moreover distribution of VEGF-targeted and non-specific IgG liposomes was the similar in the malignant tissue, but it differs from each animal in a greater degree. Thus VEGF-targeting can influence on binding and internalisation rate in vitro but do not influence on accumulation and distribution in vivo. At the same time it should be noted C6 cells incorporate liposomes greatly slowly that could be affect the result in vivo.

ACKNOWLEDGEMENTS:

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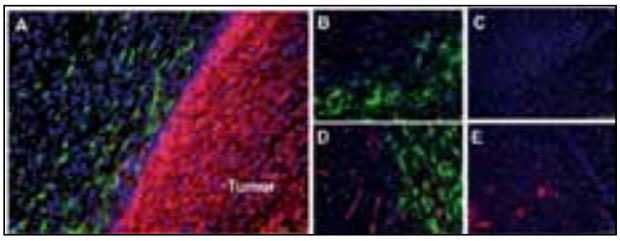
Distribution of DiI-labeled VEGF-targeted immunoliposomes. The sections were additionally stained usinga antibodies against GFAP [A, B, D] to study the liposome (red) accumulation in the peritumoral space. Visualization of the astroglial border (green) made it possible to distinguish between the tumor and nervous tissue parenchyma and to localize the fluorescent label in the peritumoral space. Figures demonstrate high level of heterogeneity of accumulation from each animal and areas of the tumor. A – tumor cells accumulation. B, C – low degree accumulation. D, E – vessels accumulation.



MULTIFUNCTIONAL GOLD NANOSTRUCTURES FOR CANCER PHOTOTHERAPY

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INTRODUCTION

Gold and silver as bulk materials for the production of nanostructures are chemically inert and highly compatible with biologic tissues; however, it is important to understand what biological changes may occur when the cells contact with those nano-scale systems. The biological environment can affect the nanostructures' characteristics, by changing their size, through aggregation or modification of the SPR band position, causing a shift the wavelength absorption and different aspect ratios. Also, the use of reducing and capping agents are toxic to cells. If not efficiently eliminated, these compounds can increase unspecific cell death1. In this scope, we describe the development of a multifunctional nanoparticle system comprising three main components: a polymeric matrix, a gold domain and a bio-therapeutic agent. Gold nanostructures (GNS) were developed, characterized and conjugated with Epidermal Growth Factor (EGF) for tumor therapy using a combined approach of site-specific drug delivery and photo-sensibilization. The addition of "green" extracts from natural sources to nanocarrier formulations is an ongoing approach, through the use of the naturally antioxidant compounds beyond their use as reducing agents and as shape modulator agents[2]. The aqueous extracts of Plectranthus saccatus Benth. are rich in antioxidant compounds (mainly rosmarinic acid, identified by HPLC-DAD), demonstrating promising results for acetylcholinesterase inhibition, DPPH free radical-scavenging and cytotoxicity assays[3]. Thus, this extract was selected as source for reducing and capping agents in GNS production, as an alternative to more toxic substances, for example, hexadecyltrimethylammonium bromide (CTAB)[4]. The aim of this study is to develop multifunctional EGF-conjugated GNS which, through active targeting and laser photo-therapy combination, can reach superficial cancers (e.g. skin cancer and some superficial lesions in breast cancer), improving the efficiency and drug concentration at those sites. EGF was selected as the ligand specific to human epidermal growth factor receptor (EGFR). Once coupled to the GNS it will promote GNS' binding, uptake and internalization medicated by EGFR5. By merging a targeting action, guided by the presence of specific ligands at the GNS´ surface; an absorption wavelength band at the near infra-red (NIR) range – also known as the "optical therapeutic window" – associated with low normal tissue absorption; and finally, an equally efficient GNS "green" production in alternative to commonly used reducing agents with great cytotoxicity, we may actually accomplish our biological outcome expectations and improve the treatment efficiency and possibly secure the patients' survival.

EXPERIMENTAL METHODS

Firstly, a P. saccatus extract's powder solution was prepared[3]. GNS were produced by a modified seed-growth method[6]. Briefly, a solution consisting of gold (III) chloride trihydrate (1 mM), P.saccatus extract, L-ascorbic acid and silver nitrate reacted for 15 min. The polymer consisting of hyaluronic acid and oleic acid (HAOA, 1:1, w/w) was then added to the GNS suspension mixture for more 15 min. In order to prepare the bioconjugates, EGF (2.5µM) was reconstituted in 20 mM HEPES buffer (pH 7.4). Then, the three solutions (peptide, GNS and coating polymer) were mixed at a 1:1:1 ratio (v/v/v) and allowed to interact for 30 min at room temperature and then overnight at 4ºC. The solution was centrifuged twice at 500 x g for 20 min in a FV2400 Microspin (BioSan, Riga, Latvia) to remove unbound peptides and the pellet was re-suspended in PBS7. Surface plasmon resonance (SPR) band of GNS and the maximum absorbance wavelength (λ_{max}) peak was determined by UV-VIS spectrometry (Evolution 600, UK). Particle size, polydispersity index (PI) and zeta potential were determined using a Coulter Nanosizer Delsa Nano™C (Fullerton, CA). Additional observations were carried out on a JEOL 5200LV scanning electron microscope and on a JEOL 100C transmission electron microscope (JEOL Ltd., Tokyo, Japan) to confirm the size and to analyze the morphology and surface features of the functionalized GNS. Co-localization with EGF conjugated Alexa Fluor 647 (λ_{em} 665 nm) and Coumarin-6 attached to GNS (λ_{em} 500 nm) was performed with a confocal microscope Leica TCS SP5 (Germany). In order to study potential EGF conformational changes upon exposure to UV light and temperature, the protein´s fluorescence properties were investigated using steady state and time-resolved fluorescence (Felix GX, Photon Technology International, USA). The fluorescence emission and excitation spectra of Tryptophan (Trp) and of likely photoproducts (N-formylkynurenine, NFK; Dityrosine, DT; and Kynurenine, Kyn) were monitored.

RESULTS AND DISCUSSION

Plain GNS showed a size around 100-150 nm (PI of 0.2), using photon correlation spectroscopy for particles characterization. Absorption wavelength was around 800 nm (Figure 1). After addition of HAOA polymer, GNS suffered a blue-shift and tuned to wavelengths of 600 nm (Figure 1), as the two longitudinal and transverse bands also merged into a single band[8]. The mean size increased to approximately 300 nm and led to the formation of structures with different shapes (octahedrons with an internal angle of ~110-120 degrees and triangles with internal angle of ~60-70 degrees), as demonstrated by TEM micrographs (Figure 2). SEM images showed that the formulation was composed of a mixture of rods and spherical nanoparticles. In terms of zeta potential, we found that plain GNS showed a neutral surface charge (0.26 mV), but after addition of the biopolymers the surface charge of the GNPs changed to -19.25 mV. Possibly, the compounds of P. saccatus extract can act as both reducing and capping agents, while oleic acid (surfactant) and hyaluronic acid (polysaccharide) are capable of modulating the GNS, like CTAB[9]. After protein conjugation, the GNS size and PI varied slightly (approx. 90% 230 nm, PI: 0.3). Co-localization confocal microscopy confirmed bioconjugation of EGF onto the GNS. Fluorescence studies showed that GNS have a quenching effect on the excitation and emission spectra of the studied proteins. Continuous UVB illumination of EGF in solution leads to the decrease of Trp/Tyr fluorescence emission and excitation intensity and to the formation of photochemical products such as NFK, Kyn and DT. Interestingly, coupling EGF to GNS protected EGF from UVB induced photochemical damage and did not induce protein denaturation.

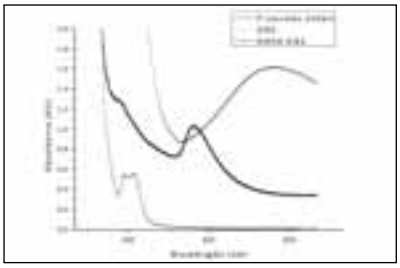
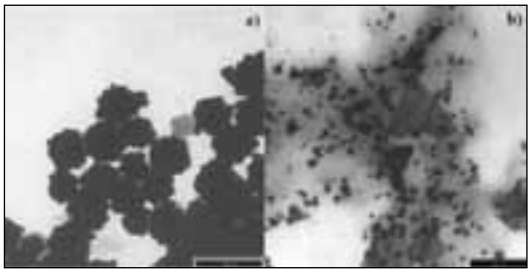


Figure 1. Spectra of P. saccatus extract, plain GNS (λmax = 820 nm) and HAOA GNS (λmax = 570 nm) after production by seed-growth method.

Figure 2. TEM micrographs. a) Plain GNS b) HAOA GNS (scale: 250 nm).



CONCLUSION

Gold nanosystems can be prepared by using biocompatible and biodegradable molecules, with modifications in terms of size, shape and localized SPR. In this work, we showed the development of GNS by using natural reduction and capping agents, conjugated with a small targeting peptide, like EGF, and a protective polymeric structure made of hyaluronic acid and oleic acid, for a local antitumor therapy. GNS showed an absorption band at the NIR spectra, which suffered a blue shift after addition of biomolecules. After bioconjugation, GNS showed a mean size between 200-300nm and increased the peptide stability. Further, we will test the antitumor drug carrier for receptor binding and internalization in cancer cells through EGFR binding and MTT cytotoxic assays.

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DITERPENE PARVIFLORON D LOADED HYBRID NANOPARTICLES FOR POTENTIAL CANCER THERAPY

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INTRODUCTION

We describe the development of hybrid nanoparticles (NPs), made of conjugated biopolymers and lipids. The aim of this study is to apply this system as a targeted anti-tumor drug delivery platform for a natural diterpene, Parvifloron D (PvD). The use of new and natural compounds for cancer treatment can be a solution for drug multiresistance problems, since different molecular mechanisms and metabolic pathways can be explored. PvD, which is extracted from Plectranthus ecklonii (Benth.), is one of the abietane diterpenes with a royleanone motif (Figure 1) that has demonstrated promising antimicrobial activity[1]. Furthermore, PvD has promoted cell death of human leukemia cells3, while other compounds of the P. ecklonii extract have demonstrated cytotoxic effects on human breast cell lines (MCF-7) and on human melanoma cell lines (SK-MEL-1)[2]. In order to understand whether PvD was selectively toxic to cancer cells, cytotoxicity studies were carried out in normal-like keratinocytes. Moreover, we studied the encapsulation of PvD into hybrid NPs as a drug delivery platform, for further combining a retention effect and accumulation at tumor site at higher concentration, as well as providing a continuous therapeutic efficacy.

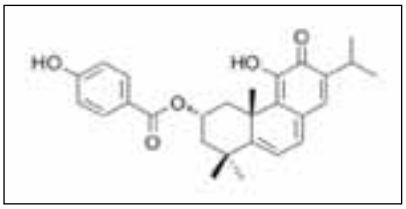

Figure 1. Parvifloron D structure.

Besides biocompatibility and biodegradability, these nanosystems may also promote the additionally surpass of cancer multiresistance, allowing an efficient targeted drug release from their inner core structure mainly due to a specific modification of the NPs' surface coating[4]. When applied to more superficial cancers such as melanoma, NPs may improve anti-tumor therapies, preventing the tumor evolution to metastatic stages, and may provide an alternative to invasive approaches, like surgery.

EXPERIMENTAL METHODS

PvD was isolated from Plectranthus ecklonii (Benth.)[1]. The cytotoxicity of PvD (0.1 – 50 µM; 24 h-incubation) was evaluated in human keratinocytes (HaCat cells) using the crystal violet assay. Empty and loaded NPs were prepared according to a solvent displacement method, described elsewhere[5], with several modifications. Briefly, an organic phase prepared by mixing 100 mg of poly-epsilon-caprolactone (PCL) dissolved in acetone, through ultrasound exposure, with stearic acid ethanolic solution (0.025%, w/v) over an aqueous solution containing Pluronic® F-127 (0.25%, w/v) and the polymer hyaluronic acid and oleic acid (HAOA 1:1, w/w) under vigorous magnetic stirring. For loaded NPs, PvD (0.0625 mg/mL in ethanol) were also dissolved in the previous described organic phase. The suspension was stirred for 15 min, concentrated under reduced pressure and then isolated by centrifugation at 13,157 x g for 15 min (Hermle Labortechnik Gmbh type Z36HK, Germany). Mean particle size, polydispersity index (PI) and zeta potential of the NPs' concentrated suspension were measured with a Coulter Nano-sizer Delsa Nano™C (Fullerton, USA). The morphology of empty and loaded NPs was obtained by Zeiss DSM-950 scanning electron microscope and Zeiss M10 100C transmission electron microscope (Carl Zeiss Microscopy GmbH, Göttingen, Germany). Empty NPs were also labeled with a hydrophilic dye (Rhodamine B, λem= 590 nm) according to a method already published[6], after some adjustments, for visualization with a Confocal Microscope Leica-Microsystems SP5 (Mannheim, Germany) with an excitation laser He-Ne 561 nm and a λemission of 569-666 nm. Study of interactions between drug and NPs were assessed by Fourier transform infrared (FT-IR) spectroscopy (KBr pellet method) in a FT-IR Spectrum 2000 (Perkin Elmer, USA) from 4000-400 cm^{-1}. Samples of bare NPs, empty HAOA coated NPs and loaded HAOA NPs were evaluated by ^{1}H NMR and ^{13}C NMR spectra were obtained by a 300 MHz spectrometer (Oxford Instruments, England), using different solvents D_2O, DMSO-d6 and CDCl3-d6, in order to identify the presence of the several components and to confirm the presence of HAOA on the NPs shell and PvD on the inner core, by comparing the different spectra. Thermal transformations and phase transitions of NPs, drug and raw components were studied by using a Mettler Toledo DSC 30 Calorimeter (Columbus, Ohio, USA), at 10ºC/min over a temperature range from 25-375ºC. Drug solubility, encapsulation efficiency into the NPs (EE, %) and in vitro drug release studies were carried out through a new developed reverse-phase HPLC chromatographic method, consisting of acetonitrile (ACN)

and Milli-Q water (60:40, v/v) as mobile phase, a Supelcosil LC-18 column (4.6 x 150 mm, 5 µm particle size) as the stationary phase. The flow rate was 1.0 mL/min and at a detection wavelength of 254 nm. Standards for PvD between 0.1µg/mL and 10µg/mL were evaluated in triplicate and a calibration curve was obtained with $R^2 > 0.998$. The chromatographic data was processed using Gold-System Nouveau software.

RESULTS AND DISCUSSION

PvD showed marked cytotoxicity to HaCat cells at low micromolar concentrations, suggesting a lack of selectivity towards cancer cells. Therefore, this drug should be delivered using a system that favors the contact with cancer tissue. We have formulated and characterized empty and loaded hybrid NPs in terms of their physical characteristics (size, charge, structural layers and morphology), drug-NPs interactions (FTIR, DSC and NMR), in vitro release profile and drug EE after loading with a hydrophobic drug. NPs showed an increase of the mean size after addition of HAOA coating polymer (about 100 nm) and zeta potential slightly increased from -18 to -13 mV. NPs' size, corona size and round shape and smooth surface were confirmed by SEM and TEM. Empty non-coated NPs showed 100-200 nm in size, low density "channels" inside the PCL core, which may be attributed to stearic acid presence. The formation of these fatty acid compartments may promote a controlled drug release as it stays stably entrapped inside them[7]. Moreover, it was perceptible the presence of a shell around the NPs' core, with a diameter around 60-100 nm. Both empty HAOA and drug-loaded HAOA NPs showed a mean size around 300-400 nm. FT-IR has demonstrated the existence of a HAOA coating, by the presence of three specific bands for amines (I, II and III) detected in both empty HAOA coated NPs and drug-loaded NPs[8]. As for the interactions between drug and NPs, we were able to differentiate the spectra of PvD-loaded NPs and the physical mixture of PvD and empty NPs (at different ratios), indicating that the drug was successfully entrapped inside the hydrophobic core of the NPs. NMR studies also confirmed the presence of HAOA in coated NPs and, in addition, of PvD for loaded NPs. DSC studies showed that, at different heating rates, PvD varied its melting points indicating the presence of polymorphic forms by incomplete crystallization. All nanosystems (core NPs, empty HAOA coated NPs and loaded HAOA coated NPs) showed a lower melting point (50-52ºC) in comparison with the raw materials. Finally, EE% was around 87%. PvD solubility was around 2-8 µg/mL and the totality of the drug was released after almost three months. Based on a review of commercially available topical formulations for skin cancer treatment, prolonged therapy is common (from 2 weeks to 7 months), with several times applications' daily or weekly[9]. By using the developed NPs, for example embedded in a skin patch, the need for regular applications could be reduced and it may keep the drug target distribution and treatment effectiveness during one month, in PBS pH 5.5 medium.

CONCLUSION

Our nanosystems appear to be promising platforms for a long-term drug release, presenting the desired structure and a robust performance for a targeting and local anti-tumor therapy. Further, we will compare the efficacy of this system after encapsulation of paclitaxel and also study the conjugation of other targeting biomolecules ligands to the NPs.

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IN VITRO AND IN VIVO EVALUATION OF NEW DOXORUBICIN-LOADED NANOPARTICLES ENGINEERED FROM A MICROEMULSION PRECURSOR

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INTRODUCTION

Numerous methods have been applied to prepare polymeric nanoparticles (NPs) for drug delivery. These methods include: (a) emulsion/solvent evaporation technology, in which evaporation of organic solvents (methylene chloride or chloroform) from an emulsion under high shearing mixing results in solid nanoparticles; (b) nanoprecipitation from polymer-drug solution in water-miscible organic solvent upon slow introduction of surfactant-containing aqueous phase; (c) salting-out method, in which a polymeric organic phase is emulsified in a saturated salt water under strong stress; and (d) spray-drying procedure, in which dried nanoparticles are created in one-step process by atomization of polymer solution into a spray droplets that dried immediately as they contact hot air. We have develop a new technique by which microemulsions is used as a template for nanoparticles. This technique was combined with a simple phase-separation process to avoid the necessity of extraction the microemulsion's oily components by organic solvents. The main purpose of this study was to investigate a process in which self-assemble doxorubicin-loaded nanoparticles are produced by using a water-in-oil (W/O) microemulsion as a reactor. The reactor microemulsion was prepared from pharmaceutically acceptable components, such as widely-used nonionic surfactants, isopropyl palmitate (as the oil), and a carbomer solution as the inner aqueous phase. This study has involved characterization of the nano-particulate system, as well as evaluation of the in-vitro drug release and the in vivo efficacy in animal model using B16 melanoma-xenografted mice. Cancer treatment with the doxorubicin-encapsulated NPs (DOX-NP) system was compared with an untreated control and a conventional injection of doxorubicin (DOX-HCl) solution.

METHODOLOGY

In vitro Drug release study: The release of DOX from NPs was evaluated by incubation of the DOX-NP dispersion at 37ºC in the donor compartment of a Franz diffusion cell system (PermeGear, Inc., Hellertown, PA). The diffusion area was 1.767 cm^2 (15 mm diameter orifice), and the receiver compartment volume was 12 ml. The solutions in the water-jacketed cells were thermostated at 37°C and stirred by externally driven, Teflon-coated magnetic bars. A synthetic membrane (SnakeSkin Dialysis Tubing, 10,000 MW cutoff, 22mm, Thermo Fisher Scientific, Rockford, USA) was placed on the receiver chambers and the donor chambers were then clamped in place. The receiver chamber was filled with phosphate buffered saline (PBS, pH 7.4). Aliquots (0.5 ml each) of DOX-NP dispersion or plain DOX solutions in water were applied over the membrane. Samples (1 ml) were withdrawn from the receiver solution at predetermined time intervals, and the receiver cell was replenished up to its marked volume with fresh buffer solution each time. Addition of PBS to the receiver compartment was performed with great care to avoid trapping air beneath the membrane. The receiver samples were taken into 1.5-ml vials and kept at -20ºC until analyzed by HPLC.

IN VIVO EFFICACY STUDY:

Cell culture: B16F0 melanoma cells were maintained in RPMI 1640 medium supplemented with 10% bovine serum and 200 µM L-glu-

tamine, 10 units/ml Penicillin, and 10 µg/ml streptomycin (Biological Industries, Beth Haemek, Israel). The cells were kept at 37 °C in a 5% CO_2 humidified atmosphere. Animals: Female C57BL/6 mice (5-6 weeks, 14-18g) old were obtained from Harlan (Rehovot, Israel). Mice were housed under humidity- and temperature-controlled conditions, and the light/dark cycle was set at 12-h intervals. The animal protocol was reviewed and approved by the Institutional Committee for the Ethical Care and Use of Animals in Research, which complies with the Israeli Animal Welfare Law. In vivo therapeutic efficacy: The tumor regression effect of the DOX-NPs was investigated in mice inoculated melanoma B16F0 cells ($1x10^6$) subcutaneously in the right flank of the mice. After tumors were visualized a week later, the mice were weighed and measured for tumor size, and were randomly sorted into three groups. The groups were assigned for untreated control animals, treated animals with DOX-HCl solution (40 µg/ml), and treated animals with DOX-NP dispersion (40 µg/ml) (n=12 mice/treatment group, n=11 mice in the control group). The dose of DOX administered to the treatment groups was 0.5 mg/kg twice a week, by subcutaneous injection proximal to the tumor at the frontal side. Tumor volumes and mouse body weights were measured routinely before each treatment. Measurement of tumor size was performed with a caliper in two dimensions, and individual tumor volumes (V) were calculated by the formula: $V = [length \times (width)^2] /2$

RESULTS AND DISCUSSION

Self-assembled polymeric NPs were engineered by using a water-in-oil microemulsion template containing water-soluble polymer in the inner aqueous phase. The carbomer in the nanodroplets was crosslinked by Ca^{++} ions to form nanoparticles which were separated from the system by a simple and direct phase separation. NPs have been commonly prepared by emulsion/solvent evaporation, nanoprecipitation by organic solvents, spray-drying, or by salting-out under high-shearing stress. In the most common latter process, a polymer is dissolved in an organic phase (e.g., acetone), which is miscible in all proportions with pure water but being separated and emulsified in salt-containing water. That emulsion is gradually diluted to create a monophasic system containing NPs while the solvent is evaporated. According to our method, we have employed a self-assembled NPs by using a microemulsion-crosslinking process that was directly purified by phase separation that removed the oily components. Figure 1 shows the morphology of the formed NPs as observed under a transmission electron microscope (TEM) using two staining techniques. The TEM picture and DLS analyses demonstrate nanoparticles with a spherical morphology, low polydispersity, and a size range of 100-200nm.

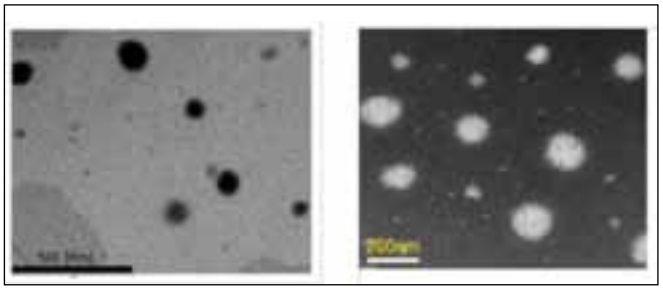


Figure 1: Morphology and size characterization of the nanoparticles using transmission electron microscopy (TEM) and negative staining: phosphotungstic acid staining (left), uranyl acetate staining (right)

To evaluate the controlled release manner of DOX-NPs, doxorubicin release was monitored following 6-h permeation through a dialysis membrane and was compared with a plain aqueous solution containing 40 µg/ml DOX-HCl. Figure 2 shows a bi-phasic pattern of DOX release from the NP dispersion, an initial burst release in the first 3 h followed by a sustained release profile. DOX solution, in comparison, wholly transported through the membrane during 3 hours followed by a plateau.

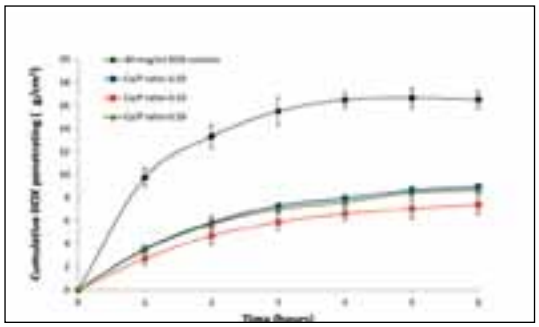


Figure 2: Doxorubicin permeation per squared cm of a dialysis membrane to the receiver medium. NPs formulations were tested demonstrating sustained release compared with 40µg/ml DOX solution. NPs contained 40.5-42.1 µg/ml DOX, prepared from microemulsions contained three different Ca++/polymer (Ca/P) ratios, 0.01% polymer concentration (0.625mg/ml in the inner phase) and 16% aqueous phase content in the microemulsion template.

It has already been clinically evidenced that liposome-encapsulated DOX-HCl products, such as PEGylated liposomes (Doxil® or Caelyx®, Janssen-Cilag International N.V., Belgium) or non-PEGylated liposomes (Myocet, Elan Pharmaceuticals, NJ), have a reduced toxicity compared with the conventional doxorubicin. This is due to the fact that the internalized DOX-HCl in the liposomes transported into the plasma at a slow rate, thus prolonging the therapeutic efficacy while reducing systemic adverse effects. Particles, especially those made of nanoscale, can more easily transport with their entrapped drug molecules through the leaky tumor vasculature (EPR effect), and due to the lack of tumor lymphatic drainage can increase drug bioavailability and accumulation in the tumor tissue. We have hypothesized that the same effect might occur upon treatment with our DOX-NP system. Therefore, an efficacy study was performed using B-16 melanoma-xenografted mice treated with DOX-NPs and compared with a conventional DOX-HCl solution injection and an untreated control. In comparison with the 40 µg/ml DOX-HCl solution treatment twice a week, the same dosage of DOX-loaded NPs dramatically reduced tumor growth (ANOVA, $p<<0.05$). Figure 3 illustrates the tumor growth inhibition by the chemotherapeutic drug in terms of volume change during the course of treatment (18 days). The significant inhibition of tumor growth by DOX-NPs clearly demonstrated the efficacy of NP encapsulation.

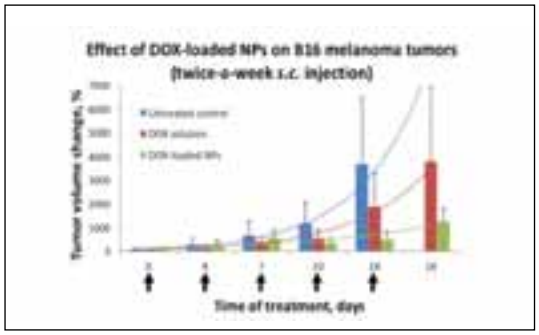


Figure 3: Inhibition of B16 melanoma tumor growth by DOX solution and DOX-loaded NPs. Comparison was made with a non-treatment control group (n=12)

PROLONGED INHIBITION OF SEMAPHORIN3A PATHWAY VIA BIODEGRADABLE IMPLANT TOWARDS A BETTER THERAPY FOR VISUAL SENSORY IMPAIRMENTS

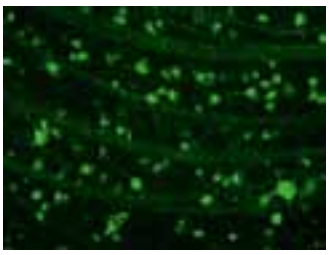
PROF. ARIEH S SOLOMON, Project Coordinator; Prof. Ari Barzilai and Prof. Itai Benhar, Tel Aviv University , Israel;Dr. Hagit Sacks and Dr. Yohann Aouat , Nicast Ltd, Israel; Prof. Angel Messeuger, CSIC – QAC , Barcelona, SpainDr .Michael Burnet, Synovo GmbH Tubingen, Germany;Dr. Mirea Coma , Anaxomics SL, Barcelona, Spain

Background: Semaphorin 3A is a leading factor in the apoptotic death program of the neural cells in CNS. It is right to suppose that inhibition of Sema3A expression in the right time window will reduce the death of a great population of neural cells following assault.

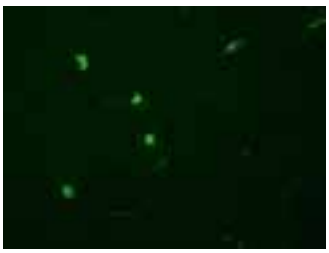
Purpose: To develop low molecular weight inhibitor of Sema3A and produce function blocking Sema3A human antibodies Manufacture a biodegradable polymeric implant for controlled release of Sema3A inhibitor.

Material and Methods: We used laboratory rats and rabbits and created acute and chronic assault to the optic nerve. Following the assault we injected the substances mentioned above in the injured eye.

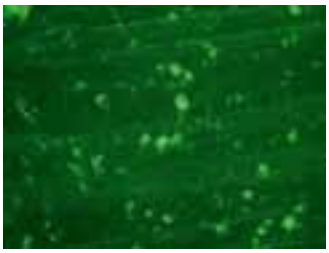
Results: We present the methods of creating the low MW inhibitors of Sema3A and the Sema3A antibodies. The creation of the implants is shown. We present here the positive inhibitory activity of these substances in vitro and in vivo experiments



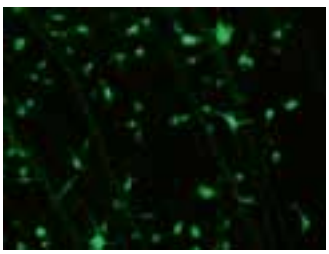
Di-10 Asp staining of normal retinal ganglion (RGC) cells in rat



Di-10 Asp staining of retina following axotomy of optic nerve



Di-10 Asp staining of RGC post axotomy and injected inhb- SM



Di-10 Asp staining of RGC post axotomy and injected inhb-Ab

CELL LOCALISATION OF RADIOSENSITISING GADOLINIUM-BASED NANOPARTICLES AND THEIR EFFECT ON DNA DAMAGE AND REPAIR

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Radiotherapy is used to fight against cancer in about 50 % of all cancer patients. Recently, the addition of nanoparticles (NPs) has been proposed as a new strategy to enhance the effect of radiotherapy particularly in the treatment of aggressive tumors. The physical processes involved in radiosensitisation by NPs have been well characterized [1, 2]. Nonetheless, further understanding of its biological impact, including the localisation of the NPs in the target cells and NPs effect on cell organels upon irradiation, is still lacking. Most localisation studies were performed with NPs tagged with fluorescent markers that can affect the NPs uptake and/or localisation. In this study a set of methods was used to unambiguously and fully characterize the uptake and localisation of label-free radiosensitising NPs, and their effect on the most harmful cellular structure - nuclear DNA, important steps in the understanding of the mechanism of NPs action.

Gadolinium-based nanoparticles (GdBN), promising theragnostic agents, were studied in U87 glioblastoma cells extracted from highly aggressive human tumor. For the first time, Synchrotron Radiation Deep UV (SR-DUV) microscopy is proposed as a new tool to track label-free GdBN. This highly innovative technique certified the localisation of the NPs in the cytoplasm of U87 cells and their absence in the nucleus (Fig 1).

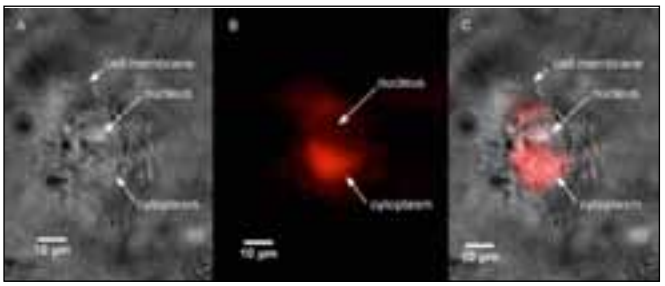

Fig 1. Localisation of GdBNs in U87 cells visualised by SR-DUV microscopy. (A) Light transmission image of U87 cell, (B) fluorescence image of label free GdBN (red), (C) merge of transmission and fluorescence images (GdBN in red).

Transmission Electron Microscopy (TEM) demonstrated that GdBN are localised in the vesicles in the cytoplasm and they are taken up by cells via endocytosis (Fig 2).

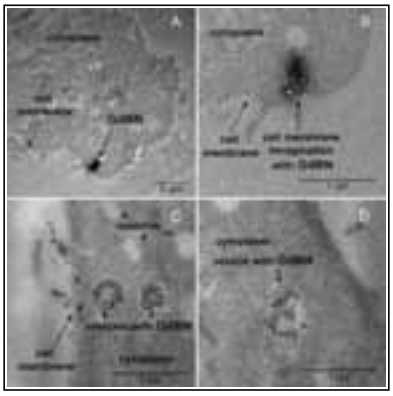

Fig 2. TEM images of U87 cells containing GdBN. (A) Image of a cell with electron dense regions located close to the membrane. (B) Zoom of the electron dense region shown in A. (C and D) Images of cells with electron dense regions located in the cytoplasm.

Confocal microscopy of living cells uncovered GdBN co-localisation with lysosomes but not with mitochondria (Fig 3).

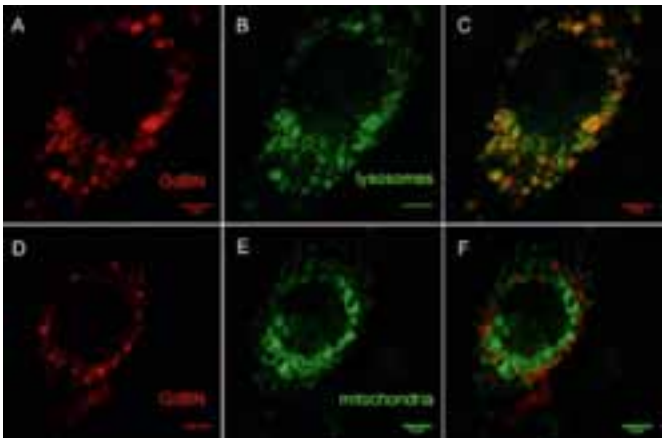

Fig 3. Fluorescence images obtained by confocal microscopy of U87 cells loaded with GdBN-Cy5.5 (red) (A, D, C and F) in the presence of Lysotracker-green (green) (B and C) or Mitotrackergreen (green) (E and F). (C) Merged image of (A) and (B). (F) Merged image of (D) and (E).

Comparison of DNA double strand break formation and repair between cells preincubated with GdBN or not and irradiated with gamma rays didn't evidence any significant differences (Fig 4).

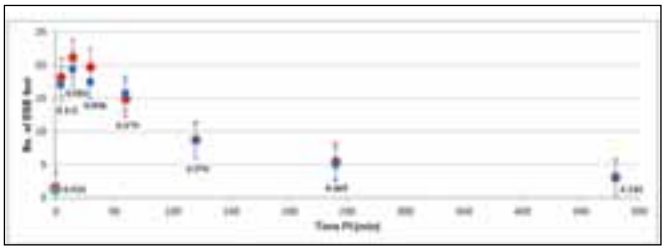

Fig 4. Number of DSBs after induction (5 min PI) and during repair process (15 min, 30 min, 1 h, 2 h, 4 h, 8 h PI) in U87 cells irradiated with 1Gy γ-IR in presence (■) and absence of GdBN (●), respectively. DSB numbers measured for non-irradiated control cells either incubated with GdBN (□) or not (○) are shown at the time "0". The numbers in the graph correspond to P values of Mann-Whitney Rank Sum Test.

Clonogenic assay measurements proved that the presence of NPs in the lysosomes induces a neat amplification of the killing of glioblastoma cells irradiated by gamma rays (Fig 5).

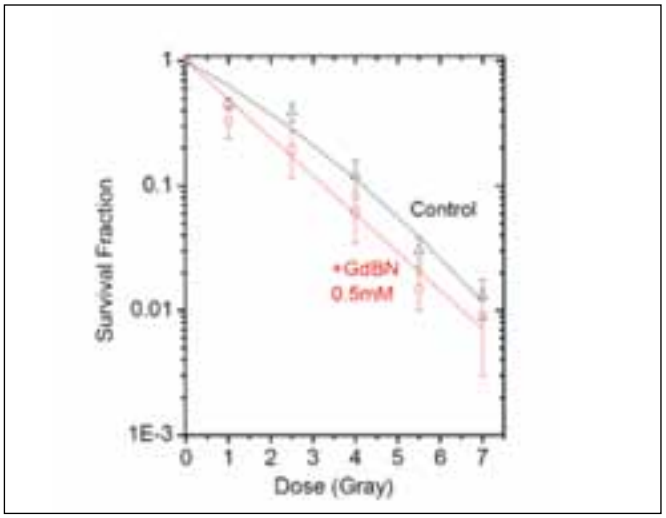

Fig 5. Surviving fraction as function of radiation dose of U87 cells free of GdBN (black) and in the presence of GdBN (red) irradiated by gamma rays (^{60}Co).

Our results suggest that the radiosenzitization of glioblastoma cells via GdBN is a cytoplasmic event originating in lysosomes.

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[2] Porcel E, Liehn S, Remita H, Usami N, Kobayashi K, Furusawa Y, Le Sech C, Lacombe S (2010) Platinum nanoparticles: a promising material for future cancer therapy? Nanotechnology 21:7

FUNCTIONALIZED GOLD NANOPARTICLES INHIBIT OLIGOMERIZATION OF THE ALZHEIMER PEPTIDE Aβ

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Neurodegenerative disorders such as Alzheimer's disease affect an ever increasing number of people in aging societies. In many of these diseases, protein misfolding is supposed to induce a cascade leading to neuronal loss and clinical symptoms. In our study, gold nanoparticles will be conjugated with multiple functional molecules as potentially active agents against misfolding of the Aβ peptide in Alzheimer's disease. These ligands include an Aβ-selector, a β-sheet breaker and a protease.[1] Hence, nanoparticles will be employed as organizational platform and transport vehicle for different, synergistically acting ligands. So far, laser-generated gold nanoparticles [2] were systematically conjugated with Aβ-selecting ligands. Conjugation efficiency and conjugate stability were analyzed as function of ligand-to-nanoparticle ratio. Thereby different ligand coverages on the nanoparticle surface were established and regimes of high colloidal stability were identified.[3] Furthermore, functionalized nanoparticles were subsequently incubated with the Alzheimer peptide Aβ. In these in vitro experiments, it was shown that Aβ binds to both, ligand-free and ligand-functionalized nanoparticles. However, only functionalized nanoparticles seem to inhibit Aβ aggregation, whereas non-functionalized nanoparticles do not have an effect. In future, we plan to synthesize even more effective bi- and trifunctional nanoparticles, which are expected to counteract Aβ aggregation in substoichiometric dose.

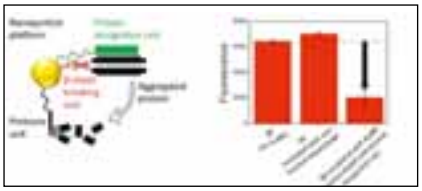

Figure 1: Underlying concept of gold nanoparticle surface functionalization with molecules, which are able to detect and reverse protein aggregation (left). Quantification of Aβ aggregates with an Aβ-specific fluorescence assay after incubation with monofunctionalized gold nanoparticles (right).

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IN VIVO STUDIES ON HEART AND LIVER REGENERATION IN ZEBRA FISH USING SILVER SYNTHESIS PARTICLE FROM TURBINARIA CONODIES

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The zebrafish, Danio rerio, is a small teleost fish originating from the rivers of northern and eastern India (Engeszer et al. 2007). It possesses a number of advantageous physical characteristics that have resulted in its common use today as a laboratory model. These include its relatively small adult size of 2–3 cm, its rapid generation time of approximately 3 months, its ability to produce large clutches of externally fertilized eggs and the ease with which fish can be kept and bred in aquaria. To date, there have been no reported studies detailing the lifespan of zebrafish in the wild. Additionally, in comparison to other established vertebrate models, e.g. the mouse, fish have simple advantages: Early developmental processes are less accessible in mammalians because they occur in utero. Other established systems such as Drosophila sp. In practice, this knowledge has been applied in aquaculture where sex steroid treatment during critical developmental stages is routinely used to produce monosex populations of fish. In nanotechnology, a particle is defined as a small object that behaves as a whole unit in terms of its transport and properties. It is further classified according to size: in terms of diameter, fine particles cover a range between 100 and 2500 nanometers, while ultrafine particles on the other hand, are sized between 1 and 100 nanometers. Similar to ultrafine particles, nanoparticles are sized between 1 and 100 nanometers. Nanoparticles may or may not exhibit area of intense scientific interest due to a wide variety of potential applications in biomedical, optical and electronic fields. Applications of nanotechnologies in medicine are especially promising, and areas such as disease diagnosis, drug delivery targeted at specific c sites in the body and molecular imaging are being intensively investigated and some products are undergoing clinical trials. Nanotechnology is relatively new. Although the full scope of contributions these technological advances will make in medicine is unexplored, recent advances suggested nanotechnology will have a profound impact on disease prevention, diagnosis, and treatment.

BIOSYNTHESIS OF SILVER NANOPARTICLES

Fresh leaves and stem of Turbinaria conoides were collected and cleaned. The collected samples were washed thrice with tap water and twice with distilled water. About 10 g of leaves and stems were taken and finely cut into small pieces and boiled with 100 ml of double distilled water separately for 5 min. The boiled extracts were filtered through Whatmann no. 1 filter paper. A total of 10 ml of collected filtrate was treated with 90 ml of 2 mM silver nitrate aqueous solution and incubated at room temperature for 10 min, resulting in the formation of brownish black colour indicating the synthesis of silver nanoparticles. After that, about 1 ml (diluted with 1:20 V/V Milli Q water) of stems and leaves silver nanoparticle solution was monitored in UV–visible spectrophotometer (at 550 nm) at different time intervals (15 min, 30 min, 4, 6 and 8 h). After the incubation period, the solution was centrifuged at 12,000 rpm for 20 min, and their pellets were re dispersed in Milli Q water. The centrifugation and redispersion was repeated three times to ensure the complete separation of nanoparticles (Syed Ali et al. 2014). Later the pellets were air dried. The air dried pellets of leaf extract was taken and mixed thoroughly with 2 ml of DMSO stored at 4ºC for future use.

ZEBRAFISH (DANIO REIO)

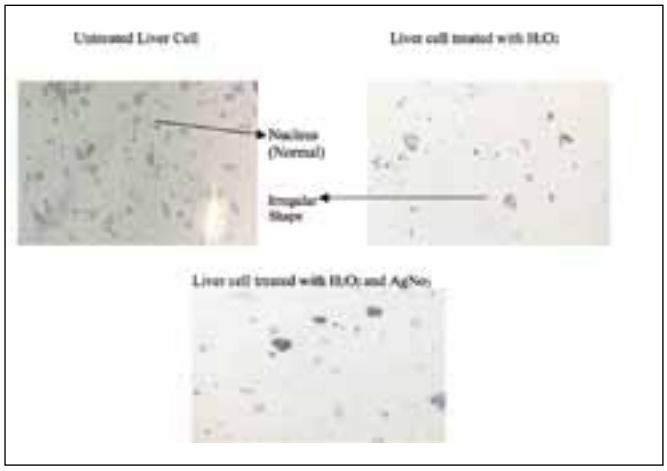
Taxonomy

Phylum : Chordata
Class : Actinopterugii
Order : Cypriniformes
Family : Cyprinidae
Genus : Danio
Species : reio

BIOACTIVITY IN ZEBRA FISH MODEL-RESULT

CARDIO AND LIVER PROTECTION ASSAY
Assay 1- Hepatocyte Viability Staining After H202 Treatment:
ASSAY 2 - CARDIOMYOCYTE RESPONSE TO CA2+
Assay 3 Alcohol Dehydrogenase activity of Liver
Assay no 4 Alanine Amino Transferase Biomarker expressions

HEPATOCYTE VIABILITY STAINING AFTER H_2O_2 TREATMENT



The recent development and implementation of new technologies have led to new era, the nano-revolution which unfolds role of plants in bio and green synthesis of nanoparticles which seem to have drawn quite an unequivocal attention with a view of synthesizing stable nanoparticles. Although nanoparticles can be synthesized through array of conventional methods biological route of synthesizing are good competent over the physical and chemical techniques. Green principle route of synthesizing have emerged as alternative to overcome the limitation of conventional methods among which plant and microorganisms are majorly exploited. Employing plants towards synthesis of nanoparticles are emerging as advantageous compared to microbes with the presence of broad variability of bio-molecules in plants can act as capping and reducing agents and thus increases the rate of reduction and stabilization of nanoparticles. Biological synthesized nanoparticles have upsurge applications in various sectors. Hence the present study envisions on biosynthesis of nanoparticles from Turbinaria conoides extract, characterisation of biosynthesised nanoparticles and further exploring its cardioprotective and liver protective ability using zebra fish as model organism.

CHEMICAL TRANSDUCTION OF SELF-ASSEMBLING PEPTIDE NANOFIBERS CONTAINING BONE MARROW HOMING PEPTIDES AND LONG MOTIF OF LAMININ ALTERS FATE OF NEUROGENESIS IN IN-VITRO AND IN-VIVO

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Spinal cord injury (SCI) in humans stayed a ruining and healless disorder. Since, longer laminin motif (CQAASIKVAV (CQIK)) better mimics conformation of native region in active site than IKVAV and resulted in improved cellular response so, for the first time in this study, CQIK bounded with 2 glycins spacer and (RADA)4 as a self-assembling peptide nanofiber backbone (-CQIK) was used and compared with bone morrow homing peptides (BMHP). The purpose of this study was to investigate the role of -CQIK and -BMHP in neural differentiation of hEnSCs in-vitro, and assess the supportive effect of this hydrogel in an animal model of chronic SCI. Results disclosed that proton concentration of -CQIK has direct effect on human endometrial derived stromal cells (hEnSCs) membrane damage but not on neuroblastoma cells. However, cell viability of neuroblastoma encapsulated into -CQIK was higher than hEnSCs at the concenteration of 0.125 % v/w . Overall it seem that BMHP nanofibers were more cell compatible than -CQIK. Gene expression analysis of cells encapsulated into -CQIK and -BMHP confirmed neurogenesis, and GFAP suppression eventually through α6 and β1 integrin site and …., respectively. However, it revealed higher level of neurogenesis by -CQIK as compared to -BMHP. Although, BBB score of chronic model of SCI in rat implanted with -CQIK nanofiber was higher than control and PBS group but significantly was less than BMHP group. However, -CQIK had induced neurite outgrowth and myelination and inhibited asterogliosis. Based on our results it might be concluded that peptidic nanofiber containing long motif of laminin although induced significantly higher level of neural differentiation than -BMHP in in-vitro but -BMHP provokes significantly stronger motor neuron recovery than -CQIK and holds great promise for spinal cord injury recovery with increment of neurogenesis and astrogliosis decrement.
Keyword: self-assembling peptide nanofiber, Long motif of laminin, Bone marrow homing peptides, Spinal cord injury.

Introduction

To date, spinal cord injury (SCI) has remained an incurable disaster that is associated to paralysis. In spite of many studies have been deeply performed in the field of neurodegenerative disorders but thus far, successful recovery of SCI has not achieved.

By improvement of biomaterial sciences and its involvement in cell biology, scientists encourage to develop more mimicking environment to extracellular matrix (ECM) for cell culture instead of Petri dish. Use of nanofibrous hydrogels as an injectable biomaterials holds great promise with minimum tissue damage and invasion in treatment of SCI 1. It is demonstrated that fiber diameter significantly influences neural differentiation and proliferation and self-assembling is one of several methods for nanofibers synthesis. Some designed oligopeptides are converted into nanofiber upon contact to ionic environment such as cell culture media and in-vivo environment. To the best of our knowledge, for the first time Gelain et al., investigated a self-assembling peptide nanofiber containing IKVAV motif and compared its neural differentiation capacity by neural stem cells seeded on bone marrow homing peptides (-BMHP). Results showed that improved neural cell attachment potential of -IKVAV as compared to self-assembling backbone but less than -BMHP nanofiber. Early studies demonstrated that long motif of laminin can better mimic laminin conformation and improve neurogenesis. Based on these data, in this study, (RADA)4 was used as a backbone and chemically linked to a long motif of laminin (-CQIK) and -BMHP.

The purpose of this study was to compare in neural differentiation of hEnSCs in-vitro by –CQIK and -BMHP and compare the supportive effects of these nanofibers in an animal model of SCI.

Material and methods

With regards to in vitro approach, hEnSCs were isolated from Human endometrial tissue and analyzed using flow cytometry (Partec, Germany). Then, hEnSCs were encapsulated into -CQIK and -BMHP at the 0.125 % v/w concentrations and cell viability and cell membrane damage were assessed. Cells encapsulated into nanofibers were treated with neural differentiation medium for 18 days, and then neural genes and protein markers were analyzed using real time-PCR and immunocytochemistry (ICC).

With regards to in-vivo approach, -CQIK and -BMHP were implanted into rats with SCI and followed for 42 days using a behavioral test. Then, histological specimens were prepared for LFB, Nissl and IHC staining.

RESULT AND DISCUSSION

It seems that mitochondria and cell membrane of neuroblastoma cells were more compatible than hEnSCs to -CQIK nanofibers. Gelain et al., investigation showed that cell attachment of -BMHP nanofiber were higher than short laminin's motif. Our study also indicated that cell viability of encapsulated cells into -BMHP nanofiber was significantly higher than long laminin's motif.

Real-time PCR disclosed that both nanofibers induced neural differentiation and suppressed GFAP gene expression. However, neurogenesis by -CQIK was significantly higher than -BMHP nanofiber and provokes over-expression of TH. Cell membrane contains ECM receptors that can bind to laminin and induces neural differentiation and neurite outgrowth via α6β1 integrin receptors 2. Besides, it might be said that nanofiber diameter plays a critical role in ECM production and neural differentiation. Fiber diameter of –BMHP nanofiber (< 10 nm) was significantly smaller than –CQIK nanofiber (36-42 nm).

It is notable that both nanofibers suppressed GFAP expression at the level of gene and protein which is involved in asteroglial scar formation. It might be due to binding to β1 integrin receptors and triggers of signaling pathway thereof. Bcl2 gene was significantly expressed in higher level by -BMHP than -CQIK nanofiber. Bcl2 through β catenin signaling promotes neurogenesis. It has been said that Bcl2 overexpression induces apoptosis resistance, neurogenesis and axonal regeneration and the reduction in BMP-4. Then, BMP-4 inhibits astrogenesis, as seen in this study.

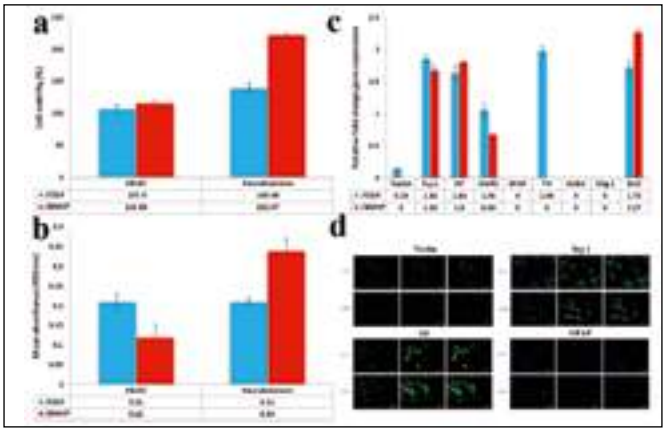

Fig.1. a) Cell viability of cells encapsulated into nanofibers after 48 h b) Flowcytometry of isolated hEnSC indicated that up-regulation of stem cell markers on the cell surface. b) LDH release of cells encapsulated into nanofibers after 24 h. c) Relative fold gene expression of cells encapsulated into nanofibers. d) ICC of cells by using Nestin, Tuj-1, NF and GFAP markers.

The BBB score of rats implanted with -CQIK nanofiber (13.75) were significantly less than -BMHP nanofibers (19) along with decreasing

of cavity size, higher extent of NF and myelination and less GFAP up-regulation (reactive astrocytes) and gliosis around the cavity as compared to -CQIK, PBS and control groups on the 42nd day.

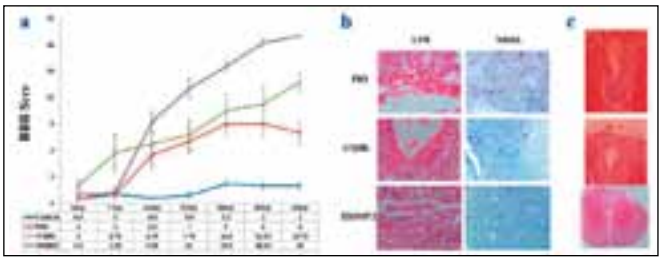

Fig. 2. a) BBB score of rats implanted with PBS, control, -CQIK and -BMHP b) Nissl and LFB staining of specimens. c) Cavity size of specimens, upper PBS, middle –CQIK and bottom - BMHP; Scale bar is 200µm.

In the light of axon regeneration with long motif of laminin in the presented study it might be said that some earlier reports disclosed that a beta amyloid precursor (110 KD) from brain-membrane-associated laminin binding protein- through binding to IKVAV site improves neurite outgrowth and others indicated that activation of c-jun directly and indirectly through activation of FAK, JNK, PI3K/Akt and ERK pathways by IKVAV- α6 integrin interaction induces neurite outgrowth. Since, BMHP motif homed to bone marrow and specifically bound to hematopoietic stem cells 3 Bjornson et al,. 4 demonstrated that eventually there is some shared adhesion receptors and differentiation pathways between neural stem cell and bone marrow stem cells and this receptors is involved in neurogenesis by BMHP nanofibers. These results disclosed the neurogenesis and anti-asterogliosis potential of these nanofibers in-vitro and in-vivo.

CONCLUSION

In conclusion based on our data, it might be said that although nanofiber containing long motif of laminin induced higher neural differentiation of hEnSCs than -BMHP nanofiber and even TH gene expression but in-vivo investigation showed that -BMHP nanofiber had stronger efficacy on SCI rats. This finding proved the critical role of chemical transduction by the self-assembling peptide nanofiber with different biological motif.

ACKNOWLEDGMENT

This work was supported by grant from Student's Scientific Research Center, Tehran University of Medical Sciences, Iran.

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NGS BASED NON-INVASIVE PRE-IMPALNTATION ANEUPLOIDIA TESTING FOR ASSESSING EMBRYO VIABILITY

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Following in vitro fertilisation (IVF), embryo assessment and therefore selection for transfer is routinely based on morphological characteristics such as blastomere number and size, degree of fragmentation, symmetry and multi nucleation (Alpha Scientists in Reproductive Medicine and ESHERE Special Interest Group of Embryology, 2011). The successful pregnancy rate of assisted reproductive technology (ART) is around 30 % (Hansen et. al, 2005). From the embryonal side, aneuploidy is one of the main risks of implantation failure. One choice to increase success rates of IVF is selecting embryos without chromosomal abnormalities and with the highest viability. Aneuploidy testing before embryo transfer involves screening of extra or missing chromosomes, and „omics" technologies through „low DNA" sequencing protocols opened the door to develop methods for routine preimplantation genetic diagnosis (PGD). PGD is usually highly regulated not only due to ethical concerns, but in practise it is connected with controversial invasive sampling, namely removal of embryonal cells such as polar bodies, blastomeres, or trophoectodermal cells. Embryonal biopsy procedure is not considered risk-free, so it is still an issue to develop a novel and less questionable methods connected with non-invasive sampling to avoid any damage of the embryo. Recently the research has turned to the investigation of the spent embryo culture media, because the profiling of released DNA content and metabolomics of the embryo could offer opportunity to conclude to embryo viability (Stigliani et al., 2013, Uyar et. al, 2014).
Alternative NGS-based non-invasive screening method was developed using the early (day 3 or 5) embryonal media to screen chromosomal abnormalities to meet both ethical and clinical needs. After whole-genome amplification (WGA), sequencing was performed on Iontorrent PGM platform and the copy number of the individual 22 chromosome were tested for the diagnosis for aneuploidy, where chromosomal differences were detected as copy number deviations. Method has been validated on one hand using samples from healthy individuals and from people diagnosed with Down and Kleinefelter syndrome and from other hand using pseudodiploid REH2 and hyperdiploid MHH-CALL2 kariotyped cell lines (Fig 1 A and B).

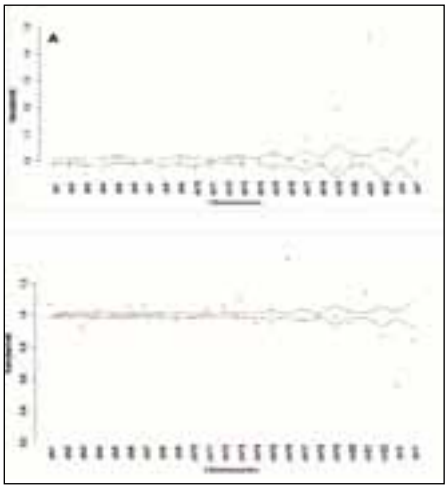

Fig 1. Graphical representation revealed equal read distribution of the WGA products derived from healthy samples. Verification of

chromosomal copy number changes observed in tested samples from (A) Down syndrome (21 trisomy) diagnosis and from (B) pseudodiploid cell line (-X, +16, del3).

Our results revealed reduced amplification bias as an outcome of the linear amplification and distribution of sequenced reads across the whole genome. Based on our findings, the chromosome ratios of each successful pregnancy are between 0.7 and 1.35. We detected significant aneuploidy events in 3 and 5-day embryos. Pregnancies started from embryos having chromosome ratios higher than 1.35 have led to spontaneous abortion (Fig.2).
After clinical validation, NGS-based aneuploidy screening appears to forecast the lethal genetic aneuploidies, and with the overcoming of invasive sampling issues it has the potential to represent useful strategy in increasing ART success.

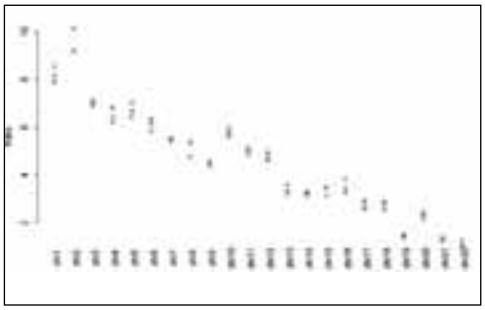

Fig 2. Visualisation of chromosome read ratios of aborted embrios (x/o) versus healthy control (+).

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ANALYSIS OF THE PATTERN RECOGNITION PEPTIDE-NUCLEIC ACID INTERACTIONS

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Pattern recognition is an ancient mechanism through which cells recognize each other and the immune system recognizes a broad range of pathogens. The human protein DMBT1 reflects these ancient functionalities by participation in differentiation, regenerative and immune defense processes. The main pattern recognition mechanism attributed to DMBT1 is the capability to recognize and bind poly-sulfated and poly-phosphorylated agents via a short peptide motif present in its repetitive ligand-binding domains. We aimed to investigate interactions of synthetic DMBT1 peptides with nucleic acids, using siRNA as a paradigm. Hypothetically, these would interact via positively charged residues with the phosphate backbone, while aromatic amino acids, such as tryptophan and tyrosine, could stabilize complexes further by pi-stacking similar to the amyloid fibril formation in Alzheimer`s disease. To this end, we report that stable binding takes place under defined in vitro conditions with both single-stranded and double-stranded nucleic acids. The complexes are stable over time and confer nuclease-resistance. Next steps include characterization of the complexes and testing stability in the presence of serum.

CURCUEMULSOMES TAILORED WITH THE S-LAYER TARGETS IMMUNOGLOBULIN G

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Curcumin is a natural polyphenolic compound with intrinsic anticancer properties. Its medical use remains limited due to its extremely low water solubility and bioavailability. Our previous study introduced novel curcumin-emulsome nanoformulations, so-called CurcuEmulsomes, where curcumin is encapsulated inside the lipid matrix of the nanocarrier. Incorporation of curcumin into emulsomes overcame the bioavailability limitation and increased its solubility by up to 10,000-folds corresponding to a concentration of 0.11 mg/ml.[1] Proceeding one step further, recently CurcuEmulsomes were modified by S layer proteins fused with two protein G domains possessing specific affinity for immunoglobulin G (IgG).[2] This study has demonstrated that the S-layer fusion proteins recrystallize on the surface of the nanocarriers and form an ordered surface layer exposing a square lattice with 13 nm unit-by-unit distance (Figure 1-A); thereby presenting the functional GG domains in a predicted orientation. This inherent control over orientation enables binding of the IgG on the S-layer with its F_c domain, whereas functional F_{ab} region of the IgG remains accessible for antigen binding, as verified by transmission electron microscopy (TEM) analysis using anti-IgG gold conjugates (Figure 1-B). The introduced system, i.e. CurcuEmulsomes tailored with the S layer, has the potential to enable targeted delivery of curcumin to abnormal cell.3

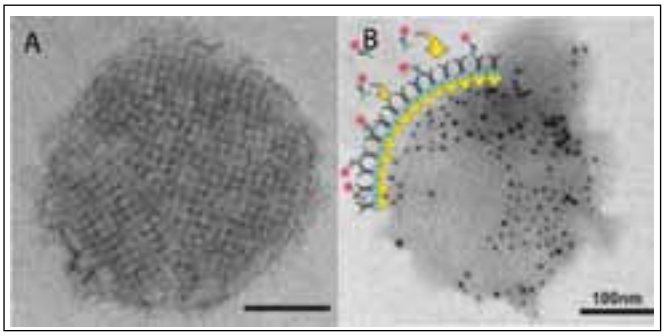

Figure 1. TEM images of (A) CurcuEmulsome coated with a functional S-layer, (B) CurcuEmulsomes specifically interacting anti IgG-Au conjugates. Bar sizes correspond to 100 nm.

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LONG-LASTING NEUROKININ-1 RECEPTOR TARGETED POLYMER-DRUG-CONJUGATES FOR POTENTIAL THERAPY OF RHEUMATOID ARTHRITIS

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The functional role of the neurokinin-1 receptor (NK-1R), a transmembrane bound G-protein coupled receptor is discussed in the context of autoimmune diseases and the genesis and perception of pain. Typical NK-1R expressing cells are related to the immune system and nerve fibers. In rheumatoid arthritis (RA), the NK-1R and its major agonist substance p are also found to be upregulated in cells of the joint fluid and cartilage. RA patients suffer from both, the persistent inflammation in the joint and the resulting pain. Great efforts were underway to develop efficient antagonists for the blockade of neurokinin-1 receptors with the aim to interrupt the immune and pain response at the same time. However, many ligands failed in their early preclinical phase because of short half-lives, poor water solubility and unspecific or rather unselective binding.
Our idea is to overcome these problems by coupling potent and selective antagonists to branched polyethylene glycols (PEGs) and thereby form multivalent receptor blockers with the ability to bind several receptors simultaneously. This should result in an improved pharmacokinetic in the joint space and increase the probability to target only morbid cells with high receptor density.
As a selective NK-1R ligand we used spantide I, a hydrophilic undecapeptide and linked it either to succinimidyl carbonate functionalized linear methoxyPEG5k (mPEG5k) or to 8armPEG20k via its amino groups. Reversed-phase HPLC analysis was performed to monitor the success of the PEGylation reaction and to verify complete removal of free unbound ligand after several ultrafiltration steps. The coupling efficiency was determined with a combination of tryptophan absorption measurement and quantification of PEG in a $BaCl_2$/iodine reaction assay. We calculated that 7.6 ligands were bound per 8armPEG20k molecule. In cell-based binding studies with a neurokinin-1 receptor positive CHO cell line we determined the IC50 values of unmodified and PEGylated spantide I by measuring the intracellular calcium release. We found that PEGylation in both cases resulted in a change of the receptor binding properties of spantide I (Figure 1). This could be confirmed on the one hand by the total loss of partial agonism of the peptide after PEGylation and on the other hand by a reduced antagonistic binding affinity. Especially the linkage to linear PEG resulted in a 1200-fold reduced antagonistic affinity of spantide I. In contrast the 8armPEG bound spantide I showed a 20-fold reduction in affinity. This affinity loss seemed to be compensated by a multivalent binding effect, which explained the 65-fold lower IC50 value of 413 nM ± 1 in contrast to the mPEG5k-spantide I of 27.4 µM ± 1.1. Changes in the Hill slope from steep to flat, indicated a tendency for a negative cooperative effect with increasing PEGylation degree.

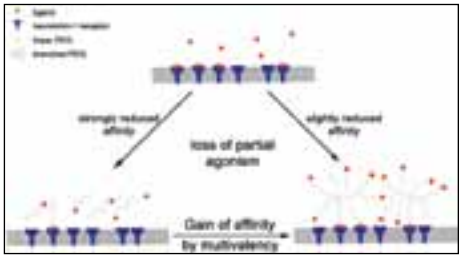

Figure 1. Schematic overview of binding study results with ligands coupled to different polyethylene glycol species.

Alltogether, coupling of spantide I to PEG leads to a full antagonist without partial agonistic potency. This implies that there are two different recognition motives within the receptor sequence for either agonistic or antagonistic intracellular signaling effects. In each case an individual neurokinin-1 receptor binding pocket has to be formed. The coupling of peptide based NK-1R ligands to branched polymers seem to be a promising strategy to get water soluble and potent receptor blockers with long-lasting characteristics for the local therapy of rheumatoid arthritis. In future experiments we will try to explore their properties in a rheumatoid arthritis in vivo model.

QM BASED CHARACTERIZATION OF NANOMATERIALS FOR INDUSTRY

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You manufacture or distribute innovative products containing nanomaterials and need customized analytical solutions for quality or safety control of your product? You prefer dealing with just one contract partner and have to rely on quality management?
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LAURIC ACID - ALBUMIN COATED HYBRID SPIONS FOR MAGNETIC DRUG TARGETING – THE ROAD TO GMP

JAN ZALOGA[1],Christina Janko[1], Johannes Nowak[2], Rainer Tietze[1], Ralf P Friedrich[1], Stefan Odenbach[2], Frank Dörje[3], Stefan Lyer[1], Geoffrey Lee[4], Christoph Alexiou[1]

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Figure1: Graphical abstract of the GMP-production of the particles as a stepstone towards clinical trials

Core-shell superparamagnetic iron oxide nanoparticle (SPION) systems have demonstrated very promising properties for biomedical applications. Magnetic Drug Targeting, which is one of the potential applications, involves local accumulation of the drug- loaded particles by a magnetic field gradient. Our group has previously shown the outstanding potential of Magnetic Drug Targeting (MDT) in animal tumour models [1]. For this application, the particles need to exhibit excellent drug binding capacity, colloidal stability even in complex biological fluids and under the influence of strong magnetic fields and good magnetic attractability. In order to realise the translation of lab-scale synthesis into clinical trials, the synthesis

has to be up-scaled and translated into a manufacturing environment under current good manufacturing practice (cGMP) regulations. Additionally, it is desirable to optimize the system in order to show maximum efficiency in human experiments.
Here, we present the synthesis of a lauric acid / serum albumin hybrid coated iron oxide nanoparticle system (SEONLA-SA). The two-step synthesis is highly reproducible and allows easy up-scaling. All products can be obtained in pharmaceutical quality. Such hybrid coated SPION systems demonstrated outstanding biocompatibility and colloidal stability in biorelevant fluids –like whole blood- before [2]. Using cryo – TEM we proved that the protein forms a distinct and compact albumin shell around the particle multicore clusters, with the fatty acid acting as anchoring groups on the particle surface.

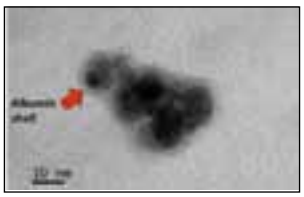

Figure2: cryo- TEM images of the lauric acid/albumin coated iron oxide nanoparticles

Purification by tangential ultrafiltration allows sterile removal of excess protein and concentration of the particles in order to enhance their magnetic attractability. Using vibrating sample magnetometry (VSM) we showed that the saturation magnetization (MS) of SEONLA-SA can be enhanced to up to 1667.9 A/m, representing an over 3.7 – fold increase. This allows additional use of hyperthermia treatments, as the maximally achievable temperature is increased from 44.4 °C to up to 64.9 °C.

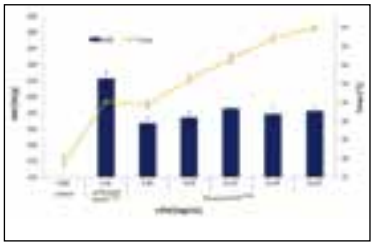

Figure3: increase of the maximally achievable heating temperature (Tmax) of SEONLA-SA after concentration by tangential ultrafiltration

We coupled the cytotoxic drug Mitoxantrone (MTO) to the particles, which exhibited high binding efficacy of up to 800 µg/ml ferrofluid. We investigated the in vitro therapeutic efficiency on prostate cancer (PC-3) and breast cancer (MCF-7) cells under the influence of a magnetic field. The MTO-loaded particles (SEONLA-HSA*MTO) show excellent dose-dependent toxicity which is locally minimised to the point of magnetic attraction.
In conclusion, we have prepared and developed a system that fulfils all the requirements for clinical use. Although it was primarily designed for targeting of cancer, it is not necessarily limited to that target and could also be used for other biomedical applications. With this product, we are confident going forward to the translation to a GMP environment in the near future.

Acknowledgement: The authors would like to thank the German Research Foundation (HI 689/7-4, AL552/5-1, TR408/4-3) and the EU project FP7-NMP-2012-LARGE-6-309820 “NanoAthero” for their financial support.

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