CLINAM

European Foundation for Clinical Nanomedicine

10/2017

Basel, May 7–10

10 YEARS CLINAM! CONFERENCE PROCEEDINGS

10TH EUROPEAN & GLOBAL SUMMIT FOR CLINICAL NANOMEDICINE AND TARGETED MEDICINE: WITNESSING THE SOLUTIONS AND TACKLING THE HURDLES

Summit under the Auspices of the Swiss Confederation in Collaboration with 31 Organizations:

FDA

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Swiss Confederation

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

Non Biological Complex Drugs

EPMA

EFGCP

E·MRS

nanomedicine
EUROPEAN TECHNOLOGY PLATFORM

epnm

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Materials Science & Technology

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Nanomedicines ALLIANCE

ESNAM
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CLINAM

European Foundation for Clinical Nanomedicine

10/2017

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EUROPEAN & GLOBAL SUMMIT FOR CLINICAL NANOMEDICINE AND TARGETED MEDICINE

10TH EUROPEAN & GLOBAL SUMMIT FOR CLINICAL NANOMEDICINE AND TARGETED MEDICINE: WITNESSING THE SOLUTIONS AND TACKLING THE HURDLES MEDICINE

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CLINAM 10/2017

10TH EUROPEAN & GLOBAL CONFERENCE AND EXHIBITION FOR CLINICAL NANOMEDICINE AND TARGETED MEDICINE: WITNESSING THE SOLUTIONS AND TACKLING THE HURDLES

The Founders in 2007

Welcome to Basel – Ten years CLINAM FOUNDATION AND TEN CLINAM SUMMITS. We use the opportunity of this Jubilee to present a programme with a strong focus on the route of nanomedicine – from basic and enabling sciences to successful clinical applications in targeted and precision medicine and related fields.

We will this year discuss critically common bottlenecks based on the experience of the past decade by our broad international expert community. Over the past decade, the CLINAM Summit evolved to an exquisite and globally unique event that brings together all stakeholders in Nanomedicine and Targeted Medicine. It builds on the principle that fundamental scientists, developers and professionals in clinical application and all to Nanomedicine related persons can mutually learn from each other to find better solutions for the medicine of the future. Based on recent groundbreaking achievements, the meeting will be a highlight.

Nanomedicine and Targeted Medicine are the catalyst disciplines for developing diagnostics and treatments accounting for the nanoscale, molecular and cellular origin of disease and one of the enabling disciplines for the knowledge-based personalized medicine. The CLINAM-Foundation's primary goal is to support the development and application from the stage of basic research all the way to the clinics for the benefit of the patient and humankind.

Again, the CLINAM Summit hosts the regulatory authorities from all continents. We are grateful that the regulators have decided for a satellite meeting to discuss nanotechnology and specifically nanomedicine related issues relevant to regulated products. The International Pharmaceutical Regulators Forum (IPRF) will give birth to a second Declaration of Basel which also serves as initial point for the open discussion in the by now traditional Plenary Session Regulatory Authorities' Voice which is open to all participants of the Summit.

This year the Nobel Laureates Prof. Dr. Jean-Marie Lehn and Prof. Dr. Ada Yonath will open the Summit. Prof. Lehn will speak about the pathways from supramolecular chemistry towards adaptive chemistry, focusing bioorganic and biomedical aspects and Prof. Yonath about the next generation of antibiotics and antiparasites. Forty following sessions will give room to 170 speakers and 100 poster presenters from the summit-community to highlight their work followed by lively discussions.

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

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

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

INTRODUCTION
ON BEHALF OF SWITZERLAND

For the tenth time the CLINAM Summit is held in Switzerland. It has become by now the international renowned Platform with not less than 31 supporting collaborating organizations to exchange the knowledge and research results in Nanomedicine and Targeted Medicine and related fields.

CLINAM is the place to debate the unmet medical needs, hurdles and novel solutions that can be offered by the application of Nanomedicine, Targeted Delivery and other related Novel Technologies. At CLINAM, the interventions are short and the discussion is long. This seems to be the good style to progress and to come to cooperation, to new co-working networks and projects.

CLINAM hosts participants form 35 countries. This neutral non-for-profit Summit has gained high value. This is proved since also the International Regulatory Authorities now take advantage of the platform. For the second time they will have their International Pharmaceutical Regulators Forum (IPRF), this year under the Lead of Canada. The Nanomedicines Working Group shall discuss nanotechnology and specifically nanomedicine related issues relevant to regulated products. There will be again a Declaration of Basel, highlighting the outcome of the meeting.

We welcome you all, dear Regulatory Authority Members from Europe, from the United States, from China, from India, from Canada, from Japan and from Africa and we are proud that you have chosen Switzerland for your present important meeting. The participants, a mix of Clinicians, Biochemists, Chemists, Physicists, Pharmacologists, Engineers, Investors and Industry has the opportunity to have face to face contact with you. It creates trust, understanding and will doubtlessly accelerate processes, leading to new medications to the benefit of the patients and humankind.

Nanomedicine and Targeted Medicine are essential building blocks in the development towards Precision Medicine. Personalized medicine is certainly the future, which to some extent has already started and is at CLINAM a most important Focus.

The Jubilee-Summit of CLINAM is under the auspices of the Swiss Confederation. We support it, because we believe that Switzerland has the political stability to actively promoting novel scientific fields as an Innovation leading country in Europe.

We wish all of you a fruitful meeting with new projects and worldwide cooperation that will accelerate the innovation process in medicine.

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

CLINAM

European Foundation for Clinical Nanomedicine

10/2017

Basel, May 7–10

CURRICULA VITAE SPEAKERS

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

Namrata Anand

Dr. Namrata Anand born on 9th April 1985 is a PhD from Postgraduate Institute of Medical Education and Research, India and is currently working as a postdoctoral fellow in Department of Zoology Panjab University, India. During my PhD, I have worked on milk protein 'Lactoferrin' (Lf) isolated from bovine and buffalo colostrum and examined its antiparasitic properties against intracellular parasites like; Plasmodium, Toxoplasma and Leishmania. I have also worked on the Lf protein interaction towards RBCs and macrophages of human origin to better understand the mechanism of action as they are the host cells for intracellular parasites. I have found that, Nanoformulation of bovine and buffalo Lf using Alginate Enclosed Chitosan nanoparticles showed better results compared to native Lf form in controlling the disease progression in mice model. The mode of mechanism of Lf protein was found to be due to production of free radicle ions and accumulation inside the macrophages of mice visceral organs which are site for parasite multiplication. My current research work is to examine the antiparasitic properties of these nanoparticles and its comparison with conventional drugs using pregnancy mouse models of disease like malaria and toxoplasmosis.

PUBLICATIONS

- Antiparasitic and immunomodulatory potential of oral nanocapsules encapsulated with lactoferrin protein against Plasmodium berghei. Anand et al., 2016. Nanomedicine.11 (1): 47-62.
- Oral administration of encapsulated iron binding bovine lactoferrin protein Nanocapsules against intracellular parasite :Toxoplasma gondii. Anand et al. International Journals of Nanomedicine. 2015.10: 6355-6369.
- Effect of lactoferrin protein on red blood cells and macrophages-mechanism of parasite host interaction. Drug Design Development and Therapy. Anand et al., 2015 :9 3821–3835.
- Standardization of Plaque Assay of Japanese Encephalitis Virus (Nakayama NIH Strain) on BHK-21 (Cl-13) Cell Line 2010. Anand, N., Kumar,S., and Gowal,D. Am. J. Biomed. Sci. 2010, 2(1), 43-50.
- Lactoferrin nanocapsules prevent malaria infection via immunomodulation and regulate miRNAs involved in iron metabolism. Anand et al., 2016. In communication with Journal of Infectious Disease. (Pre Reviewed)

Priya Anand

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EDUCATIONAL QUALIFICATIONS

- Ph.D. (Biosciences), University of Heidelberg, Germany (01/2010-11/2013); Thesis: Structure based design of protein linkers for Zinc finger nucleases. (Magna cum Laude)
- M.Sc. (Biophysics Hons.), Panjab University, Chandigarh, India (04/2002–04/2004); Thesis: Modulatory Effects of Nootropic Drug Piracetam and PP-20/DPJ on the Aluminum Induced Neurotoxicity in Developing and Developed Rat Brain.
- B.Sc. (Biophysics Hons.), Panjab University, Chandigarh, India (04/1999 – 03/2002).

WORK EXPERIENCE

- Postdoctoral Research Associate, November 2013 – Current; Research Group: Prof. (apl.) Dr. Wolfgang Wenzel, Karlsruhe Institute of Technology, Germany. Rational Development of Peptide-Surface Interactions to design biomolecular peptide tags for magnetic particles using Monte Carlo simulations in combination with Umbrella Sampling and Metadynamics (patent application in process).
- Ph. D. Graduate , January 2010 – November 2013; Advisor: Prof. Dr. Jörg Langowski, DKFZ, University of Heidelberg, Germany. Structural-based design of possible protein linker sequences that can be introduced between the individual zinc finger protein (ZFP) motifs to skip upto 10 DNA base pairs between adjacent ZFP recognition sites in the DNA.

AWARDS

- Awarded "DAAD (DEUTSCHER AKADEMISCHER AUSTAUSCHDIENST)" Fellowship (May 2007). Worked at John von Neumann Institute for Computing, Forschungszentrum Jülich, Germany.
- Awarded "A. R. Gopala Ayengar Award" from the Indian Biophysical Society, New Delhi, India (2007).
- Awarded "Silver Jubilee Merit Scholarship" for excellent academic records, twice by the Government of Haryana, Chandigarh, India (2002–04).

PUBLICATIONS

- S. Blank-Shim, S. Schwaminger, M. Borkowska-Panek, P. Anand, P. Fraga García, K. Fink, W. Wenzel, S. Berensmeier. Selective and Reversible Binding of Short Peptides to Magnetic Iron Oxide Nanoparticles. ACS Nano 2017 (Submitted).
- Kyungsoo Ha, P. Anand, J. Lee, J. Jones, C. Kim, D. Bertola, J. Labonne, W. Wenzel, L. Layman, H.G. Kim. Steric Clash in the SET domain of Histone Methyltransferase NSD1 as a Cause of Sotos Syndrome and its Genetic Heterogeneity in a Brazilian Cohort (2016) Genes, Nov 9;7(11).
- P. Anand, M. Borkowska-Panek, F. Gussmann, K. Fink, W. Wenzel. Modeling Peptide Adsorption on Inorganic Surfaces. Springer International Publishing AG (2016), E. Bartocci et al. (Eds.): CMSB 2016, LNBI 9859, pp. 339–340.

- J. Labonne, M. J. Chung, J. R. Jones, P. Anand, W. Wenzel, D. Lacoboni, L. C. Layman1, H.G. Kim. Concomitant Partial Exon Skipping by a Unique Missense Mutation of RPS6KA3 causes Coffin-Lowry syndrome. (2016) Genes, 575(1):42-7.
- C Schmidt, F. Wiedmann, C. Langer, F. Tristam, P. Anand, W. Wenzel, P. Lugenbiel, PA. Schweizer, HA. Katus, D. Thomas. Cloning, functional characterization, and remodeling of K2P3.1 (TASK-1) potassium channels in a porcine model of atrial fibrillation and heart failure. (2014) Heart Rhythm, 11(10):1798- 805.

PATENTS

- "Magnetic particle-binding peptides" European Patent (Submitted).

Ulf G Andersson

NanoMed North

Ulf G Andersson is the founder and chairman of NanoMed North, a nanomedicine consortium with more than 150 members, mainly from Scandinavia.
He is presently the CEO of Medeon Science Park & Incubator, Malmö, Sweden in addition to board membership in various Life Science companies. He is also a member of the Research Education Committee at the Medical Faculty of Lund University, Sweden.
Andersson has a basic education from the Technical Faculty of Lund University within Applied Biochemistry and Biotechnology (1982-86).
Since 1986, Andersson has a professional career as a CEO, President, VP Marketing and Sales and board memberships in more than 15 Life Science companies and has worked in various fields of Life Science including nanomedicine, biotech, pharma development, medtech, diagnostics and medical imaging, all with significant global activities.

Elke Anklam

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Elke Anklam is a chemist, with specialisation in food, organic and radiation chemistry. After obtaining her PhD from the University Hamburg, Germany, she worked in various European Research Institutions and was a Teaching Professor at the Applied University of Fulda, Germany.
Since 1991, she has been working at the European Commission's Joint Research Centre (EC-JRC). From 2006-2012, she was Director of the JRC-Institute for Health and Consumer Protection (IHCP) in Ispra, Italy and from 2012 – July 2016, Director of the JRC-Institute for Reference Materials and Measurements in Geel, Belgium.
Since July 2016, she is the Director of the JRC-Geel site and the new JRC Directorate F: Health, Consumers & Reference Material (a merger of the former IHCP and IRMM), located at the JRC-Geel and JRC-Ispra site.

Marianne Ashford

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

Anthony Amaechi Attama

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

PUBLICATIONS:

- Attama A.A., Reginald-Opara J.N., Uronnachi E.M., Onuigbo E.B. (2016). Nanomedicines for the Eye: Current Status and Future Development. In: Nanoscience and Dermatology. MR Hamblin, P

Avci and TW Prow (Eds.), Elsevier, pp. 323-335.
- Nnamani P.O., Ugwu A.A., Ibezim E.C., Kenechukwu F.C., Akpa P.A., Ogbonna J.D.N., Obitte N.C., Odo A.N., Windbergs M., Lehr C.M., Attama, A.A. (2016). Sustained-release liquisolid compact tablets containing lumefantrine-artemether as alternate-day-regimen for malaria treatment to improve patient compliance. International Journal of Nanomedicine 11, 6365-6378.
- Attama A.A., Kenechukwu F.C., Onuigbo E.B., Nnamani P.O., Obitte N., Finke J.H., Pretor S., Muller-Goymann C.C. (2016). Solid lipid nanoparticles encapsulating a fluorescent marker (coumarin 6) and antimalarials – artemether and lumefantrine: evaluation of cellular uptake and antimalarial activity. European Journal of Nanomedicine 8(3) 129-138.
- Umeyor C.E., Attama A.A., Uronnachi E.M., Agbo C.P., Reginald-Opara J.N., Kenechukwu F.C. (2016). Formulation of gentamicin as surface modified self-nanoemulsifying formulations (SNEFs) improves its anti-pneumococcal activity. European Journal of Nanomedicine 8(2) 101-110.
- Umeyor C.E., Attama A.A, Uronnachi E.M., Kenechukwu F.C., Nwakile C.D., Nzekwe I.T., Okoye I.E., Esimone C.O. (2016). Formulation design and *in vitro* physicochemical characterization of surface modified self-nanoemulsifying formulations (SNEFs) of gentamicin. International Journal of Pharmaceutics 497, 161–198.

Gilad Bachrach

The Institute of Dental Sciences, the Hebrew University-Hadassah School of Dental Medicine, Jerusalem P.O.B 12272, Israel 91120, giladba@ekmd.huji.ac.il

Prof. Bachrach began his academic career at the Hebrew University of Jerusalem where he received his B.S. in Biology (1987), M.S. in Microbiology (1989) and his Ph.D. in Microbiology (1995). In his Ph.D. he investigated the highly virulent Brucella melitensis that causes Malta fever, a disease still found in Israel.
He then followed to widen his experience in work with virulent bacteria as a post doctorate fellow at the National Institute of Medical Research in London where he investigated molecular mechanisms involved in virulence of Mycobacterium tuberculosis (1996-1998). He then continued for a year at the National Institute of Dental and Craniofacial Research (NIH, USA) to gain experience in the field of Oral Microbiology prior to his return to Israel in October 1999 as a staff member in the Hebrew University-Hadassah School of Dental Medicine in Jerusalem where he is an associate professor today and head of the Biomedical Sciences Graduate Program.
During his year at NIH, Prof. Bachrach became interested in Fusobacterium nucleatum, an oral anaerobic bacteria involved in the development of periodontal disease. His group created unique genetic tools for working with fusobacteria and is one of few capable of their genetic manipulation. These tools prove very useful in investigation of the recently discovered involvement of fusobacteria in colon cancer.
Prof. Bachrach is married to Daniela and is they are the parents of Maya, Adi and Emanuel.
Prof. Bacharach's Scientific Interests include BcateriOncology, OncoBacteriology, Oral Microbiology

PUBLICATIONS

- Abed, J., Emgard, J. E., Zamir, G., Faroja, M., Almogy, G., Grenov, A., Sol, A., Naor, R., Pikarsky, E., Atlan, K. A., Mellul, A., Chaushu, S., Manson, A. L., Earl, A. M., Ou, N., Brennan, C. A., Garrett, W. S., and Bachrach, G. (2016) Fap2 Mediates Fusobacterium nucleatum Colorectal Adenocarcinoma Enrichment by Binding to Tumor-Expressed Gal-GalNAc. Cell Host Microbe 20, 215-225
- Gur, C., Mandelboim, O., and Bachrach, G. (2015) "Messieurs, c'est les microbes qui auront le dernier mot": Gentlemen, it is the microbes who have the last word (Louis Pasteur)- protect tumors from killing by immune cells. Oncoimmunology 4, e1038690
- Gur, C., Ibrahim, Y., Isaacson, B., Yamin, R., Abed, J., Gamliel, M., Enk, J., Bar-On, Y., Stanietsky-Kaynan, N., Coppenhagen-Glazer, S., Shussman, N., Almogy, G., Cuapio, A., Hofer, E., Mevorach, D., Tabib, A., Ortenberg, R., Markel, G., Miklic, K., Jonjic, S., Brennan, C. A., Garrett, W. S., Bachrach, G.,* and Mandelboim, O.* (2015) Binding of the Fap2 Protein of Fusobacterium nucleatum to Human Inhibitory Receptor TIGIT Protects Tumors from Immune Cell Attack. Immunity 42, 344-355
- Coppenhagen-Glazer, S., Sol, A., Abed, J., Naor, R., Zhang, X., Han, Y. W., and Bachrach, G. (2015) Fap2 of Fusobacterium nucleatum Is a Galactose-Inhibitable Adhesin Involved in Coaggregation, Cell Adhesion, and Preterm Birth. Infect Immun 83, 1104-1113
- Sol, A., Skvirsky, Y., Nashef, R., Zelentsova, K., Burstyn-Cohen, T., Blotnick, E., Muhlrad, A., and Bachrach, G. (2014) Actin Enables the Antimicrobial Action of LL-37 Peptide in the Presence of Microbial Proteases. J Biol Chem 289, 22926-22941

* Equal contribution of authors

Simon Baconnier

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

Lajos (Lou) P. Balogh

Dr. Lajos (Lou) P. Balogh, Ph.D., is the Executive Editor of Manuscript Clinic, (www.manuscriptclinic.us) supporting scientists and students to successfully publish research results. He is also the Chief Scientific Advisor of AA Nanomedicine & Nanotechnology USA (AA Nanomed). AA Nanomed promotes nanomedicine and nanotechnology research, assists R&D projects and organizes nanotechnology events. It offers expert consultation, project evaluation, and technology due diligence for institutions, companies, and government agencies in nanomedicine, nanobiotechnology, and nanotechnology. Past and current clients include NIH, NSF, EPA, DoD, DTRA, CAS, SNUH, etc.

Dr. Balogh received his Ph.D. in Hungary and held faculty positions at the Kossuth L University, Hungary, the University of Massachusetts Lowell, MA, the Michigan Molecular Institute, Midland, MI, the University of Michigan, Ann Arbor, MI, and the Roswell Park Cancer Institute, Buffalo, NY. Between 2008-2016 Lou made the journal Nanomedicine: Nanotechnology, Biology and Medicine (Elsevier) successful as Editor-in-Chief (JIF=6.9, 5yr IF=7.5) Dr. Balogh published 219 scientific papers, gave >220 invited lectures, and was awarded 12 patents in chemistry, dendrimers, drug discovery, materials science, nanomedicine, and nanotechnology. Lou has 6 papers with more than 200 and 14 papers with more than 100 citations (a total of > 6000 today). Lou is one of the five Founders of the American Society for Nanomedicine, board member of the International Society for Nanomedicine, scientific board of CLINAM, member of the Nanobusiness Alliance USA, the Steering Committee of the ANSI Nanotechnology Panel, the US Technical Advisory Committee to ISO on Nanotechnology (TC-229), in addition to numerous USA, European, and International expert committees. He is Recipient of the KOFST Brain Pool Program Award for Renowned Foreign Scientists, Visiting Professorship for Senior International Scientists of the Chinese Academy of Sciences, and member of the External Body of the Hungarian Academy of Sciences, etc.
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Yechezkel Barenholz

Head of Membrane and Liposome Research Lab, Hebrew University-Hadassah Medical School, POB 12272, Jerusalem 91120, Israel,
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chezyb@ekmd.huji.ac.il

Professor Emeritus Barenholz (Daniel G. Miller Professor in Cancer Research) received his Ph.D. at the Hebrew University-Hadassah Medical School, Jerusalem in 1971. He has been on the faculty of the Hebrew University since 1968 and was promoted to a Professor in 1981. He was a Visiting Professor it the Department of Biochemistry, University of Virginia School of Medicine, Charlottesville VA, USA from 1973 to 2005. He has been a Visiting Professor at the following universities: University of Utrecht, The Netherlands, 1992; the University of Kyoto, Japan, 1998; La Sapeinza University, Rome, 2006; Jaiotung University, Shanghai, China, 2006; Kings College, University of London, UK, 2006; and, the Danish Technical University DTU, Copenhagen, 2010.

The basic research of Professor Barenholz focuses on the biophysics of lipid assemblies such as liposomes and micelles, and on the composition-structure-function relationships of biological membranes (with special focus and contributions related to sphingolipids).

His applied research centers around the development of drug delivery systems (DDS) and drugs based on such DDS including low molecular weight anti-cancer, anti-inflammatory, anti-bacterial, and local anesthetic drugs, as well as delivery systems for peptides, proteins, nucleic acids, and vaccines. This is best exemplified by Doxil®, which was based on Barenholz invention and was developed to an FDA- and world-wide-approved anti-cancer drug by Professor Barenholz together with the oncologist Professor Alberto Gabizon, and SEQUUS Pharmaceuticals, Menlo Park CA, USA. Doxil® (Caelyx® in Europe) is the first FDA-approved nano drug and the first FDA-approved liposomal drug (1995). It is distributed today all over the Globe by Johnson and Johnson. More than 600,000 people were treated so far with Doxil, and its sales exceeds half a billion dollars a year. Professor Barenholz, with the help of others, based on his inventions, founded the following start-up companies: 1. Moebius Medical, for the development of a liposome-based treatment of osteoarthritis. Moebius finished successfully first clinical trial and are now preparing for a large pivotal clinical trials; recently this technology was licensed Sun-Pharma 2. Polypid LTD which develops local, prolonged, controlled release drug delivery system based on a combination of lipids and polymers for broad spectrum of active agents including low molecular weight drugs, proteins, and nucleic acids. Polypid finished successfully her first clinical trial in the local bone treatment by BonyPid an antibiotic used for the treatment of bacterial infection of bones. Polypid is now preparing for more advanced clinical trials 3. LipoCure Ltd that focuses on the development of liposomal nano drugs based on Professor Barenholz' inventions for treatment of cancer and inflammatory diseases [rheumatoid arthritis (RA) and multiple sclerosis (MS)], as well as for highly prolonged local anesthetics based on special bupivacaine remotely loaded large multi-vesicular liposomes embedded in hydrogel. Two of the liposomal drugs under development at LipoCure are in final preparation for clinical trials; 4. Ayana Pharma LTD, which focuses on large scale production and FDA registration of liposomal nano-drugs.

Professor Barenholz is a coauthor of more than 405 scientific publications having altogether more than 25,000 citations. He is a co-inventor in 50 approved patent families. He was an executive editor of Progress in Lipid Research, an editor of 4 Special Issues, and is on the editorial board of 5 scientific journals.

Professor Barenholz was awarded the following prizes and awards: the Donders Chair Professor at the Faculty of Pharmacy, University of Utrecht, The Netherlands {1992); the Kaye award for innovation, twice (1995 & 1997) at the Hebrew University, Jerusalem, Israel; the international Alec D. Bangham (the founder of the Liposome field) award (1998); the Teva Founders Prize (2001), Israel; an Honorary Doctor degree for "outstanding contributions to lipid membrane research and highly innovative achievements in nanomedicine" from the Technical University of Denmark (DTU) in 2012, (Copenhagen, Denmark); the international Controlled Release Society's (CRS) most prestigious CRS Founders Award for 2012 and The Israeli chapter of the international Controlled Release Society's (ICRS) Award of 2014, for Outstanding Achievements in Drug Delivery (pioneering work in the field of Liposome Science and Liposome-Based Drug Delivery Systems).

In 2003 Professor Barenholz founded (from Doxil royalties) the "Barenholz Prizes" for Israeli Ph.D. students to encourage excellence and innovation in applied science.

Jack Barokas

Running and owner of private audio visual equipment maintenance Company over 20 year's. Computer and network maintenance services at TAU-CD, Head of the Educational Digital Media Applications team at TAU, BA degree in Learning Technologies and Instructional Design, HIT 2010 Israel.

Online courses development to replace Face2Face learning at School of Medicine, Higher Education Reform Expert (HERE) in the framework of Israel National Tempus Office (NTO).

Life webcasting and lecture/course recording services for European projects such as: Nano2Life, NanoEl, NanoSkills, QNano. Horizon 2020 Up2U, ERASMUS+ NanoEl-Asia. Currently Local coordinator of TEMPUS EduNano project,

RECENT PUBLICATIONS

- Danilo Demarchi, Gianluca Piccinini, Mariagrazia Graziano, Jack Barokas, Silvia Schintke, Philippe Morey-Chaisemartin, and Slavka Tzanova, HANDS-ON LABORATORIES IN THE NANOEL PROJECT, 9th European Workshop on Microelectronics Education EWME'12, Grenoble, France, May 11, 2012.
- Philippe Morey Chaisemartin, Slavka Tzanova, Silvia Schintke, Danilo Demarchi, Jack Barokas, Fabian Wleklinski, Jean-Marc Melique, and Eric Beisser, Industry Needs Analysis for Developing New Skills in Nano-Electronics, 9th European Workshop on Microelectronics Education EWME'12, Grenoble, France, May 10, 2012.

• Barokas, J., Ketterl, M. & Brooks, C. (2010). Lecture Capture: Student Perceptions, Expectations, and Behaviors. In J. Sanchez & K. Zhang (Eds.), Proceedings of World Conference on E-Learning in Corporate, Government, Healthcare, and Higher Education 2010 (pp. 424-431). Chesapeake, VA: AACE

François Berger

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

EDUCATION

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

PROFESSIONAL CAREER

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

RECENT PUBLICATIONS

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

Kunal Bhattacharya

Dr. Kunal Bhattacharya is currently employed as an Assistant Professor (2013–ongoing) in the research group of Prof. Bengt Fadeel at the Division of Molecular Toxicology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden. He is doing research in the field of nanotoxicology, and nanomedicine application of biodegradable carbon nanotubes. Dr. Bhattacharya had done his PhD from the University of Duisburg-Essen, Germany (2008) in the field of nanotoxicology. Following which he had worked as a Postdoctoral researcher at the Dublin Institute of Technology, Dublin, Ireland (2008–2011) and the Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden (2012–2013).
Dr. Bhattacharya has 14 years' experience in the field of nanotoxicology / nanomedicine. He is an European Registered Toxicologist and member of UK register for Toxicologists and European Society of Toxicology In Vitro. He is also reviewer for several prestigious scientific journals and himself published several peer-reviewed articles in the field of nanotoxicology and more recently nanomedicine.

Patrick Boisseau

Patrick Boisseau is graduated in biological engineering from the French Elite Schools Institut National Agronomique (1983) and Ecole Nationale du Génie Rural, des Eaux et des Forêts (1985). His career is fully dedicated to academic research, research and development and research management. He started his career in 1987 at CEA, a French public research organisation where he occupied several functions in biological research and later in medical technologies.
Patrick Boisseau's current position is VP Healthcare responsible for the Strategic Planning in Life Sciences and Healthcare Technologies, at CEATech, a public non-for-profit Research & Technology Organisation, based in Grenoble, France.
He has acquired a large expertise of coordination of EU projects like the Network of Excellence Nano2Life (2004–2008), EuroNa-

noBio, BIBA, TARGET-PDT, and recently the EU-NCL infrastructure on nanocharacterisation. His field of technical expertise is nanomedicine, drug delivery, medical imaging and innovative medical technologies.
Patrick Boisseau is solicited for the numerous expertise for the European Commission and other European and national funding agencies.
He is elected Chairman of the European Technology Platform on Nanomedicine since December 2012.
He is chairing the ESTHER Task Force designing and implementing this European Industry Driven Initiative on Emerging and Strategic Technologies for Healthcare since May 2015.

Gerrit Borchard

PharmD, Ph.D.
Gerrit Borchard is a licensed pharmacist and obtained his Ph.D. in pharmaceutical technology. After holding several academic posts at Saarland University (Germany) and 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). Prof. Borchard has published more than 135 scientific paper and book chapters (7015 citations, h-factor 45), and is named as inventor on 9 patents. Since 2014, he is president of the Swiss Academy of Pharmaceutical Sciences (SAPhS).

Sven Even Borgos

Sven Even Borgos (born 1976) earned both his undergraduate and PhD degrees at the Norwegian University of Science and Technology in Trondheim, which is the main technical university of Norway. His undergraduate was from the Faculty of Physics and Mathematics, specialization in Biophysics and Medical Technology. His PhD, however, was in molecular biology. More specifically, it was concerned with genetic engineering of the antibiotic-producing soil bacterium Streptomyces noursei in order to develop mutants producing derivatives of the clinically important antifungal antibiotic nystatin and related compounds, with improved pharmacological properties. His post doc was earned in systems biology, developing and validating a genome-scale metabolic model of the alginate-producing bacterium Pseudomonas fluorescens. Since 2006, he has been working in SINTEF (Norway), which is one of the largest independent research institutes in Northern Europe with more than 2000 employees. Here, he has been working with advanced analytical chemistry, mainly based on mass spectrometry coupled to chromatography in the Research Group Mass Spectrometry. The last years, he has been specializing in physicochemical characterisation of nanomaterials, with an emphasis on nanomedicines, using various modalities of mass spectrometry and novel separation methods such as field flow fractionation (FFF). He is working in the European Nanomedicine Characterisation Laboratory H2020 project leading the chemical part of the characterisation cascade, as well as leading the work package that identifies novel nanomedicines characterisation technologies. Since 2017, the SINTEF Mass Spectrometry group is also a main analytical partner in the B-SMART H2020 project developing RNA-based nanomedicines against neurodegenerative disorders. He also has a keen interest in the novel field of mass spectrometry imaging (MSI), and is leading this activity within SINTEF. The use of MSI for label-free, spatially resolved analysis of drug biodistribution in tissues is particularly fascinating, e.g. in terms of targeted drug delivery by nanomedicines.

RELEVANT RECENT PUBLICATION (1):

1. Borgos, S.E.F. (2016) Characterization Methods: Physical and Chemical Characterization Techniques. Pharmaceutical Nanotechnology: Innovation and Production, 2 Volumes.

Massimo Bottini

Ph.D.
Department of Experimental Medicine and Surgery, University of Rome Tor Vergata, Rome, (Italy), and Infectious and Inflammatory Diseases Research Center, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, (USA).

Massimo Bottini is Associate Professor of Biochemistry at the Department of Experimental Medicine and Surgery at the University of Rome Tor Vergata (UTV, Rome, Italy) since 2015. He also holds a position as Affiliate Associate Professor at the Sanford Burnham Prebys Medical Discovery Institute (SBPMDI, La Jolla, USA) since 2016.
Dr. Bottini has been working for more than 10 years on the fabrication and characterization of nanoparticles and their use for the transport of drugs into specific tissues for the cure of diseases[1]. He has coordinated an international research project funded by the Juvenile Diabetes Research Foundation for the development of a nanosystem for the cure of diabetes[2]. He has also coordinated an international research project funded by the Arthritis National Research Foundation for the develpment of nanoparticles to deliver cargo inside a T cell sub-population residing in arthritic joints. These funds enabled Dr. Bottini, in collaboration with the La Jolla Institute for Allergy and Immunology and the University of California San Diego (La Jolla, USA), to develop innovative nanoparticles to transport model drugs into specific cell sub-populations[3]. He also investigated the biologic interactions of nanoparticles with bodily fluids in collaboration with groups at the UTV, the SBPMDI, the Karolinska Institutet (Stockholm, Sweden) and the University of Pittsburgh (Pittsburgh, USA)[4]. Recently, he has been recently awarded with the President's International Fellowship Initiative (PIFI) from the Chinese Academy of Science (CAS) to visit the Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety (CAS) in Beijing to develop new types of multifunctional nanoparticles for the cure of arthritis. In collaboration with the SBPMDI, Dr. Bottini has also been investigating the nanomechanical properties of a special class of extracellular vesicles, responsible for bone mineralization, by means of innovative techniques based on atomic force microscopy[5].

PUBLICATIONS

- Sacchetti C., Rapini N., Magrini A., Cirelli E., Bellucci S., Mattei M., Rosato N., Bottini N., Bottini M. In vivo targeting of intra-tumor regulatory T cells using PEG-modified single walled carbon nanotubes. Bioconjugate Chemistry, 2013; 24 (6): 852-858.
- Delogu L.G., Magrini A., Bergamaschi A., Rosato N., Dawson M.I., Bottini N., Bottini M. Conjugation of antisense oligonucleotides to PEGylated carbon nanotubes enables efficient knock-down of PTPN22 in T lymphocytes. Bioconjugate Chemistry. 2009; 20 (3): 427-431.
- Sacchetti C., Liu-Bryan R., Magrini A., Rosato N., Bottini N., Bottini M. Polyethylene-glycol-modified single-walled carbon nanotubes for intra-articular delivery to chondrocytes. ACS Nano, 2014; 8 (12): 12280-12291.
- Sacchetti C., Motamedchaboki K., Magrini A., Palmieri G., Mattei M., Bernardini S., Rosato N., Bottini N., Bottini M. Surface PEG conformation influences the protein corona of PEG-modified single-walled carbon nanotubes: potential implications on bio-

logical performance. ACS Nano, 2013; 7 (3):1974-1989.

- Yadav M,* Bottini M,* Cory E, Bhattacharya K, Kuss P, Narisawa, S, Sah RL, Beck L, Fadeel B, Farquharson C, Millán JL. Skeletal mineralization deficits and impaired biogenesis and function of chondrocyte-derived matrix vesicles in phospho1-/- and phospho1/Pi t1 double-knockout mice. Journal of Bone and Mineral Research. 2016; 31 (6): 1275-1286. * = equal contribution. (volume cover page)

Angela Brand

Professor Angela Brand is Full Professor in the Department of International Health at the Faculty of Health, Medicine and Life Sciences (FHML) as well as Professorial Fellow at UNU-MERIT (United Nations University – Maastricht Economic and Social Research Institute on Innovation and Technology) at Maastricht University, The Netherlands. She is also Director of the European Centre for Public Health Genomics (ECPHG) as well as Dr. T.M.A. Pai Endowment Chair on Public Health Genomics and Adjunct Professor at the School of Life Sciences at Manipal University, India. Before she worked in the clinics, at various academic institutions and in governmental bodies in the USA and Germany. She is Paediatrician, Specialist in Public Health Medicine, holds a PhD in pathology, a Master of Public Health (MPH) from Johns Hopkins University, USA, and received her habilitation focusing on Health Technology Assessment. She has been the pioneer of Public Health Genomics in Europe and coordinated and established successfully this field in more than 15 EU Member States within the last years (PHGEN, www.phgen.eu). She is Fellow of the Rockefeller Foundation, USA, of the 21st Century Trust of the Wellcome Trust, UK, and Member of the New York Academy of Sciences.

Professor Brand is Editor-in Chief of the international Journal Public Health Genomics. She is Expert on „Big Data“ of the International Consortium Personalised Medicine (IC PerMed) jointly coordinated by the European Commission, over 26 European Member States and Canada. She serves as Expert for the European Medicines Agency (EMA), the OECD, WHO, the EC, the German Robert Koch-Institut (RKI), Genome Canada, Science Europe, AXA Research among others. She collaborates with industry partners such as IBM, Roche, Pfizer, AMGEN, Novartis, MSD, Bayer HealthCare. Her research focus is on Public Health Genomics, Personalised Medicine, Big Data Analytics for Public Health, PPP, Data Cooperatives and Innovation Management.

Donald Bruce

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

He is a member of the UK Animals in Science Committee. He was a former member of the Scottish Science Advisory Committee, the Societal Issues Panel of Engineering and Physical Sciences Research Council, the Public Affairs advisory group of Biotechnology Research Council, and of the Advisory Board of the Institute of Nanotechnology.

Reto Brun

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Reto Brun is a well-known parasitologist who mainly worked on malaria, African sleeping sickness and other protozoan diseases. He studied biology at the University of Basel and received his Ph.D. in 1973. Thereafter, he worked as a post-doctoral fellow 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 many 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 550 research articles and reviews, and 15 book chapters.

Christoph Brutschin

Christoph Brutschin (59) is Head of the Department of Economic, Social and Environmental Affairs of the Canton of Basel-Stadt since 2009, with the specialist fields of economics, of social insurance and social support and of environmental and energy policy. He represents the interests of the Canton within a variety of affiliated organisations, in-cluding: Member of the Board of EuroAirport Basel-Mulhouse-Freiburg, of MCH Group AG (exhibition centre) and of Swiss Rhine Ports. Since june 2016 Christoph Brutschin presides the Conference of the Cantonal Directors of Public Economy.

William J. Burlingham

William J. Burlingham received his B.A. from Livingston College in 1974, and his Ph.D. (in Biochemistry) from Syracuse University in 1979. He completed his postdoctoral training as a transplant immunologist with Dr. David Steinmuller at the Mayo Clinic(Rochester, MN) in 1983. He was an academic staff member (Research Scientist) in the University of Wisconsin-Madison Dept. of Surgery from 1983-1993, after which he was promoted to Assistant Professor. He became Associate Professor of Surgery in 1998, and a full professor in 2002. His laboratory is focused on basic and translational studies in transplant immunology. His goal is to develop strategies to improve transplant outcomes by inducing immunologic tolerance based on chimerism, in the great UW tradition of Ray Owen. Ray discovered mixed chimerism in red blood cells of dizygotic cattle twins in 1945, launching the fields of modern immunogenetics and transplantation. Dr. Burlingham's laboratory studies two types of clinical transplants: kidney, where organs of living donors are the focus, and lung, where the organs are all from deceased donors. Although the specific antigens driving tolerance or rejection in each organ are different, developing a favorable ratio of regulatory-to-effector-T cells both within the host and in the microenvironment of the donor organ is a common theme of his research in both. His overall goal is to use the knowledge we have gained in our studies of kidney and lung transplant recipients and donors to find tolerance–prone donor-recipient pairs. According to this research so far, the recipe for successful tolerance involves exploitation of "immune privilege" within in the donor organ, including tissue-resident Treg and tolerogenic DC, combined with anergy, clonal deletion, and regulation in the host. His focus is on adaptive and innate T cells which create the conditions for, and stabilize the tolerant state. The specific adaptive and innate T cells required appear to differ in each organ system, depending on the barrier and metabolic functions of the organ. Dr. Burlingham's lab was the first to identify in lung transplant patients, "autoimmune" Th17 responses that were critical for the chronic failure of human lung transplants, known as bronchiolitis obliterans. His lab also was the first to describe the "split" tolerance effect of non-inherited maternal antigens(NIMA) in sibling kidney transplantation (early acute rejection, followed by long-term freedom from chronic rejection), and to recognize the importance of bi-directional regulation in living-related kidney transplantation. We have recently discovered what we think is the mechanism of split tolerance to NIMA, namely the differential antigen presentation contexts (CD86/co-stimulatory vs PD-L1/co-inhibitory) arising from the interaction of maternal microchimerism-derived extracellular vesicles (EV) with a single host dendritic cell.

Will has been principal investigator on >20 R01 & R21 grants; recently co-PI on a P01 with pulmonologist and immunologist Dr. David Wilkes, and is currently co-PI on a non-human primate U01 grant with Dr. Dixon Kaufman. He is the Director of the Humanized Mouse Core of the UW-Madison School of Medicine and Public Health, and has served until recently as co-editor in chief of the journal Chimerism, launched in 2010. He is the recipient of the Outstanding Service Award from the Autumn Immunology Conference, Chicago, IL in 1994, a Career Development Award from NIAID National Institutes of Health, K02-AI01452-01 "Soluble Forms of HLA-A, B and Chronic Rejection" from 1997-2002. He also served on two NIH study sections Surgery, Anesthesiology, and Trauma[Ad Hoc] 1993- 96, Tumor, Transplantation, and Tolerance [TTT] study section, NIH-NIAID 2007-2011. His lab recently completed a subcontract from the EU One Study and begun a new subcontract from the Immune Tolerance Network to develop *in vitro* diagnostic test(s) for donor-specific regulation to support trials of cellular therapy. Finally, he has served on AST Awards and Grants Committee from 2004-2007. He has been an active member of AST and ASTS since 1990, and is a regular reviewer and editorial contributor to the American Journal of Transplantation.

David R. F. Carter

Dave graduated from York University with a BSc in Biochemistry, which included a year working on the human genome project at the Sanger Institute in Cambridge. He completed his PhD at Cambridge University under the supervision of Dr Peter Fraser. During his PhD he developed a novel assay, 'RNA-tagging and recovery of associated proteins', to demonstrate a physical interaction between a locus control region and the β-globin gene. He then worked at Oxford University as a postdoctoral researcher in Prof Peter Cook's lab, investigating the structure of transcription factories. He was appointed as Senior Lecturer in Biomedical Science in October 2009 (and recently promoted to Reader) in the Faculty of Health and Life Sciences at Oxford Brookes University (OBU). Here he established a lab to study the effects of non-coding RNAs and extracellular vesicles in stress response.

Werner Cautreels

PhD, President and CEO
Selecta Biosciences Inc.

Prior to joining Selecta Biosciences in 2010, Dr. Cautreels was Chief Executive Officer of Solvay Pharmaceuticals, the pharmaceuticals division of the Solvay Group, in Brussels, Belgium, from 2005 until Solvay Pharmaceuticals was acquired by Abbott Laboratories in 2010. Before becoming the CEO of Solvay Pharmaceuticals, Dr. Cautreels was its Global Head of R&D from 1998.

Prior to joining Solvay, he was employed by Sanofi, Sterling Winthrop from 1979 to 1994, and Nycomed Amersham from 1994 to 1998 in a variety of R&D management positions in Europe and in the United States. Dr. Cautreels is a director of Galapagos NV in Mechelen, Belgium. He held director positions at Seres Therapeutics, Inc. in Cambridge Massachusetts from 2013 to 2016, at Innogenetics NV in Gent, Belgium from 1999 to 2006 and at ArQule Inc. in Woburn, Massachusetts each from 1999 to 2006. He was the President of the Belgian Luxemburg Chamber of Commerce for Russia and Belarus until June 2010. Dr. Cautreels received his Ph.D. in Chemistry, specializing in Mass Spectrometry, from the University of Antwerp (Antwerp, Belgium), and his financial and business training from the Advanced Management Program at Harvard Business School.

Carl Cerniglia

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Dr. Carl E. Cerniglia is a Senior Biomedical Research Service (SBRS) Research Microbiologist, Director of the Division of Microbiology at the National Center for Toxicological Research (NCTR), US Food and Drug Administration (FDA) and elected member of the American Academy of Microbiology. He is also an adjunct Professor in the Department of Pharmacology and Toxicology at the University of Arkansas Medical Sciences, Little Rock, AR. Dr. Cerniglia leads a team at the NCTR that has impacted public health in a variety of research areas including food safety, antimicrobial resistance, environmental biotechnology, nanotechnology, women's health and human intestinal microbiome-host interactions. Dr. Cerniglia's

research has resulted in over 400 scientific publications and numerous book chapters and review articles. His research has been frequently highlighted in the scientific and popular press. Dr. Cerniglia has made more than 400 invited presentations at national and international conferences and meetings and is also an ASM Foundation of Microbiology lecturer. The research achievements of Dr. Cerniglia has been recognized by national and international awards from the Food and Drug Administration, American Pharmaceutical Association, International Society of Toxicity Testing, American Society for Microbiology, and American Academy of Microbiology and U.S. Department of Health and Human Services. Dr. Cerniglia was recently awarded the Silver Medal by the World Health Organization for outstanding scientific contribution to the Joint Expert Committee on Food Additives (JECFA) in advancing science-based risk assessments on evaluating the effects of veterinary drug residues and other food contaminants on the human intestinal microbiome, the FDA Lifetime Achievement Award, the FDA Commissioner's Award Merit, the DHHS Outstanding Leader Award in providing mentoring, training and career advancement opportunities to employees in a diverse workforce and Distinguished Alumnus Award at North Carolina State University.

Insung S. Choi

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

Patrick Couvreur

Prof Patrick Couvreur is Full Professor of Pharmacy at the Paris-Sud University and holder of the chair of "Innovation Technologique" (2009-2010) at the prestigious « Collège de France ». He is appointed as a Senior Member of the "Institut Universitaire de France" since 2009. He is also the recipient of an "ERC Advanced Grant" (2010-2015) and of an "ERC Proof of Concept" (2015-2016). He has hold many important national and international academic positions as Director of the UMR CNRS 8612 (a CNRS associated department gathering together more than 120 researchers in the drug delivery field), Director of the Doctoral School "Therapeutic Innovation" (over 300 PhD students at Paris-Sud University), founder member of the pole of competitivity MEDICEN, Extraordinary Professor at the University of Louvain (Belgium), member of the board of governors of many international scientific organizations (ie. The International Pharmaceutical Federation FIP, the Controlled Release Society CRS, the European Federation of Pharmaceutical Scientists, APGI etc.). He was a member of the "Conseil National des Universités" and of the "Comité national du CNRS" in France. Patrick Couvreuris currently a member of the "Conseil Académique" of the new Paris Saclay University and a member of the "Collège des Conseillers Scientifiques" at Institut Pasteur.

Prof Patrick Couvreur's contributions in the field of drug delivery and targeting are highly recognized around the world with more than 500 peer review research publications (H-index 84 and over 29,000 citations, Web of Science), some of them in prestigious journals (Nature Nanotechnology, Nature Materials, PNAS, Angewandte Chemie etc.). His research is interdisciplinary, at the interface between Physico-Chemistry of Colloids, Polymer Chemistry, Material Science, Cellular and Molecular Biology and Experimental Pharmacology. He supervised 72 PhD students.

Patrick Couvreur's research has led to the funding of two start-up companies (Bioalliance and Medsqual). Bioalliance (now ONXEO) entered the stock market in 2005 and a nanomedicine (the LivatagR) invented in Couvreur's lab is currently at the end of phase III clinical trial for the treatment of the hepatocarcinoma.

The major scientific contribution of Patrick Couvreur to the Pharmaceutical Sciences is also recognized by numerous international (the "2004 Pharmaceutical Sciences World Congress Award", the prestigious "Host Madsen Medal", the "European Pharmaceutical Scientist Award" of the European Federation of Pharmaceutical Sciences, the European Inventor Award 2013 given by the European Patent Office, the Speiser award 2014 and the Higuchi Award 2015, Japan) and national awards (the "Prix Galien 2009" and the "Médaille de l'Innovation 2012 of the CNRS). His appointment as a member of eight academies (Académie des Sciences, Académie des Technologies, Académie de Médecine and Académie de Pharmacie in France, as well as the Académie Royale de Médecine in Belgium, the Royal Academy of Pharmacy in Spain, the United States National Academy of Medicine and the United States National Academy of Engineering) is another recognition of major scientific and scholarly contributions of Patrick Couvreur.

Daan J.A. Crommelin

PhD

Prof. Daan Crommelin is professor emeritus from 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 edited a number of books. He is Editor-in-Chief of the AAPS book series 'Advances in the Pharmaceutical Sciences'. He advises venture capital groups and acts as a consultant for several big pharma companies and SME's. He chaired the Board of the UCAB Foundation: the Utrecht Center of Excellence for Affordable Biotherapeutics, a WHO supported initiative and the Board of Pharmaceutical Sciences of the International Pharmaceutical Federation (F.I.P.). He 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).

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Luisa De Cola

Luisa De Cola is since September 2013 Professor Exceptionnelle at the University of Strasbourg (ISIS) as chair of Supramolecular and Bio-Material Chemistry, and part time scientist at the INT-KIT, Karlsruhe, Germany. Since 2016 she is also Professor at the Institut Universitaire de France (IUF).
She was born in Messina, Italy, where she studied chemistry. After a post-doc at the Virginia Commonwealth University, USA, she was appointed Assistant Professor at the University of Bologna (1990). In 1998 she was appointed Full Professor at the University of Amsterdam, The Netherlands. In 2004 she moved to the University of Muenster, Germany.
She is recipient of several awards, the most recent being the IUPAC award as one of the Distinguished Women in Chemistry and Chemical Engineering (2011), the international Prize for Chemistry "L. Tartufari" from Accademia dei Lincei (2015) and the Catalan -Sabatier prize from the Spanish Royal Academy of Science (2015). She was elected in 2014 member of the German National Academy of Sciences Leopoldina and in the same year has been Nominated "Chevalier de la Légion d' Honneur" by the President of the French Republic, François Hollande. In 2016 she was elected member of the Akademie der Wissenschaften un der Literatur of Mainz. She i salso in the board of several Institutes and Universities as well in evaluation committees. Her main interests are luminescent and electroluminescent complexes and their assemblies and nano-, porous and degradable structures for bio-applications. In particular in the biomedical area she has developed different type of stimulus responsive silica materials and hybrid hydrogels. She has published 330 articles in international peer reviewed journals and filed 36 patents (H index = 62).

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EDUCATION AND POSTDOCTORAL TRAINING

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

SELECTED PEER REVIEWED PUBLICATIONS (UNDERLINED = FIRST AUTHOR; * = CORRESPONDING AUTHOR)

1. S. Edmonds, A. Volpe, H. Shmeeda, A. C. Perente-Pereira, R. Radia, J. Bagunya-Torres, I. Szanda, G. W. Severin, L. Livieratos, P. J. Blower, J. Maher, G. O. Fruhwirth, A. Gabizon and R. T. M. de Rosales*, Exploiting the Metal-Chelating Properties of the Drug Cargo for In Vivo Positron Emission Tomography Imaging of Liposomal Nanomedicines, ACS Nano, 2016, IN PRESS
http://pubs.acs.org/doi/abs/10.1021/acsnano.6b05935
2. X. Cui, D. Mathe, N. Kovács, I. Horváth, M. Jauregui-Osoro, R. T. M. de Rosales, et al., Synthesis, Characterization, and Application of Core–Shell Co0.16Fe2.84O4@NaYF4(Yb, Er) and Fe3O4@NaYF4(Yb, Tm) Nanoparticle as Trimodal (MRI, PET/SPECT, and Optical) Imaging Agents. Bioconjugate Chemistry 2016 27 (2), 319-328.
http://pubs.acs.org/doi/abs/10.1021/acs.bioconjchem.5b00338
3. L. Sandiford, A. Phinikaridou, A. Protti, L. K. Meszaros, X. Cui, Y. Yan, P. A. Williamson, N. Gaddum, R. M. Botnar, P.J. Blower, M. A. Green and R. T. M. de Rosales*, Bisphosphonate-anchored PEGylation and Radiolabeling of Superparamagnetic Iron Oxide: Long-circulating Nanoparticles for In Vivo Multimodal (T1 MRI-SPECT) Imaging. ACS Nano. 2013, 500
4. R. T. M. de Rosales*, R. Tavaré, Rowena L. Paul, Maite Jauregui-Osoro, A. Protti, G. Varma, I. Szanda and P. J. Blower*, Synthesis of 64Cu(II)-Bis(dithiocarbamate-Bisphosphonate) and Conjugation with Superparamagnetic Iron Oxide Nanoparticles: In Vivo Evaluation as Dual-Modality PET-MRI Agent, Angew. Chem. Int. Ed., 2011, 50, 5509-5513.
5. R. T. M. de Rosales*, R. Tavaré, A. Glaria, G. Varma, A. Protti and P. J. Blower, 99mTc-Bisphosphonate-Iron Oxide Nanoparticle Conjugates for Dual-Modality Biomedical Imaging, Bioconjugate Chem., 2011, 22, 455-465.

Tapas K. De

Dr. Tapas K De has extensive industrial experience with both proteins/enzymes and small molecules in formulation and process development, including scale-up/technology transfer. His scientific specializations are in parenteral and ocular drug delivery and release kinetics, nanoparticle (ceramic, polymeric, bioadhesive, hydrogel) formulation development, reverse micellar enzymology, enzyme kinetics and reaction mechanism. He is an expert on nab formulation development and characterization. He has developed Abraxane nanoparticle formulation at Abraxis Bioscience, as well as nab-17-AAG, nab-docetaxael, nab-rapamycin, nab-ABI011 etc. He was the lead formulation scientist for the nab business venture with National and international pharmaceuticals companies.
He has authored and co-authored more than 10 USA and world patents, 20 research articles and written chapters in CRC Handbook of Surface and Colloid Chemistry, second Edition and Encyclopedia of Surface and Colloid Sciences.
He is one of the co-founders of the lipid-based nanoparticle formulation company LipoMedics Inc, Fort Worth, TX, USA and currently serving as Chief Executive Officer of the Company.

Paolo Decuzzi

Paolo Decuzzi is a senior researcher and director of the Laboratory of Nanotechnology for Precision Medicine at the Italian Institute of Technology in Genova – Italy. Dr. Decuzzi earned his M.Sc. degree in Mechanical Engineering from the Politecnico di Bari (Italy) in 1997 and his Ph.D. degree in Mechanical Engineering from the University of Naples – Federico II (Italy) in 2000, with a thesis on friction and adhesion at the nanoscale. In 2002, he was nominated Assistant Professor of Machine Design at the Politecnico di Bari and, in 2005, he became Associate Professor in the School of Medicine of the University 'Magna Graecia'. There, he co-founded BioNEM - the laboratory of BioNanotechnology and Engineering for Medicine

- one of the first nano-engineering laboratories built in a School of Medicine. In October 2007, he joined The University of Texas Health Science Center in Houston as an Associate Professor of Biomedical Engineering. In October 2010, he moved to the Houston Methodist Hospital where he served as a Professor of Biomedical Engineering till July 2015. There, he founded the Center for the Rational Design of Multifunctional Nanoconstructs, with the financial support of the Cancer Prevention and Research Center of Texas and the US National Cancer Institute; and served first as the co-chair of the Nanomedicine Department and then as the interim chair of the Translational Imaging Department. In July 2014, Dr. Decuzzi was awarded a 5-year European Research Council "Consolidator Grant" to design, synthesize and develop nanoconstructs for imaging and therapy in brain cancer.

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

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

Ángel Del Pozo

Angel Del Pozo is a Bachelor of Science in Chemical Engineering 2003, from the University of Salamanca (Spain). He also holds an Advanced Studies Diploma 2006 in Chemical Engineering, obtained based on a Thesis about microencapsulation of stem cells for regenerative medicine at the University of Salamanca. In 2007 he started to work for private research institutions, like Tecnalia, in Environemt and Health related regional, national and international research projects. He has been working at Biopraxis (Praxis Pharmaceutical) since 2011 as IPR and EU projects manager, and is currently participating in five EU research projects (Fp7 and H2020).

He has been also nominated as Expert by the European Commission, acting as evaluator for different Work Programmes, including Societal Challenge one and NMBP proposals. In his work experience, he has experience in R&D&I consorciated projects (both at national and European level), identification and exploitation of new business opportunities in pharma, bio and nanotechnology, including startegies of IP protection and exploitation.

Working for a private company limits publications, while promotes patenting some relevant examples are the following:

- European Patent application: 12382476.5 and PCT/ES 2013/070833 (Microparticles with EGF, method of preparation and use).
- European Patent application 13382275.9 (Lipid nanoparticles for wound healing).
- European Patent application: 13382268.4 (Lipid nanoparticles of Polymyxin for CF infections tretament).
- M.Pastor, A del Pozo et alt, Sodium colistimethate loaded lipid nanocarriers for the treatment of Pseudomonas aeruginosa infections associated with cystic fibrosis, International Journal of Pharmaceutics 477 (2014) 485–494
- A Fernandez Villegas, A del Pozo et alt, parasite biomarker set for evaluating Benznidazole treatment 1 efficacy in 2 chronic Chagas disease patients, Journal of Antimicrobial Chemotherapy (in press)

Neil P. Desai

PhD

Neil Desai is Founder/CEO of AADi, LLC, a clinical stage start-up developing targeted mTOR therapeutics for oncology/cardiovascular applications and Founder/CEO of Aadigen, LLC, a company focused on delivery of nucleotide therapeutics. He was formerly VP of Strategic Platforms at Celgene Corp and also SVP of Global R&D at Abraxis Bioscience (Los Angeles, California, USA, acquired by Celgene in 2010 for approximately $3B) where he led the development of Abraxane®, the company's flagship nanotechnology product. Dr. Desai is an inventor of the nanoparticle-albumin bound (nab®) drug-delivery platform and Abraxane, which is approved for metastatic breast cancer, non-small cell lung cancer and pancreatic cancer with sales of $973M in 2016. Prior to Abraxis, Dr. Desai held positons of increasing seniority at American Bioscience, Inc., VivoRx, Inc. and VivoRx Pharmaceuticals, Inc. (predecessor companies of Abraxis), where he worked on the early discovery and development of Abraxane, developed novel encapsulation systems for living cells and was part of the team that performed the world's first successful encapsulated islet cell transplant in a diabetic patient. Dr. Desai has over 25 years of experience in novel therapeutic delivery systems with over 100 issued patents, over 40 peer-reviewed publications and book chapters, and over 200 presentations at scientific meetings. He is reviewer for several scientific journals, an active participant in FDA and EU Nanotechnology initiatives and a member of the Steering Committee for the National Cancer Institute (NCI) Alliance for Nanotechnology in Cancer. He holds board and advisory positions in various start-ups. Dr. Desai recieved a M.S and Ph.D. in Chemical Engineering from the University of Texas at Austin, USA, and a B.S. in Chemical Engineering from the University Institute of Chemical Technology in Mumbai, India.

László Dézsi

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PhD, DrHabil., Senior Research Associate, leading scientist of the *in vivo* small animal laboratory, Nanomedicine Research and Education Center, Institute of Pathophysiology at Semmelweis University since 2012 in Budapest, Hungary.

He obtained his MSc degree in biology at Eötvös Loránd University and his PhD in physiology at Semmelweis University Medical

School, Budapest, Hungary. He conducted teaching and research activities at Semmelweis University (1981-1999), and meanwhile he received fellowships at Albert Ludwigs Universität, Freiburg, Germany (Prof. E. Bassenge) working in the field of local regulation of blood flow in skeletal and cardiac muscle; as well as at the University of Pennsylvania, Philadelphia, USA, Cerebrovascular Research Center (Prof. M. Reivich, Dr. J. H. Greenberg) working in the field of cerebral blood flow/metabolism and cerebral ischemia/reperfusion in stroke models in animals. He had been head of laboratory, CRO monitor and research project manager in vascular and safety pharmacology at Gedeon Richter Pharmaceutical Plc. (1999-2012). He was manager of Analgesic Research Laboratory (2006-2012), a joint venture of Gedeon Richter and University of Pécs, Department of Pharmacology (Prof. J. Szolcsányi). He was involved in curriculum development and had been Secretary of Biomedical Engineering (BE) Course Committee (1994-2000), now member of the MSc BE Committee at Technical University, Budapest. He made his habilitation at Semmelweis University in 2005 and became Adjunct Professor of physiology in 2006. He established his own teaching course in 2008 entitled "Cardiorespiratoric and neurophysiological measuring techniques" at the Department of Human Physiology and Clinical Experimental Research (Prof. Z. Benyó). He participates in Postgradual Education in Nanomedicine (Prof. L. Rosivall).
Currently he is working in the field of nanomedicine investigating cardiopulmonary and immunological effects of nanoparticles in various *in vivo* models of complement activation related pseudoallergy (CARPA) and participates in the development of new models under the supervision of Prof. J. Szebeni. He works as a member of the EU FP7 "NanoAthero" Consortium (head: Didier Letourneur).

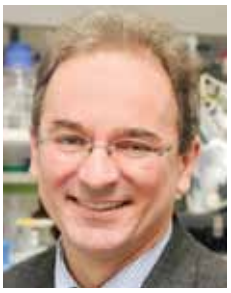
Gilles Divita

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

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

Daryl Drummond

Daryl Drummond currently serves as the Head of Research and Senior Vice President of Discovery for Merrimack Pharmaceuticals, where he oversees the discovery efforts for Merrimack's Nanotherapeutics and Biologics-based therapeutics. Dr. Drummond received a Ph.D. degree in Biochemistry from Indiana University in 1997, with an emphasis on membrane biochemistry and biophysics, and later did a postdoc under the renowned father of lipid-based drug delivery systems, Demetrios Papahadjopoulos at UCSF and California Pacific Medical Center.
Dr. Drummond was one of two principle inventors for many of Merrimack's nanotechnology-based drugs and platform technologies, most notably Onivyde, a highly stabilized liposomal formulation of irinotecan. He joined Merrimack in October of 2009 following the merger of Merrimack with Hermes Biosciences. Dr. Drummond received a Ph.D. degree in Biochemistry from Indiana University in 1997, with an emphasis on membrane biochemistry and biophysics. He later joined Hermes Biosciences in 2000 as an Associate Director of Liposomal Research and Development following a postdoc in the laboratory of the renowned father of current liposome drug delivery systems, Demetrios Papahadjopoulos. Overall, Dr. Drummond has more than 20 years of experience in the research and development of advanced drug delivery systems, including four unique drugs that have been tested in various clinical trials, >40 issued patents or patent applications, and more than 65 peer reviewed publications focused on lipid-based nanotherapeutics.
The focus of his research is in developing targeted nanotherapeutics for treating a wide range of solid tumors. He successfully developed novel platform technologies for targeting lipidic nanocarriers such as liposomes using a range of novel ligands, but most notably Fab' or scFv antibody fragments. He has also developed platform technologies for dramatically improving the in vivo drug retention of difficult to stabilize small molecule drugs, and for systemic delivery of nucleic acids. Three of their nanotherapeutics are being studied in clinical trials, including an ErbB2-targeted liposomal doxorubicin which is currently being evaluated in a Phase II study in ErbB2-overexpressing breast cancers and a nanoliposomal formulation of irinotecan which recently showed promising results in a Phase III trial in gemcitabine-refractory pancreatic cancer. A fourth antibody targeted lipososomal drug (MM-310) is scheduled to enter the clinic in the second half of 2016.



Albert Duschl

Albert Duschl studied biology at the University of Gießen, where he received his PhD in 1986 for a thesis on bacterial membrane proteins. He was a Postdoc at the University of California, Irvine, and at the Max Planck Institute for Biochemistry, Martinsried. From 1990 to 2001 he was University Assistant and Group Leader at the Dept° of Physiological Chemistry, University of Würzburg, where he started working on cytokines and on molecular regulation of immune responses, in particular with respect to allergic diseases. During this time he also began to study particle effects on the immune system, stimulated by reports that Diesel exhaust particles may promote the development of allergies. He soon switched to engineered nanoparticles, which are much nicer to work with in the lab.
Albert Duschl was appointed Full Professor (Chair) of Biochemistry at the University of Salzburg in 2001 where he also held the post as Vice-Rector for Research from 2003 to 2011. His group today works on signal transduction within cells of the immune system and on the effects of nanoparticles on immunity. Both fields inter-

act intensely and among the specific research directions are indeed effects of co-exposure towards nanoparticles and allergens, a subject that is of substantial interest for improved allergen immunotherapy. This ties in with numerous studies performed by the group on properties, consequences and evolution of the protein corona that attaches to nanoparticles in biological matrices. The group of professor Duschl is a member of the FWF-funded international PhD program "Immunity in Cancer and Allergy (ICA)" and a member of the excellence cluster "Allergy / Cancer / Bio/Nano-Interaction (ACBM)" within the University of Salzburg. He is European Co-chair of the Community of Research for Human Toxicity within the US-EU bridging nano-EHS research efforts

Starting with FP5, Albert Duschl has participated to six finished and to two on-going EU-projects, two of which he has coordinated (FP7 NanoTOES, FP7 NanoEIS). He has through these efforts developed an interest in the European training landscape in nanotechnology. According to a study performed within FP7 NanoEIS, employers report Health and Safety as the top area in which they intend to recruit skilled personnel in the future (http://nanoeis.sbg.ac.at/industry.html). Interestingly, other non-technical subjects also rate quite high, including regulation, standardization and environment. The training contents of European university curricula were investigated in another NanoEIS study, which revealed a poor correlation with skill needs in the job market (http://nanoeis.sbg.ac.at/sites/nanoeis.eu/files/downloads/D32-corrected-public.pdf). An effort to improve matters is the establishment of a focus network on education and training, headed by Albert Duschl, within H2020 EC4SafeNano.

Reach out to the public is supported via the BMBF-funded project DaNa 2.0, which maintains the website http://nanoobjects.info/, featuring research-based information on "nano"-topics for a general audience. Albert Duschl is a member of the DaNa team and recommends this excellent website. Naturally, the website of the group is also to be recommended for anyone interested in research on immune response modification by nanoparticles and other agents, at www.uni-salzburg.at/tapir.

http://orcid.org/0000-0002-7034-9860; http://www.researcherid.com/rid/E-5872-2011

Eldad Elnekave

Eldad Elnekave, MD serves as the director of the Clinic for Interventional Oncology at the Davidoff Cancer Institute, Rabin Medical Center, Israel. He obtained his medical degree from Tufts University in Boston, Massachusetts and spent two years as a Howard Hughes Medical Institute Research Scholar at the National Institutes of Health in 2003-2005. Dr. Elnekave completed radiology training at Albert Einstein Medical Center and trained in Vascular and Interventional Radiology at Memorial Sloan-Kettering Cancer Center. His clinical and research focus is on the combination of anatomical and molecular targeting to treat disease in the most precise and minimally invasive method possible. Dr. Elnekave also serves as the founding Chief Medical Officer of Zebra Medical Vision, LTD.

Noam Emanuel

Ph.D.

Dr. Emanuel has vast experience in biotechnology projects, including development of drug delivery systems and immunology. His extensive expertise includes immunotherapy, vaccines, immunodiagnostics, systemic and local drug-delivery, and medical devices. Dr. Emanuel has a number of approved patents in the field of drug delivery and diagnostics. Dr. Emanuel is a co-founder of PolyPid and served as its CEO during the company's first three years. He received his Ph.D. degree from the Faculty of Medicine at the Hebrew University of Jerusalem.

Mike Eaton

Mike Eaton worked in research in the Pharma industry for more than 35 years. Initially at GD Searle, where he built the first synthetic gene for Urogastrone and sequenced human fibroblast interferon. He was a founding member of Celltech in 1980; later acquired by UCB. He has worked on a number of marketed drugs - Mylotarg in 2000, the first Antibody drug conjugate and certolizumab pegol in 2009, the first PEGylated antibody. Unusually he has worked with both small molecules and large molecules, including DNA. 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 cheesemaking. He has worked on low molecular weight drugs including the first non-emetic PDEIV inhibitor. Mike is a special professor at Nottingham University. Currently he is involved with the Translation Advisory Board of ENATRANS, a free translational tool funded by the EC to assist SMEs and academics. He left UCB in February 2010 and is now a strategic and technical adviser to a number of companies. His particular interest is commercial translation of nanotechnology research to nanomedicines – medicines to help patients.

Bengt Fadeel

Bengt Fadeel is Professor of Medical Inflammation Research at the Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden, and Adjunct Professor of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA. He received his M.D. and Ph.D. degrees from Karolinska Institutet. He served as Vice Chairman of the Institute of Environmental Medicine from 2009-2013 and he was elected as a Fellow of the Academy of Toxicological Sciences in 2012. He is a current or past member or coordinator of several EU-funded projects on nanosafety, including NANOMMUNE, MARINA, NANOREG, SUN, and NANOSOLUTIONS, and member of the Flagship Project GRAPHENE as well as the national MISTRA Environmental Nanosafety consortium. Dr. Fadeel is chair of the scientific panel of the national nanosafety platform SweNanoSafe and member of the WHO-IPCS panel on immunotoxicity testing of engineered nanomaterials. He is co-editor of Adverse Effects of Engineered Nanomaterials: Exposure, Toxicology, and Impact on Human Health (Elsevier, 2012, 2017) and editor of Handbook of Safety Assessment of Nanomaterials: From Toxicological Testing to Personalized Medicine (Pan Stanford Publishing, 2015). Dr. Fadeel was awarded the national Environmental Medicine Prize (2011) by the Cancer and Allergy Foundation for his research on the opportunities and risks of the emerging nanotechnologies.

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 established and directs the Center for Nanomedicine at BWH. He is a faculty member of the Brigham Research Institute Cancer Research Center. He is additionally a member of the Dana Farber/Harvard Cancer Center Programs in Prostate Cancer and Cancer Cell Biology. Dr. Farokhzad's research is focused on the development of therapeutic nanoparticle technologies; most notably, he pioneered the high throughput combinatorial development and screening of multifunctional nanoparticles for medical applications. Dr. Farokhzad has authored approximately 135 papers (~28,000 citations; H-Index 68) and holds more than 146 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 five biotechnology companies: BIND Therapeutics (NASDAQ: BIND; acquired by Pfizer), Selecta Biosciences (NASDAQ: SELB), Tarveda Therapeutics (formerly Blend Therapeutics), Placon Therapeutics (formerly Blend Therapeutics), and Koan Biotherapeutics, which are translating the aforementioned academic innovations toward commercialization and societal impact. Dr. Farokhzad has served in various capacities on the Board of Directors and the Scientific Advisory Board of these companies. He was a recipient of the 2013 RUSNANOPRIZE, one of the largest international nanotechnology prizes, for the development and industrialization of nanoparticle technologies for medical applications. In 2014, he received the Golden Door Award from the International Institute of New England for his societal and economic impact as a naturalized USA citizen. In 2015, he was named as one of The Worldview 100 by Scientific American, which recognized visionaries who shape biotechnology around the world. In 2016, he was among the recipients of the Ellis Island Medal of Honor for his scientific, societal and economic contributions to America as an immigrant. Dr. Farokhzad was elected to the College of the Fellows of the American Institute of Medical and biological Engineering. He was selected by Thomson Reuters among the Highly Cited Researchers in 2014, 2015 and 2016. The Boston Globe selected him among the top innovators in Massachusetts and the Boston Business Journal selected him among the Health Care Champions for his innovations. In 2012, he was among the regional Ernst & Young Entrepreneur of the Year awardees. Dr. Farokhzad completed his post-graduate clinical and post-doctoral research trainings, respectively, at the BWH/HMS and MIT in the laboratory of Institute Professor Robert Langer. He received his M.D. and M.A. from Boston University School of Medicine and his M.B.A. from the MIT Sloan School of Management.

PRESENT POSITIONS AND AFFILIATIONS:

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

CURRENT RESEARCH: NANOBIOMEDICINE

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

ACADEMIC BACKGROUND

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

POSITIONS HELD

November 2001–November 2006: 5-year tenure track Ramón y Cajal Position. Research Center for Bioelectronics and Nanobioscience. Barcelona Science Park, Universitat de Barcelona, Spain.
May 1999–November 2001: Postdoctoral position. Plant Biotechnology Group. Department of Biochemistry and Molecular Biology, School of Pharmacy, Universitat de Barcelona, Spain.
April 1993–April 1999: Postdoctoral position. Novartis AG-Friedrich Miescher Institut, Basel, Switzerland, and Marine Biological Laboratory, Woods Hole, USA.
October 1992–March 1993: Postdoctoral position. Institute of Agroalimentary Research and Technology (IRTA), Cabrils, Spain.
February 1987–September 1992: PhD Thesis. Department of Biochemistry and Molecular Biology, Universitat Autònoma de Barcelona, Spain.
July–October 1985 and July–December 1986: Trainee student. Zentrale Forschungslaboratorien, CIBA-GEIGY AG, Basel, Switzerland.
PEER-REVIEWED PUBLICATIONS: 89; **CONFERENCE CONTRIBUTIONS**: 140

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).

Alke Fink

Prof. Alke Fink received her Ph.D. in Chemistry from the University of Ulm, Germany in 2000. 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.

Andreas Fisch

Senior Fellow, Novartis Pharma AG, Basel, Switzerland

Andreas Fisch holds a position as a Senior Fellow in the Pharmaceutical Development Unit at Novartis Pharma AG in Basel, dealing with Parenteral, Topical & Ophthalmic dosage forms. There he is a project leader in early phase development of parenteral dosage forms and is also involved in technology platforms focusing on nanotechnology and extended release formulations. He received his Ph.D in Pharmacy from Mainz University in Germany in 1992, for which he performed his research at the Institute of Immunology in Mainz on MHC class II restricted antigen presentation. He continued his academic career in Clinical Pharmacology at the University Hospital of Mainz on blood cell and tissue interactions for three years. After a two year research fellowship in the Biomedical Research Center of Baxter Bioscience in Vienna, Austria, he lead the Pharmaceutical Development Center for parenteral colloids for plasma volume replacement of B.Braun in Crissier, Switzerland, from 1997–2007 before joining Novartis.

Beat Flühmann

Dr. Beat Flühmann Pharmacist, MBA (Ph.D.-molecular biology) and, now is Director of Vifor Pharma and Steering committee member of the Non-Biological Complex Drugs Working Group hosted at Lygature a non for profit organization. Dr Flühmann's current main interest is in regulatory science aspects of nanomedicines. Before that Dr. Flühmann was working at Hoffman La Roche R&D in the area of diabetes and lipid metabolism.

Gerd Folkers

Science Studies in Chemistry and Pharmaceutical Sciences, ETH Zürich
Prof. Dr. Gerd Folkers Gerd Folkers is head of the Critical Thinking Initiative at the ETH Zurich and elected Member of the Swiss Academy of Engineering Sciences. He has published more than 250 papers and 22 books, his handbook of Drug Design (together with H.-D. Höltje) being among the Top Ten of Wiley Publishers for years. "Schmerz – Innenansichten eines Patienten und was die Wissenschaft dazu sagt." (together with A. Wittwer) is his recent public science book, "that every general medical practitioner should have in its library" (spectrum.de).

ACCOMPLISHMENTS

- Pharmacist, researcher, author, editor
- President of the Swiss Science and Innovation Council
- Former Director of the Collegium Helveticum a joint project of ETH Zurich and University of Zurich for the study of new scientific perspectives in transdisciplinary processes.
- Former Professor for Medicinal Chemistry at the ETH Zurich
- Habilitation at University of Tübingen, Germany
- Dissertation at the University of Bonn, Germany

Fabio Rocha Formiga

Fabio Rocha Formiga (Natal, Brazil, 1979) studied Pharmaceutics and Biochemistry, obtaining his bachelor's degree in 2004 from the Federal University of Rio Grande do Norte in Brazil, where he also concluded a master's course in Health Sciences in 2007. He obtained a PhD in Pharmacology in 2011 from the University of Navarra in Spain, with distinctions. During his PhD, he developed protein-loaded nanoparticles for targeted delivery into the myocardium. In 2013, Fabio received the National Research Award from the Spanish Society of Industrial and Galenic Pharmacy (SEFIG). He is a member of the Controlled Release Society (CRS) and the Tissue Engineering and Regenerative Medicine International Society (TERMIS). His research has focused on the development of nanobiomaterials for drug delivery applied to regenerative medicine and neglected diseases.

PUBLICATIONS

- Rebouças JS, Santos-Magalhães NS, Formiga FR. Cardiac Regeneration using Growth Factors: Advances and Challenges. Arq Bras Cardiol. 2016 Sep;107(3):271-275. doi: 10.5935/abc.20160097.
- Formiga FR, Pelacho B, Imbuluzqueta I, Garbayo E, Abizanda G, Gavira JJ, Simón-Yarza T, Tamayo E, Prósper F, Blanco-Prieto MJ. Controlled delivery of fibroblast growthfactor-1 and neuregulin-1 from biodegradable microparticles promotes cardiac repair in a ratmyocardial infarction model. J Control Release. 2014 Jan 10;173:132-9. doi:10.1016/j.jconrel.2013.10.034
- Formiga FR, Garbayo E, Díaz-Herráez P, Abizanda G, Simón-Yarza T, Tamayo E, Prósper F, Blanco-Prieto MJ. Biodegradation and heart retention of polymeric microparticles in a rat model of myocardial ischemia. Eur J Pharm Biopharm. 2013 Nov;85 (3 Pt A):665-72. doi: 10.1016/j.ejpb.2013.02.017.
- Formiga FR, Tamayo E, Simón-Yarza T, Pelacho B, Prósper F, Blanco-Prieto MJ. Angiogenic therapy for cardiac repair based on protein delivery systems. Heart Fail Rev. 2012 May;17(3):449-73. doi: 10.1007/s10741-011-9285-8.
- Formiga FR, Pelacho B, Garbayo E, Abizanda G, Gavira JJ, Simon-Yarza T, Mazo M, Tamayo E, Jauquicoa C, Ortiz-de-Solorzano C, Prósper F, Blanco-Prieto MJ. Sustained release of VEGF through PLGA microparticles improves vasculogenesis and tissue remodeling in an acute myocardial ischemia-reperfusion model. J Control Release. 2010 Oct 1;147(1):30-7. doi: 10.1016/j.jconrel.2010.07.097.

Sieghard Frischmann

Sieghard Frischmann, studied biology, mircobiology, biochemistry and criminalistics at the Friedrich-Alexander-University in Erlangen, Germany; degree: Dipl. Biol. At the University of Hamburg, University Hospital Eppendorf he worked in the department of Physiological Chemistry on gene regulation of glucose metabolizing enzymes in the liver. He finished his doctoral thesis with the Dr. rer. nat. degree in 1991. In 1992 Sieghard Frischmann joined Mast Diagnostica GmbH in Reinfeld, Germany, in the department of scientific product management. In 1999 he moved into R&D and production at Mast where he is involved in the company's R&D projects. He is leading a team of scientists for the development of serological and molecular diagnostic assays based on immunoassay and LAMP technologies. As a regulatory board member at Mast he works as a Medical Product Safety Manager in the terms of the EN ISO 13485 standard and the German Medical Product Law.

Gregor Fuhrmann

Dr Gregor Fuhrmann was born in Berlin (Germany) where he studied Pharmacy. He received his PhD in 2013 in Pharmaceutical Sciences from the Swiss Federal Institute of Technology (ETH) Zurich (Switzerland) under the supervision of Prof Jean-Christophe Leroux. For his dissertation he received both the ETH Silver Medal for an Outstanding Doctoral Thesis and the "Rottendorf Europapreis für Pharmazie" for Excellent Pharmaceutical Research. From 2013-2016 Gregor worked as Postdoctoral Research Associate at the Department of Materials and Department of Bioengineering at Imperial College London (United Kingdom) in the research group of Prof Molly M. Stevens. For this work he received a Marie Curie Intra-European Research Fellowship from the European Comission and a Postdoctoral Fellowship from the German Academic Exchange Service (DAAD). Gregor was member of the Research Group of the Year 2014, an award from the European Life Science Awards granted to a group with unparalleled ability of excellence in life sciences. Since 2016, Gregor is Head of the independent Junior Research Group „Biogenic Nanotherapeutics" at the Helmholtz-Institute for Pharmaceutical Research Saarland, a branch of the Helmholtz-Centre for Infection Research Braunschweig (Germany). His research is supported by the young investigators programm NanoMatFutur funded by the German Federal Ministry of Education and Research (BMBF).

Gregor's research interest is focussed on engineering smart biomimetic drug carriers utilising principles established in nature. In particular, he is currently extending the idea of protecting and delivering therapeutic agents by exploring the potential of extracellular vesicles as smart drug carrier systems. These cell-derived particles are produced by almost all cells *in vitro* and *in vivo* and they are known to be conveyors of cell-to-cell communication. His lab is dedicated to using cutting-edge methodologies in nanoparticle formulation and imaging combined with tangible pharmaceutical know-how in the field of targeted drug delivery.

Gregor has authored several peer-reviewed publications in high-impact journals such Nature Chemistry, PNAS or Journal of Controlled Release and he has been invited reviewer for various international journals, funding initiatives and scientific venues. He has given oral presentations at international and national conferences such as the Annual Meeting of the Controlled Release Society, the Annual Meeting of the German Pharmaceutical Society (DPhG) and the International Coeliac Disease Symposium. Gregor is actively involved in undergraduate teaching in Pharmaceutical Sciences at the Saarland University (Germany).

Robert Geertsma

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

Robert Geertsma has worked at the Dutch National Institute for Public Health and the Environment (RIVM) for almost twenty-three 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 co-chairing the ISO/TC194/WG17 on Biological Evaluation of Medical Devices – Nanomaterials, and he is a member of the Nanomedicines WG of the International Pharmaceutical Regulators Forum. Furthermore, he frequently represents the Dutch competent authority in European Commission's working groups such as the New & Emerging Technologies WG, of which he was appointed co-Chair in 2009. He is a member of the European Society for Nanomedicine and the European Technology Platform Nanomedicine. Since 2011, he coordinates the National Platform Nanomedicine in the Netherlands.

RECENT PUBLICATIONS

- Giannakou C, Geertsma RE, Jong WH de, Loveren H van, Vandebriel RJ, Park MVDZ. Immunotoxicity Testing of Nanomedicinal Products: Possible Pitfalls in Endotoxin Determination. Current Bionanotechnology 2016; 2:95-102.
- Giannakou C, Park MVDZ, Jong WH de, Loveren H van, Vandebriel RJ, Geertsma RE. A comparison of immunotoxic effects of nanomedicinal products with regulatory immunotoxicity testing requirements. International Journal of Nanomedicine 2016; 11: 2935–2952.
- Geertsma RE, Park MVDZ, Puts CF, Roszek B, Stijl R van der, Jong WH de. Nanotechnologies in medical devices. RIVM Report 2015-0149, 2015. http://www.rivm.nl/en/Documents_and_publications/Scientific/Reports/2015/december/Nanotechnologies_in_medical_devices
- Bleeker EAJ, Evertz S, Geertsma RE, Peijnenburg WJGM, Westra J, Wijnhoven SWP. Assessing health & environmental risks of nanoparticles: Current state of affairs in policy, science and areas of application. RIVM Report 2014-0157, 2015. http://www.rivm.nl/en/Documents_and_publications/Scientific/Reports/2015/april/Assessing_health_environmental_risks_of_nanoparticles_Current_state_of_affairs_in_policy_science_and_areas_of_application
- Noorlander CW, Kooi MW, Oomen AG, Park MV, Vandebriel RJ, Geertsma RE. Horizon scan of nanomedicinal products. Nanomedicine 2015; 10:1599-1608.

Prahlad C. Ghosh

Professor, Department of Biochemistry
University of Delhi South Campus, New Delhi-110021

B. Sc (Chemistry Honours) Calcutta University 1975, M. Sc. (Biochemistry) Calcutta University 1977, Ph.D. (Biochemistry) Indian Institute of Chemical Biology 1982, Jadavpur, Kolkata **Title of Thesis:** Ligand-Mediated Uptake of Biologically Active, Molecules and Drugs by Different Tissues **Post-Doctoral Studies:** Armed Force Medical School, Washington D.C., USA., (1982–1986)

TEACHING EXPERIENCE:

Joined in the Department of Biochemistry, University of Delhi South, Campus, New Delhi-10021, as Lecturer in the year 1986, selected as Reader in1993 and promoted to professor in 2001 the same department.

RESEARCH INTEREST:

Liposome and nanoparticle mediated delivery of drugs for the treatment of infectious and cancerous diseases.

Some of the Contributions Made in the Field of Biomedical Sciences

- Prof. Ghosh was able to demonstrate for the first time that incorporation of a specific sugar moiety onto the liposomal surface it was possible to direct the liposomes towards different cell types of liver. His contribution in the field of liposomes technology, sugar mediated targeted delivery in particular, is well recognized at national and international level and was invited to write three chapters for three books in the field of liposome technology, published by well-known publishers like CRC Press, London, Marcel Dekker Inc, New York
- He has developed a novel lipid formulation of amphotericin B using cholesterol hemisuccinate and demonstrated that this formulation is less toxic and very effective for the treatment of fungal infection.
- He has also demonstrated for the first time that liposomes can be used for the delivery of monensin for the treatment of tumor in combination with immunotoxins. Numbers of laboratories throughout the world have employed this technique for the delivery of monensin to tumor cells for their selective elimination in combination with immunotoxins.
- He has also shown that liposomal monensin is very effective as an antimalarial agent.
- He has developed a colorimetric method for the estimation of polyethylene glycol (PEG) in protein and lipid conjugated forms which can be used for estimation of PEG in biological samples like blood and tissues.
- Prof Ghosh has supervised the Ph.D. work of 20 students and currently 6 Ph.D. students have been working under him for their degree and have completed 20 major research projects funded by CSIR, DST, DBT and ICMR, Govt. of India.

- He has published more than 70 research papers in international journal of repute.

AWARD:

Awarded Prof. M.L. Khorana Memorial Prize on June 05, 2015 by the Indian Pharmaceutical Association (IPA) for publishing best paper in the field of Pharmacology & Clinical Pharmacy in IJPS for the year 2013.

MEMBERSHIP OF ACADEMIC BODIES:

- Fellow of National Academy of Sciences, India.
- Life Member, International Liposome Society
- Life Member of all Academic bodies of India

Piotr Grodzinski

Ph.D

Dr. Piotr Grodzinski is a Director of NCI Alliance for Nanotechnology in Cancer at the National Cancer Institute in Bethesda, Maryland. He coordinates program and research activities of the Alliance which dedicates around $150M over funding period of 5 years to form interdisciplinary centers as well as fund individual research and training programs targeting nanotechnology solutions for improved prevention, detection, and therapy of cancer.

Dr. Grodzinski graduated from the University of Science and Technology (AGH) in Krakow, Poland and continued his studies at the University of Southern California in Los Angeles, where he researched novel semiconductor materials used in low threshold lasers. In mid-nineties, Dr. Grodzinski left the world of semiconductor research and got interested in biotechnology. He built a large microfluidics program at Motorola Corporate R&D in Arizona. The group made important contributions to the development of integrated microfluidics for genetic sample preparation with its work being featured in Highlights of Chemical Engineering News and Nature reviews. After his tenure at Motorola, Dr. Grodzinski was with Bioscience Division of Los Alamos National Laboratory where he served as a Group Leader and an interim Chief Scientist for DOE Center for Integrated Nanotechnologies (CINT). At the National Institutes of Health (NIH), in addition to his programmatic responsibilities, he co-chaired Trans-NIH Nanotechnology Task Force, which is coordinating the nanotechnology efforts across 27 institutes of the agency with the budget over $300M/year. Dr. Grodzinski received Ph.D. in Materials Science from the University of Southern California, Los Angeles in 1992. He is an inventor on 17 patents and published over 60 peer-reviewed papers and 10 book chapters. Dr. Grodzinski has been recently elected a Fellow of the American Institute for Medical and Biological Engineering (AIMBE).

Weisheng Guo

Ph.D
Assistant Professor, CAS Lab for Biological effects of Nanomaterials and Nanosafety, National Center for Nanoscience and Technology of China,No. 11, First North Road, Zhongguancun, Beijing, 100190, P.R.China, Tel: +86-10-82545615,
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Dr. Weisheng Guo got Ph.D at Tianjin University. He studied in Professor Xiaoyuan Chen's group for 1.5 year as a joint Ph.D candidate in National Institute of Biomedical Imaging and Bioengineering (NIBIB), National Institutes of Health (NIH, USA). He currently is working as an Assistant Professor with Professor Xingjie Liang at National Center for Nanoscience and Technology of China since graduation from Ph.D in July 2015. He is a lifetime member in Chinese American Society of Nanomedicine and Nanobiotechnology (CASNN). Dr. Guo's research interests are focused on inorganic nanoparticle preparation, the biodistributions and metabolism profile of the imaging guided nanomedicines for tumor theranostics, albumin namodicines for arthritis diagnosis and therapy. As first/corresponding author, Dr. Guo has published more than 15 research papers on ACS Nano, Theranostics, Nano Research, ACS Applied Materials & Interface.

REPRESENTATIVE PUBLICATIONS:

- Weisheng Guo,# Shaobo Zhang,# Jie Wei, Chan Li, Meizhen Yin,* and Xing-Jie Liang*. Terrylenediimide-Based Intrinsic Theranostic Nanomedicines with High Photothermal Conversion Efficiency for Photoacoustic Imaging-Guided Cancer Therapy, ACS Nano, 2017, Just Accepted.
- Weisheng Guo,#,* Guoxian Lv,# Wei Zhang, Tingbin Zhang, Shuyi Li, Shizhu Chen, Ahmed Shaker Eltahan, Dongliang Wang, Yuqing Wang, Jinchao Zhang, Jin Chang,* and Xing-Jie Liang*. Near-Infrared Emission CuInS/ZnS Quantum Dots: All-in-One Theranostic Nanomedicines With Intrinsic Fluorescence/Photoacoustic Imaging for Tumor Phototherapy, ACS Nano, 2016, 10(10): 9637.
- Weitao Yang,# Weisheng Guo,# Wenjun Le, Guoxian Lv, Fuhe Zhang, Lei Shi, Xiuli Wang, Jun Wang, Sheng Wang, Jin Chang,* and Bingbo Zhang*. Albumin-Bioinspired Gd:CuS Nanotheranostic Agent for In Vivo Photoacoustic/Magnetic Resonance Imaging-Guided Tumor-Targeted Photothermal Therapy. ACS Nano, 2016, 10(11): 10245.
- Weisheng Guo, Xiaolian Sun, Orit Jacobson, Xuefeng Yan, Kyunghyun Min, Avinash Srivatsan, Gang Niu, Dale O. Kiesewetter, Jin Chang*, and Xiaoyuan Chen*. Intrinsically Radioactive [64Cu] CuInS/ZnS Quantum Dots for PET and Optical imaging: improved radiochemical stability and controllable Cerenkov luminescence. ACS Nano. 2015, 9(1), 488–495.
- Weisheng Guo, Na Chen, Yu Tu, Chunhong Dong, Bingbo Zhang*, and Jin Chang*. Synthesis of Zn-Cu-In-S/ZnS Core/Shell Quantum Dots with Inhibited Blue-Shift Photoluminescence and Applications for Tumor Targeted Bioimaging. Theranostics. 2013, 3(2): 99-108.

Heinrich Haas

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

Heinrich Haas has more than 20 years of experience in academic research and industrial pharmaceutical development. After he received his Ph.D. in physical chemistry, Dr. Haas researched lipid membranes and organized biomolecular systems. His professional focus is on colloidal/nanoparticulate formulations for targeted drug delivery with therapeutic and diagnostic applications. Before joining BioNTech RNA Pharmaceuticals GmbH (former Ribological GmbH), he was responsible for a variety of projects in biopharmaceutical research and development, ranging from the exploration of novel colloidal therapeutic and diagnostic carriers to up-scaling and development of market-compliant manufacturing methods for liposome products. 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 has an active record of publications in peer-reviewed journals and patent applications in the field of drug delivery.

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.

David Haerry

David Haerry has been a treatment writer and conference reporter since 1996 and co-authors a database on travel and residency restrictions for people living with HIV, www.hivrestrictions.org
David has been involved with health care professionals' education projects since 2007 and, in 2015, became Secretary General for the Swiss Academic Foundation on Education in Infectious Diseases (SAFE-ID). He has been a Work-Package Co-leader and a member of the Executive Committee for the European Patients' Academy on Therapeutic Innovation Innovative Medicines Initiative project (EUPATI-IMI) until February 2017. David has also involved in the working group updating the Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines for Health-Related Research involving Humans published in December 2016, and in several European and global research networks and research collaborations, including the European Network of Centres for Pharmacoepidemiology and Pharmacovigilance (ENCePP) Steering Group. From 2013 to 2016, he co-chaired the Patient and Consumer Working Party at the European Medicines Agency and has served the European AIDS Treatment Group (EATG) in various positions since 2004.
In Switzerland, David Haerry is the co-chair of the Swissmedic working group for patient and consumer organisations. He is a member of the ELSIag at the Swiss Personalised Health Network. He is the founder and current vice-chair of Positive Council Switzerland and responsible for its regular newsletter directed at patients and he is coordinating the pricing working group within the Swiss Experts in Viral Hepatitis SEVHep.
David has been involved in HIV and hepatitis C virus (HCV) drug development since 2005 and has specific interests in personalised medicine, risk communication, pharmacovigilance, observational studies, biomedical prevention and HIV eradication research. David has been living with HIV since 1986.

Stefan Halbherr

Ph.D.
Manager Research and Development
InnoMedica

Studied Biochemistry at the University of Bern/Switzerland. At the University Institute for Immunology in the Insel hospital in Bern he investigated disease-specific antibody signatures in Hemophilia patients using Designed Ankyrin Repeat Protein (DARPin) technology. During his PhD, he developed genetically engineered RNA vectors for vaccination of poultry against avian influenza A (e.g. H5N1). During his doctoral studies already, he joined in 2013 the biomedical research team of InnoMedica and contributed to the initiation of the lead project "Talidox", a novel targeted liposomal formulation of the widely used cytostatic drug doxorubicin. In his role as Manager Research and Development he brought the research concepts of the acquired Yamazaki DDS, Ltd. to a marketable product, introducing many innovations in the processes of industry-scale liposome assembly, drug loading, and especially design of biologically functional liposome surfaces. 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 now leading InnoMedica to create and clinically translate new types of targeted liposome-nanodrugs. This offers new ways to approach key medical applications like chemotherapeutic treatment and diagnosis of cancer, but also management of bacterial infections, and control of diseases of the central nervous system.

Ansgar Hebborn

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Ansgar is Roche Pharma's Head of Global HTA & Payment Policy based in Basel, Switzerland. In this role he focuses on current approaches to HTA, pricing and reimbursement decision making frameworks, their impact on pharmaceutical innovation and patient access as well as their future evolution.

Ansgar is a member and active contributor to national and international pharmaceutical policy forums as well as professional associations. He currently represents Roche in relevant industry associations e.g. in Brussels as a vice-chair of EFPIA's HTA Working Group. During the past couple of years, Ansgar has taken an active role as advisor and stakeholder representative in various HTA collaboration networks e.g. EUnetHTA and the HTAi Policy Forum Committee, and also has been involved in the foundation of other initiatives in this field e.g. the Green Park Collaborative, the HTAi Asia Policy Forum and SwissHTA. He has been a member of the ISPOR Board of Directors in the past and has led the research work stream of ISPOR Vision 2020 project with focus on future ISPOR research priorities for the years ahead. During his career with Roche, Ansgar has had a range of different global roles related to pricing, market access, health economics and outcomes research, based in Switzerland and the US. He led small and large multidisciplinary teams in all major disease areas tasked with the development of global pricing and reimbursement strategies as well as the accompanying clinical, health economic and other outcomes research programs.

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, campus 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, bio-pharmaceutical research, unstructured data analytics, genomics and nanomedicine. His efforts have been documented in about 50 publications and book chapters. At the end of 2013, Dr. Hehenberger retired from IBM Research and started the HM NanoMed Partnership where he is focused on writing books, co-organizing conferences and pursuing nanomedical and genomic research topics.
His first book "Nanomedicine: Science, Business, and Impact", published in 2015, covers both the underlying science and the steps needed to take a new biomedical breakthrough all the way from concept to patient benefit.

Clemens Helmbrecht

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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

Inge Herrmann

Inge Herrmann (1985) studied Chemical and Bioengineering at the ETH Zurich with a stay at the TU Delft in 2007. During her PhD studies in the Stark lab at the ETH Zurich, she pioneered a nanomagnet-based blood purification technology in collaboration with the University Hospital Zurich. After her PhD in biomedical engineering (2010) and postgraduate studies in clinical trials management, she held various positions at the Centre of Clinical Research at the University Hospital in Zurich, the University of Illinois and in the Stevens group at the Imperial College London. Since 2015, she heads the Particles 3D group at the Swiss Federal Laboratories for Materials Science and Technology (Empa). Her research interests include the design of particle-based approaches for diagnostics and therapy, the development of point-of-care devices and translational nanomedicine.
Website: https://www.empa.ch/web/s403/particles-3d

Heinrich Hofmann

Professor for Powder Technology, Ecole Polytechnique Fédérale Lausanne, Institute of Material Science
HHofmann 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 keynote lectures on particles synthesis, modification and nanoparticles in biomedical applications in EU, US Australia and Asia. He supervised 30 PhD students in the period 1994 – 2016.
Main topics of his research today are synthesis and functionalisation of superparamagnetic iron oxide nanoparticles for application like molecular imaging, stem cell tracking, protein separation and hyperthermia. Additionally he is working in the field of colloidal behaviour of nanoparticles in complex environment including transport phenomena. Development of nanocomposites, mostly based on polymers is an other topic where he is using the core competencies of his lab: colloidal chemistry.

Patrick Hunziker

Patrick Hunziker has studied Medicine the University of Zurich, Switzerland. He received a doctoral decree based on thesis work in experimental immunology from the University of Zurich and did further research in experimental haematology at University Hospital in Zurich, Switzerland. He earned specialist degrees in Internal Medicine, Cardiology and Intensive Care Medicine. As a fellow the Massachusetts General Hospital, Harvard Medical School, worked on cardiac imaging in a joint project with the Massachusetts Institute of Technology, Cambridge.
His professional activities in Europe, the U.S., Africa and China gave him a broad insight into the needs for the medicine of the future in a variety of settings. Hunziker became involved in medical applications of Nanoscience in the late nineties and has been the pioneer physician in Nanomedicine in Switzerland since then. With improved prevention, diagnosis and cure of cardiovascular disease as his main research topic, he worked in the nanoscience fields of atomic force microscopy, nanoptics, micro/nanofluidics, nanomechanical sensors and polymer nanocarriers for targeting.
He is the co-founder and past president of the European Society for 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. He is president of the International Society for Nanomedicine.

Oihane Ibarrola

Ing. Oihane Ibarrola is a Bachelor of Science in Chemical Engineering 2008, from the University of the Basque Country (Spain). She also holds a Master in integrated management and a Master in Synthetic and Industrial Chemistry. In 2009, she started to work for BioPraxis (Praxis Pharmaceutical) on the design and development of the Praxis Pharmaceutical drug manufacturing plant. At this stage, her responsibilities were the control of the civil works, the design of the GMPs areas, and their certification by the Spanish Medicines Agency. She has great experience in engineering and maintenance, validation and qualification of pharmaceutical equipment, validation of analytical techniques, as well as in the manufacturing of lyophilized sterile drugs and other pharmaceutical forms, like medical devices and cosmetic products. She is currently the responsible for the design of the new pharmaceutical areas for the production under GMPs of medicines, sanitary products, nutraceuticals and cosmetics in research and she is participating in five EU research projects (Fp7 and H2020).

Working for a private company limits publications, while promotes patenting some relevant examples are the following:

- European Patent application: 12382476.5 and PCT/ES 2013/070833 (Microparticles with EGF, method of preparation and use).
- European Patent application 13382275.9 (Lipid nanoparticles for wound healing).
- European Patent application: 13382268.4 (Lipid nanoparticles of Polymyxin for CF infections tretament).
- Spanish patent P20143189.4. (Lipid nanoparticles of Tobramycin)

Dhifaf Jasim

Dr Jasim Dhifaf obtained her BSc in Pharmacy from the College of Pharmacy, University of Baghdad in 2001 where she worked as a teaching assistant in the Department of Pharmaceutics for several years. In 2011, she completed her MSc in Drug Delivery with Distinction in the School of Pharmacy, University of London. Her research project was carried out in the Nanomedicine Lab and involved the development of liposome-gold nanoparticle hybrids for optical imaging and theranostic applications. In 2012, she was awarded the Overseas Research Scholarship (ORS) Award from the University of London for her PhD studies at the UCL School of Pharmacy. She relocated with the Nanomedicine Lab at the University of Manchester to continue her PhD. Her thesis focused on the development of graphene oxide derivatives for biomedical applications. Since January 2016 she is the Imaging Scientist of the Nanomedicine Lab focusing on the utilisation of various whole-body imaging modalities available.

Wenlei Jiang

Dr. Wenlei Jiang is currently a Senior Science Advisor in the Office of Research and Standards (ORS)/Office of Generic Drugs (OGD)/Center for Drug Evaluation and Research (CDER). She is mainly responsible for coordinating post-market generic drug safety investigation, representing ORS on OGD's new international harmonization activities, and developing opportunities for scientific outreach. Previously she served as the Acting Deputy Director of ORS, where she provided oversight on Generic Drug User Fee Act (GDUFA) regulatory science research programs. Her research interest has been focused on bioequivalence standard development for generic complex drug products containing nanomaterials, solid oral modified release drug products, and narrow therapeutic index drugs, as well as post-market surveillance of generic drugs. 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 advanced parenteral drug delivery systems. She received her PhD in Pharmaceutics and Pharmaceutical Chemistry from The Ohio State University in 2001.

Michael Johnston

Dr. Michael Johnston completed his PhD in the Department of Biochemistry and Molecular Biology at the University of British Columbia in 2006, where his research focused on regulated drug release from liposomal delivery systems. Dr. Johnston subsequently joined Health Canada as a post-doctoral fellow and was then hired as a research scientist in 2007 to establish a nanomedicines research program. Since then Dr. Johnston has further developed his research program to focus on understanding parameters affecting critical quality attributes of nanoscale drug delivery systems with a particular interest in nanoparticles generated with recombinant protein expressed in plants. Dr. Johnston is also concerned with nanoscale vaccine adjuvants. He also currently chairs the International Pharmaceutical Regulators Forum (IPRF) working group for nanomedicines.

Jan Kamps

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Jan Kamps obtained his PhD at the University of Leiden, Biopharmaceutical Sciences, section Biopharmacy in 1992. His thesis was on the "Interaction of (atherogenic) lipoproteins with human liver parenchymal cells and Kupffer cells".
Since 1992 he works at the University of Groningen, initially as post-doctoral fellow at the departments of Physiological Chemistry (Medical Sciences) and Pharmacokinetics and Drug Delivery (Pharmacy) and later as senior researcher at the section Liposome Research of the department of Cell Biology. In 2003 he was appointed assistant professor in the Laboratory for Endothelial Biomedicine and Vascular Drug Targeting research of the department of Pathology & Medical Biology.
The research in the Laboratory of Endothelial Biomedicine & Vascular Drug Targeting research (head: prof. dr. G. Molema), focuses on understanding organ specific, microvascular endothelial cell behavior in specific (inflammatory) diseases and development of therapeutic strategies to interfere with endothelial cell (dys)function.
Two strategies are followed:
1 Unraveling the molecular pathways leading to microvascular capillary endothelial cell activation *in vivo*, and study the consequences of (targeted) drugs on these molecular functions .
2 Development of targeted drug delivery strategies that selectively interfere with endothelial cell (dys)function in diseased tissues/organs.

Specific research interests of Jan Kamps include:

- Carrier mediated targeted drug delivery
- Liposomes, including liposome technology and "drug"formulation.
- Interaction of liposomes and/or other (lipid-based) particles with (endothelial) cells, both *in vitro* and *in vivo*, including intracellular processing of the particles and of particle derived components.
- In vivo behavior and targeting of drug containing lipid-based carriers or carrier components in disease (e.g. inflammatory diseases, liver fibrosis, cancer).
- Effects and efficacy of carrier mediated targeted drug delivery on cellular molecular mechanisms involved in the disease process.

Jan Kamps is (co)author of more than 90 peer-reviewed publications and (co)organizer of several national and international symposia and workshops. Furthermore he was secretary of the Dutch Society of Pharmaceutical Sciences (NVFW) and is coordinator of the, The Netherlands Platform for Targeted Nanomedicine.

SELECTED PUBLICATIONS

- Kamps JA, Krenning G. Micromanaging cardiac regeneration: Targeted delivery of microRNAs for cardiac repair and regeneration. World J Cardiol. 2016 Feb 26;8(2):163-79.
- Ganesh Ram R. Visweswaran, Shima Gholizadeh, Marcel H.J. Ruiters, Grietje Molema, Robbert J. Kok, Jan. A. A. M Kamps. Targeting rapamycin to podocytes using a vascular cell adhesion molecule-1 (VCAM-1)-harnessed SAINT-based lipid carrier system. PLoS One. 2015 Sep 25;10(9):e0138870.
- Piotr S. Kowalski, Praneeth R. Kuninty, Klaas T. Bijlsma; Marc C.A. Stuart; Niek G.L. Leus; Marcel H.J. Ruiters; Grietje Molema; Jan A.A.M. Kamps SAINT-Liposome-Polycation particles, a new carrier for improved delivery of siRNAs to inflamed endothelial cells. Eur J Pharm Biopharm. 2015 Jan;89:40-7.
- Dickinson MG, Kowalski PS, Bartelds B, Borgdorff MA, van der Feen D, Sietsma H, Molema G, Kamps JA, Berger RM. A critical role for Egr-1 during vascular remodelling in pulmonary arterial hypertension. Cardiovasc Res. 2014 Sep 1;103(4):573-84.
- Piotr S. Kowalski, Peter J. Zwiers, Henriëtte W.M. Morselt, Joanna M. Kuldo, Niek G.J. Leus, Marcel H.J. Ruiters, b, Grietje Molema, Jan A.A.M. Kamps, Anti-VCAM-1 SAINT-O-Somes enable endothelial-specific delivery of siRNA and downregulation of inflammatory genes in activated endothelium *in vivo*. J. Control. Rel. 2014, 176: 64–75

Keon W. Kang

Dr. Keon W. Kang, a nuclear medicine physician, is a professor and the Chairman of the Department of Nuclear Medicine, Seoul National University College of Medicine. He received an M.D. degree from Seoul National University College of Medicine (1991). He was trained as an intern and a resident for Internal Medicine at Seoul National University Hospital (1991–1996). He received Ph.D. in Medical Science at Seoul National University College of Medicine (2001). He has worked as Chief, Department of Nuclear Medicine, National Cancer Center, Korea (2000–2007). He studied molecular imaging at Prof. Sam Gambhir's lab as a visiting scientist of Molecular Imaging Program at Stanford (2003–2004). His research areas are *in vivo* molecular imaging of cancer using PET & nanoparticles. He is studying (1) clinical trials using angiogenesis PET for cancer, (2) translational research using multifunctional nano-particles for optical/PET/MRI imaging, (3) *in vivo* cell trafficking of stem cells or immune cells using bioluminescence, fluorescence, radio-labeled technology, and (4) test biodistribution, pharmacokinetics, and efficacy of nano drug delivery systems using *in vivo* imaging. He published more than 160 articles in peer reviewed journals. He is currently President of the Korean Society for Nanomedicine.

Fabian Kiessling

Since 2008 Professor Dr. Fabian Kiessling is leading the Institute of Experimental Molecular Imaging at the Helmholtz Institute for Biomedical Engineering at the RWTH-University in Aachen. Aim of his research is the development of novel diagnostic, theranostic and therapeutic probes as well of advanced imaging technologies and image analysis tools. In this context, the main focus of his research is the investigation and diagnostic assessment of vascular and microenvironmental tissue properties and the exploration of its impact on disease progression and therapy response.
Fabian Kiessling studied Medicine and did his thesis at the University in Heidelberg. Until the end of 2002, he worked as resident in the Department of Radiology at the German Cancer Research Center (DKFZ) in Heidelberg. In 2003 he changed to the Department of Medical Physics in Radiology of the DKFZ as leader of the Molecular Imaging group. In parallel, he did his clinical training at different Departments of the University of Heidelberg and received the board certification as Radiologist in 2007. Fabian Kiessling did his habilitation in experimental radiology in 2006. In 2008 he founded the invivoContrast GmbH together with Matthias Braeutigam.
Fabian Kiessling is author of more than 250 scientific publications and book chapters, edited three books and received many research awards, among those the „Emil Salzer Price for Cancer Research" and the "Richtzenhain Price".
Professor Kiessling was in the Editorial board of several scientific journals including Radiology, European Radiology, European Radiology Experimental, and the American Journal of Nuclear Medicine and Molecular Imaging.
He is founding member of the European Society for Functional and Molecular Imaging in Radiology (ESMOFIR), currently treasurer of the European Society for Molecular Imaging (ESMI), founding member of the ESMI working group "Image Guided Therapy and Drug Delivery (IGTDD)" and he was chairman of the "Molecular Imaging" subcommittee of the European Society for Radiology (ESR). Furthermore, he was program chair of the European Molecular Imaging Meeting (EMIM) in Antwerp in 2014 and the World Molecular Imaging Conference (WMIS) in New York in 2016.

Ingrid Klingmann

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

European Forum for Good Clinical Practice (EFGCP), PHARMAPLEX bvba
Physician, specialized in General Medicine, Clinical Pharmacology and Pharmaceutical Medicine with over 30 years of experience in different senior operational and managerial functions in pharmaceutical companies, CROs and academic sites, focussing on clinical trial management, ethical and regulatory aspects.
Since January 2003 she has her own pharmaceutical development and site management support consulting company.
Dr. Klingmann is Chairman of the Board of the European Forum for Good Clinical Practice (EFGCP). On behalf of EFGCP she was and is involved in different FP7- and IMI-funded projects (ICREL, PatientPartner, PharmaTrain, EUPATI, Combacte-Magnet) and with her company in the FP7-funded paediatric LENA project and the IMI-project SPRINTT. Currently Dr. Klingmann is also President of PharmaTrain Federation.
Dr. Klingmann is Module Chair in the post-graduate Master Course in Regulatory Affairs at the University of Bonn, Germany, and is lecturer in the Diploma Course in Clinical Trial Practices and in the ECPM course at University of Basel and in the Pharmed course at Université Libre de Bruxelles, Belgium.

Ewelina Kluza

Ewelina Kluza received her PhD in biomedical engineering from Eindhoven University of Technology, the Netherlands, in 2011. Her project was focused on the development nanomedicine-based strategies for molecular imaging of angiogenesis and therapy monitoring in cancer. One of her key achievements was to pioneer a strategy of synergistic targeting of αvβ3-integrin and galectin-1, which significantly improved the molecular recognition of tumor angiogenesis. Furthermore, by using therapeutic/diagnostic liposomes and multiparametric MRI, she investigated the systemic activity and vascular effects of anti-inflammatory therapy in cancer.
During her postdoctoral fellowship at the Radiology Department of Maastricht University Medical Center, the Netherlands, in 2010-2012, she established a clinical research line on the vascular imaging in rectal cancer patients. Her work on the intrinsic gradient of vascular function in rectal tumors and the enhanced vasculature in the tumor-surrounding mesorectum received great attention from the international scientific community. Moreover, her studies demonstrated a high diagnostic performance of vascular imaging and quantitative morphological imaging in patients with rectal cancer.
Subsequently, she worked at the Weizmann Institute of Science in Israel (2012-2015), where she returned to the filed of nanomedicine. The primary goal of her research was to develop hyaluronidase-responsive contrast agent for MRI- and fluorescence-based detection. This work resulted in the establishment of nanoparticle formulation applicable for the inflammatory cell-targeting and hyaluronidase detection.
From 2015, she works in the Experimental Vascular Biology group at the Academic Medical Center in Amsterdam, the Netherlands and part-time at the Translational and Molecular Imaging Institute, Mount Sinai School of Medicine, New York, USA. Her research projects focus on the development of polymer- and lipid-based nanomedicine for imaging and treatment of atherosclerosis. She is particularly interested in the interplay between the nanomedicine and inflammation, and dysfunctional endothelium.

Andrey Klymchenko

Andrey Klymchenko was born in Kherson, Ukraine, in 1976. He started his research with chemistry and photophysics of new fluorescent dyes, which was a subject of his PhD degree from Kiev National University in 2003. Then, he worked in the University of Strasbourg, where he could combine synthesis of new dyes with their bioimaging applications. In 2005, in order to extend his expertise towards supramolecular chemistry and nanotechnology, he moved to Catholic University of Leuven. Then, he joined CNRS in 2006, received CNRS Bronze Medal in 2010 and was promoted to Director of Research in 2014. In 2015, he obtained ERC consolidator grant BrightSens to work on fluorescent nanoparticles for ultrasensitive detection of cancer markers. He is a leader of "Nanochemistry and Bioimaging" group. His research interests include functional fluorescent molecules and nanomaterials for biosensing, imaging and theranostics. He has already developed a number fluorescent probes for cellular imaging, notably a probe for apoptosis detection, which is currently on the market. Moreover, he recently introduced new concepts for development of ultrabright dye-loaded nanoparticles based on

polymers and lipids for bioimaging applications. He is a co-author of over 140 per-reviewed articles.

SELECTED PUBLICATIONS

1. Bouchaala, R.; Mercier, L.; Andreiuk, B.; Mély, Y.; Vandamme, T.; Anton, N.; Goetz, J.G. Klymchenko, A.S. Integrity of lipid nanocarriers in bloodstream and tumor quantified by near-infrared ratiometric FRET imaging in living mice. J. Controlled Release 2016, 236, 57.
2. Reisch, A.; Klymchenko, A.S. Fluorescent Polymer Nanoparticles Based on Dyes: Seeking Brighter Tools for Bioimaging. Small 2016, 12, 1968.
3. Reisch A, Runser A, Arntz Y, Mély Y, Klymchenko AS. Charge-controlled nanoprecipitation as a modular approach to ultrasmall polymer nanocarriers: making bright and stable nanoparticles. ACS Nano 2015, 9, 5104.
4. Kilin, V. N.; Anton, H.; Anton, N.; Steed, E.; Vermot, J.; Vandamme, T. F.; Mely, Y.; Klymchenko, A. S. Counterion-enhanced cyanine dye loading into lipid nano-droplets for single particle tracking in zebrafish. Biomaterials 2014, 35, 4950.
5. Reisch, A.; Didier, P.; Richert, L.; Oncul, S.; Arntz, Y.; Mély, Y.; Klymchenko, A. S. Collective fluorescence switching of counterion-assembled dyes in polymer nanoparticles. Nature Commun. 2014, 5, 4089.

Silke Krol

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Since 2011 Silke Krol is with the 2009 funded Center of Nanotechnology@Fondazione I.R.C.C.S. Istituto Neurologico "Carlo Besta" in Milan, Italy. Recently she joined as senior scientist the Istituto tumori "Giovanni Paolo II" in Bari, Italy and funded a laboratory for translational Nanotechnology with focus on early diagnosis and advanced therapy of cancer.

At Besta, she is 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. In collaboration with her Prof. Stellacci in Lausanne, Switzerland, they discovered the role of nanoparticles in vaccine stabilization, antiviral action and enhancer for viral infectivity for gene delivery. Additionally her group develops multifunctional polymer/nanogold based drug or drug delivery systems as well as diagnostic tool for medical applications such as a hand-held point-of-care device to measure chemotherapeutic drugs in real-time at the bedside of the patient. Moreover, the multilayer-nanocoating was 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.

In Bari, she focussed her research on the development of drug delivery systems for lung cancer and melanoma as well as the role of exosomes and the tumor secretome in cancer and predictive diagnosis.

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. She worked as project technical advisor in 3 EU-FP7 projects and is external expert for the evaluation of EU project. 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. 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.

Tony Lahoutte

Prof Dr Tony Lahoutte is head of the department of nuclear medicine at UZ Brussel and head of the molecular imaging research unit at the Vrije Universiteit Brussel (VUB) in Belgium. He obtained his medical degree in 1998 and started his research activities in combination with a residency program in nuclear medicine. His current research is focused on the development and clinical translation of molecular imaging probes and targeted radionuclide therapies for the detection and treatment of cancer. In 2014 he co-founded the company Camel-IDS NV that is developing a pipeline of radio-immuno therapeutics.

Twan Lammers

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

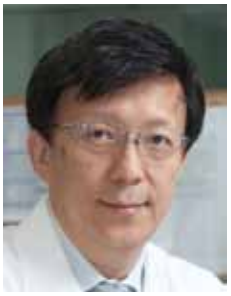
Anna Leczkowska

Anna obtained her PhD in Chemical Biology from the University of Birmingham, UK in 2011 as a Marie Curie Early Stage Research Fellow. Her PhD project involved the study of the recognition of nucleic acids structures by small molecules using a variety of spectroscopic methods.

After completing her PhD Anna moved to Imperial College London, UK, as a research associate to continue her research in medicinal chemistry. During her post-doctoral work Anna studied the interaction of metal complexes with G-quadruplex DNA structures and their effect in cancer cells.

In 2015 Anna started her work at Applied Photophysics Ltd. where she held the position of Applications Scientist. Her work was fo-

cused on using spectroscopic techniques in medicinal and biopharmaceutical research. These included studying the structural features of natural biomolecules, both proteins and nucleic acids, as well as exploring synthetic systems for medicinal applications.
Anna joined Malvern Instruments as a Product Technical Specialist – Biophysical Characterisation in April 2016. She works closely with customers within the Life Sciences sector and provides both technical and applications support primarily within the fields of Dynamic and Electrophoretic Light Scattering (DLS/ELS), Taylor Dispersion (TD) and Nanoparticle Tracking Analysis (NTA).



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. He acquired the M.D. from Seoul National University in 1982 and the Ph.D. in 1990. He has been serving in the Editorial Board of Journal of Nuclear Medicine, European Journal of Nuclear Medicine and Molecular Imaging, Journal of Nuclear Cardiology and is Editor-in-Chief of Nuclear Medicine and Molecular Imaging. He is Member of Korean Academy of Science and Technology (KAST) and Member of National Academy of Medicine of Korea. He is President-elect of World Federation of Nuclear Medicine and Biology and will serve as President in 2019-2020. His current main interest is to establish radionanomedicine as combined nuclear and nanomedicine and its plausible clinical translation.

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- Professor at the University of Strasbourg
Institute for Advanced Study (USIAS),
Chair of Chemistry of Complex Systems
- Honorary Professor at the Collège de France, Paris
- Emeritus Professor at the University of Strasbourg, Director
- Director of ISIS (Institut de Science et d'Ingénierie Supramoléculaires), Strasbourg, 1997–2004.
- Director of the Laboratoire de Chimie Supramoléculaire, ISIS, Université de Strasbourg
- Director at the Nanotechnology Institute of the Karlsruhe Institute of Technology, since 1998
- Honorary Director, "Lehn Institute of Functional Materials", Sun Yat Sen University, Guangzhou, since 2010

EDUCATION

Undergraduate Studies, University of Strasbourg: Licence ès-Sciences (Bachelor of Sciences), Strasbourg, 1960; Graduate work on "Conformational Studies of Triperpenes" with Professor Guy OURISSON, University of Strasbourg; Doctorat-ès-Sciences (Ph.D.), University of Strasbourg, 1963; Post-Doctoral Research Fellow at Harvard University, 1964: work on Vitamin B12 total synthesis with Professor Robert B.WOODWARD.

AWARDS

Nobel Prize in Chemistry, 1987; Sigillum Magnum, University of Bologna, 1988.

DECORATIONS

Ostereischiches Ehrenkreuz für Wissenschaft und Kunst, Erste Klasse, 2001 ; High Officer in the Order of Cultural Merit in Romania, section Scientific Research, 2004 ; Grosses Verdienstkreuz mit Stern der Bundesrepublik Deutschland, 2009 ; Grand Officier de la Légion d'Honneur, 2014 ; Officer of the Order of Merit of the Republic of Poland, 2015.

SCIENTIFIC WORK

985 publications ; 3 books

- "Chemia Supramolekularna", Collection of publications by J.-M. LEHN, organised and translated into Polish under the direction of Janusz Lipkowski, Institute of Physical Chemistry, Polish Academy of Sciences, 1985.
- B. DIETRICH, P. VIOUT, J.-M. LEHN, "Aspects de la chimie des composés macrocycliques", InterEditions/Editions du CNRS, 1991. English Version: "Macrocyclic Chemistry – Aspects of Organic and Inorganic Supramolecular Chemistry", VCH, Weinheim, 1993.
- J.-M. LEHN, "Supramolecular Chemistry – Concepts and Perspectives", VCH, 1995. French Version : "La chimie supramoléculaire : Concepts et perspectives", Translated from the English original text by A. Pousse, De Boeck Université, Bruxelles, 1997. Portuguese Version, translated by M.J. Calhorda, R. Delgado, A.M. Martins, V. Gageiro Machado, N. Miranda, 2007; Japanese Version, translated by Y. Takeuchi, Kagaku Dojin, Tokyo, 1997; Russian Version, translated by E.V. Boldyreva ; coeditors, V.V. Vlassov and A.A. Varnek; Nauka, Novosibirsk, 1998; Chinese Version, translated by X. Shen, Peking University, Beijing, 2002.

Claus-Michael Lehr

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Claus-Michael Lehr is Professor at Saarland University as well as cofounder and head of the department "Drug Delivery" at the Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), which was established as a branch of the Helmholtz Centre for Infection Research (HZI) Braunschweig in 2009 The Helmholtz Association of German Research Centers is the largest public research organization in Germany. The combination of expertise in infection research and pharmaceutical sciences at HZI and HIPS is a unique setting in Germany and Europe, especially in the development of new anti-infectives. Since October 2015, the institute is located in a brand new state-of-the-art research building on the university campus in Saarbrücken, the capital of Saarland. Prof. Lehr has also been cofounder of Across Barriers GmbH and acts as CEO of PharmBioTec GmbH, a non-for-profit contract research subsidiary of Saarland University.
Prof Lehr has studied pharmacy in Mainz and Hamburg (Germany) and received his PhD (1991) from Leiden University (Netherlands). After postdoctoral training at USC (Los Angeles, USA, 92), and Leiden University (93), he was appointed as professor in Marburg University (Germany, 94) until he moved to Saarland University (Saar-

brücken, Germany, since 95). The research theme of Prof. Lehr's team is non-invasive drug delivery across biological barriers, in particular the epithelia of the gastrointestinal tract, the skin and the lungs, as well as some barriers peculiar for microbes, such as the bacterial cellular envelope, biofilms and host cell membranes. A substantial part of the lab's activities is dedicated to predictive cells and tissue models, capable to study these barriers also in state of disease in order to facilitate the translation of novel therapeutic concepts into the clinic. Complementary, innovative carriers systems, often based on nanotechnology, are being investigated, capable of safely crossing the barriers and to efficiently deliver the active cargo to the target.
Prof. Lehr is (co)author of more than 300 papers with >10.000 citations (h-index = 59). He was the recipient of the CRS Young Investigator Award (2001), the APV Research Award 2006 for outstanding achievements in the Pharmaceutical Sciences and the biannual International Price 2008 of the Belgian Society for Pharmaceutical Sciences. In 2011, his team was awarded the German national research award on alternatives to animal testing. Prof. Lehr is Fellow of the American Association of Pharmaceutical Scientists (AAPS, 2010) and corresponding honorary member of the French Academy of Pharmaceutical Sciences (2012). He serves on different national and international scientific and editorial committees and is co-editor of the European Journal of Pharmaceutics and Biopharmaceutics. He is regularly involved in the organization of international conferences. In particular, he has been the initiator of an international workshop and conference on "Biological Barriers" at Saarland University, which took place in March 2016 for the 11th time with more than 200 participants. Recently, the British magazine "The Medicine Maker" rated him, for the second time, as one of the top 100 most influencing drug researchers in the world.

Hans Lehrach

Prof. Dr. Hans Lehrach obtained his Ph.D. at the Max Planck Institute for Experimental Medicine and the Max Planck Institute for Biophysical Chemistry in 1974. Next he moved on to Harvard University, Boston (1974–1978) for a postdoc and then became group leader at EMBL, Heidelberg (1978–1987). He then moved to the Imperial Cancer Research Fund, London (1987–1994) to become head of the Genome Analysis Department. In 1994 he returned to Germany to become Director at the Max Planck Institute for Molecular Genetics (since 1994, em. 2014).
His expertise lies in genetics, genomics, systems biology and personalized medicine. Highlights include his key involvement in several large-scale genome sequencing projects, such as the human, rat, and Schizosaccharomyces. His group was part of the team which identified the Huntington's disease gene. Dr. Lehrach also performed key work on technologies such as protein microarrays, protein interactome analysis, yeast artificial chromosomes and RNAseq. He has been pioneer in the application of next generation sequencing techniques and systems medicine for the development of personalized therapies in cancer (Virtual Patient Model). He was partner in two German ICGC projects and partner in the European IHEC project BLUEPRINT, a steering committee member of the 1000 Genomes project, leader of the managing entity of the IMI OncoTrack project and co-ordinated the FET Flagship pilot initiative IT Future of Medicine (ITFoM, www.itfom.eu), a finalist of the FET Flagship Call, which has established a strong technological roadmap and network of partners from 33 countries. In this current initiative, Hans Lehrach represents a growing network of research institutions, industry representatives, scientists and patient groups that share a long-term vision for sustainable health care (www.heathcarecompactforeurope.eu).
Dr. Lehrach has founded several biotechnology companies such as Sequana Therapeutics, GPC Biotech, Scienion, Prot@gen, PSF Biotech, Atlas Biolabs. Dr. Lehrach is founder of the Berlin-based company Alacris Theranostics GmbH, specialising in the development of new approaches for personalised medicine for cancer patient diagnosis, treatment and drug stratification. He is chairman of the Supervisory Board and scientific advisor of the company since 2008. In 2010 he founded the non-for-profit research institute The Dahlem Centre for Genome Research and Medical Systems Biology (DCGMS).

Didier Letourneur

Didier Letourneur, Engineer, PhD in Chemistry, is Research Director at CNRS. He is the Director of the Laboratory for Vascular Translational Science (LVTS–Inserm U1148- University Paris Diderot – University Paris 13; http://www.u1148.fr; about 160 persons). He also leads the team of Cardiovascular Bioengineering at U1148.
D Letourneur is actively involved in several national grants, in Health regional cluster Medicen, and since 2013 as European coordinator of NMP "NanoAthero" large scale project (16 partners, 10 countries – http://www.nanoathero.eu). He was also involved in several FP7 projects (Health 2007–2013 "FAD" Large scale coordinated by its Research Unit, Health 2010-2014 Prestige (WP2 co-leader), and NMP 2009–2012 "Nanoantenna").
D Letourneur is the author of 141 international publications (h-index 31), inventor of 16 patents, and won several prizes "Coup d'Elan for Research" Bettencourt Foundation 2001, Diderot Innovation Award 2009 CNRS-University Paris 7, Cardiovascular Innovation Award 2011 from Medical Research Foundation, and OSEO/BPI emergence 2012 & Creation-Dev 2013 for start-up creation. In 2016, he found the start-up SILTISS for the development of innovative orthopedic implants. In 2016 he obtained the G Winter Award, the highest recognition from the European Society for Biomaterials.
He has more than 100 invited lectures and seminars and is the co-organizer of numerous national and international conferences (India, Tunisia, Canada) and of two Inserm training workshops for Regenerative Medicine (2009 and 2012). He serves from 2013 at AVIESAN-ITMO for Health technologies in the scientific council. He was vice-chairman for Regenerative Medicine at the European Technology Platform for Nanomedicine and is now General Secretaire. Since 2009, he is President of BIOMAT, French Society for Biomaterials.

Lada Leyens

Lada Leyens works for the Swiss Therapeutics Agency (Swissmedic) as clinical study reviewer in the clinical trials division. She is also part of the Nanomedicines Expert Team within Swissmedic, represents the agency at national and international meetings and has experience as GCP inspector. Lada worked at the European Medicines Agency (EMA) as a National Expert on Secondment in the Scientific Support Team with a special focus on clinical pharmacology and pharmacogenomics. During her time at the EMA she also participated in several joint EMA-HTA Scientific Advice procedures. She has experience in the medical devices industry in the set up and management of clinical trials. Lada obtained her MSc. in International Health Economics from London School of Economics and Political Sciences (LSE) and her BSc. Hons. in Human Genetics from University College London (UCL). She is currently an external PhD candidate at UNU-MERIT (Prof. Angela Brand) at Maastricht University, The Netherlands.

Dong-Kwon Lim

Dr. Dong-Kwon Lim is an assistant professor at KU-KIST Graduate School of Science and Technology in Korea University (Seoul, South Korea) (2015 – current). After he finished his BS and MS degree of Chemistry from Kyungpook National University (1996), he worked for more than 10 years in the pharmaceutical research institutes of the company in Korea. He focused on the development of new chemical entity and incrementally modified drugs such as Amlostar® (antihypertensive drug) which is successfully marketed in Korea. After he received his Ph. D. degree of Chemistry from Seoul National University in 2011, he started his postdoctoral research at MIT (David H Koch Institutes, Advisior: Prof. Robert Langer Lab) and Harvard Medical School (Children's Hospital Boston) (2011 – 2013). Dr. Lim has made pioneering contributions to the field of DNA-based nanostructure synthesis for single molecule surface-enhanced Raman scattering (SERS) and the developments of new bio detection & therapeutic strategies based on organic/inorganic hybrid nanomaterials. His recent research interests include the synthesis of plasmonic nanomaterials with graphene for improved photothermal therapy and photoacoustic imaging. He also focused on developing new imaging technology with Raman scattering for high resolution and high speed live cell imaging. He has authored or co-authored a number of peer-reviewed publications including recent publications in Nano Letters(2016, 2015, 2013), NPG Asia Materials (2016), ACS Nano (2015), Nature Materials (2010), Nature Nanotechnology (2011). He is a member of Korean Chemical Society (KCS), Korean Nanomedicine Society, and the Polymer Society of Korea.

Beat Löffler

Beat Löffler, MD h.c. MA studied after a learning-visit in the USA Philosophy, Communication Sciences and Politics at the Freie Universität Berlin, graduating with a Master of Arts. In 2014 he received an MD h.c. from the University of Basel. 1984 he co-founded an Agency for New Media. From 1988 to 1994 he was Managing Director of the International Hightech Forum Basel organizing congresses on new technologies in mobility, energy, CFD and medical technology. 1994 he founded his company "L & A Concept Engineering" for translation of science-based visions in the application and establishment of worldwide networks (mission and strategy for realizing projects out of visions of clients). He was for 6 years was secretary general and coach of the trinational BioValley Promotion Team, with the mission of establishing the trinational Upper-Rhine Biotechnology network. From 2003 to 2006, he worked for NEC Hightech Performance Computing as Lead Consultant Life Sciences Business Development in Biology and Medicine. He founded the European Foundation for Clinical Nanomedicine in 2007 together with Patrick Hunziker. The aim of the foundation is the research and development of nanomedicine with regard to its use as an innovative technology, better medical care in the future and the establishing an international network in nanomedicine and related fields. Today is his tenth programme and organization of the scientific summit on clinical nanomedicine under the name CLINAM 10 /2017 (Clinical Nanomedicine). CLINAM shaped a neutral high-level debate platform organized by the nonprofit foundation, which serves also as meeting place for the international regulatory authorities in the field of nanotechnologies in health. Presently CLINAM is the largest network for worldwide clinical nanomedicine debates and has become a meeting between all stakeholders in Nanomedicine and related fields. The foundation launched the European Journal of Nanomedicine of which he is Managing Editor. He co-founded the European Society for Nanomedicine and the International Society for Nanomedicine, which realizes every year a Nanomedicine Summer school. He is head of dissemination the EU-funded NanoAthero project. He cooperates regularly in Projects of the European Materials Research Society.

Imre Mäger

Imre Mäger is a postdoctoral researcher and Exosome Team Leader in Professor Matthew Wood lab in the Department of Physiology, Anatomy and Genetics at the University of Oxford. He also holds a personal research grant of the Estonian Research Council as a starting P.I. at University of Tartu, Estonia. His primary research is focused on understanding various aspects of extracellular vesicle (EV) biology such as extracellular RNA species and functions, and comparing EV proteome of various cell sources and linking it to EV properties. He also explores strategies for using extracellular vesicles for targeted delivery of biotherapeutics and other types of bioactive drugs.

Volker Mailänder

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

PUBLICATIONS

- Schöttler S, Becker G, Winzen S, Steinbach T, Mohr K, Landfester K, Mailänder V, Wurm FR.: Protein adsorption is required for stealth effect of poly(ethylene glycol)- and poly(phosphoester)-coated nanocarriers. Nat Nanotechnol. 2016 Apr;11(4):372-7
- Schöttler S, Klein K, Landfester K, Mailänder V.Protein source and choice of anticoagulant decisively affect nanoparticle protein corona and cellular uptake. Nanoscale. 2016, 8: p. 5526-5536
- Hofmann, D., et al., Drug delivery without nanoparticle uptake:

delivery by a kiss-and-run mechanism on the cell membrane. Chemical Communications, 2014. 50(11): p. 1369-71.
- Paven, M., et al., Super liquid-repellent gas membranes for carbon dioxide capture and heart-lung machines. Nature Communications, 2013. 4.
- Lerch, S., et al., Polymeric nanoparticles of different sizes overcome the cell membrane barrier. European Journal of Pharmaceutics and Biopharmaceutics, 2013. 84(2): p. 265-274.

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 25 years as a senior scientist covering several roles in the R&D organization. Currently he is the Director of the Global R&D at Bracco Imaging. From 2004-2010 he served as contract professor at the Dept. of Biotechnology and Molecular Sciences at the University of Varese in Italy. His current research activity is focused on the design and development of small and nanosized probes for molecular imaging applications in combination with therapies using
Magnetic Resonance, Ultrasound, Photoacustic and Nuclear 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). Recently, the activities are extended to oncologic research focusing new metabolic risk profiles of pancreatic ductal carcinomas. Further, Harald Mangge holds since October 1, 2014 the position of an interim Head of the Clinical Institute of Medical and Chemical Laboratory Diagnosis and the function of a Vice speaker of the Cardiovascular Research Field of the Medical University of Graz. He is also deputy for the BioTechMed Graz initiative, an interdisciplinary strategic joint project of the three large universities (Technical-, Medical-, and Comprehensive-University) at the location of Graz, Austria.

Mira Marcus-Kalish

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Dr. Mira Marcus-Kalish is currently the Director of International Research Collaborations at the Tel Aviv University. Her main areas of interest are mathematical modelling, converging technologies and data mining.
Dr Kalish holds a Ph.D. in Operations Research from the Technion - Israeli Institute of Technology, where she developed one of the first computerized systems for electrocardiogram (ECG) diagnosis. She did her postdoctoral training at Harvard University, the MBCRR (Molecular Biology Computer Research and Resource) laboratory and at the Dana Farber Cancer Institute. Her B.Sc. is in Statistics and Biology from the Hebrew University of Jerusalem. Upon her return to Israel, she joined the Tel Aviv University Business School establishing the Medical Management Program focusing on Medical Informatics. Then joined the Weizmann Institute of Science, working with Prof Ephraim Katzir, mainly on protein interactions, specificity and sensitivity. She moved with Prof Katzir back to Tel Aviv University, to the Biotechnology Department taking active part in NBIC, Converging Technologies and contributing to the recent EU-US Wtec-NBIC2 activities & publication.
She was involved in a private business enterprise and served as the scientific advisor and later as the head of the Enterprise Marketing Department of IBM Israel. Dr. Kalish took an active part, mainly in the data science research, in many of the EU various framework projects, such as the Nano2Life Network of Excellence, being the joint research WP leader, SkinTreat, ReNaChip, EpoCan, etc. Current active EU projects are NanoAthero, GLAM and ENATRANS.
Nowadays she takes part in the Human Brain Flagship Project (HBP), as the Medical Informatics Sub Project leader. Her main focus is Disease Signature identification based on targeted analysis of broad band of scientific and clinical Knowledge & Data. The newly developed approach & analytical tools are trying to meet the challenges of big versus small data, missing values, various data sources, etc. towards reliable, replicable reproducible personalized and precise medicine. Other area of research are rehabilitation of the discrete sensory motor, learning function, cerebellar motor learning, protein- protein interactions, drug toxicity, learning machine systems, data mining and medical informatics and recently a broad band project on Healthy Aging.

Sylvain Martel

Prof. Sylvain Martel, Fellow of the Canadian Academy of Engineering as well as IEEE Fellow, is Chair of the IEEE Technical Committee on Micro- Nanorobotics and Automation, and Director of the NanoRobotics Laboratory at Polytechnique Montréal, Campus of the University of Montréal, Canada, and Adjunct Professor in the Department of Bioengineering at McGill University, Montréal, Canada. He received many awards mostly in interdisciplinary research and he is a recipient of a Tier 1 Canada Research Chair in Medical Nanorobotics. He developed several biomedical technologies including platforms for remote surgeries and cardiac mapping systems when at McGill University, and new types of brain implants for decoding neuronal activities in the motor cortex when at MIT. Among other achievements, Dr. Martel's research group is also credited for the first demonstration of the controlled navigation of an untethered object in the blood vessel of a living animal. Prof. Martel's interdisciplinary team is recognized worldwide as a pioneered and leading authority in the development of navigable therapeutic agents and interventional platforms for cancer therapy.

Pascal Mäser

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Pascal Mäser graduated from the University of Basel in 1998 with a Ph.D. in microbiology. He moved on to the University of California San Diego for postdoctoral research in molecular plant physiology, and in 2002 returned to Switzerland as an assistant professor of the University of Bern. In 2009 he joined the Swiss TPH. Currently he is associate professor for parasitology and protozoology at the University of Basel, and head of the parasite chemotherapy unit of the Swiss TPH. His research focuses on drug discovery for trypanosomatid parasites and for malaria.

Yasuhiro Matsumura

Dr. Yasuhiro Matsumura is the Director of the Division of Developmental Therapeutics at the Exploratory Oncology Research & Clinical Trial Center, National Cancer Center, Japan. He has been involved in basic research on drug delivery systems (DDS) and in the clinical development of drugs used in DDS.

After graduating from Kumamoto University Medical School in 1981, he received training in the Department of Surgery at the same medical school. He then moved to the Department of Microbiology at Kumamoto University (Prof. H. Maeda). There, he and Prof. Maeda discovered the enhanced permeability and retention (EPR) effect. He then joined Dr. D. Tarin's lab at the Nuffield Department of Pathology, University of Oxford, where he worked on the molecular pathology of cancer. It was during this period that he discovered the abnormal splicing of CD44 mRNA in various cancers and successfully developed a monoclonal antibody against CD44 v2. In 1994, he commenced his career as an oncologist at the National Cancer Center Hospital, Japan. Beginning in 1999, as the Head of the Special Therapy Division, he introduced clinical trials for DDS and conducted translational studies on DDS. In 2002, he was appointed as the Director of the Investigative Treatment Division of the National Cancer Center Hospital East (present laboratory). To date, he has succeeded in developing several mAbs. Among them, he is mainly involved with research on anti-insoluble fibrin mAb, which reacts with fibrin clots in the stroma of solid tumors, particularly invasive tumors such as pancreatic cancer, stomach cancer, and glioblastoma.

Based on his clinical experiences and observed data, he began to question why DDS was not a mainstream strategy for oncological treatment, and he also felt that a divergence in efficacy existed between non-clinical and clinical data. Careful and long-term basic and translational studies have led him to believe that drug penetration within the tumor tissues should be considered, in addition to the EPR effect, during the treatment of clinical cancers. In this context, he proposed the concept of cancer stromal targeting (CAST) therapy for the first time in 2011. The use of anti-insoluble fibrin mAb conjugated to an anticancer agent (ACA) is one example of CAST therapy.

Mariarosa Mazza

Mariarosa is Research Fellow in Nanomedicine within the Nanomedicine Lab, Faculty of Bology, Medicine and Health, University of Manchester. Mariarosa obtained her first degree (with distinction) in Pharmaceutical Chemistry and Technology from the University of Calabria, Italy. She is a registered pharmacist with the General Pharmaceutical Council and an Associate Member of the Royal Society of Chemistry. She first joined the UCL School of Pharmacy in 2006 as an Erasmus student, working on the micromanipulation of liposome membranes and tether formation at the Centre for Drug Delivery Research. She then started her PhD studies at the UCL School of Pharmacy in October 2007 and completed her thesis on 'Peptide Nanofibres for Drug Delivery' in November 2011. Her thesis work was endorsed with a patent application. Mariarosa joined the Nanomedicine Lab in February 2012 working on the development of nanofibre and 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) o the development and pharmacological evaluation of these novel nanoparticles for applications that range from drug and gene delivery, to biomedical imaging.

Kelley McCabe

Kelley McCabe, is currently an applications engineer at Microfluidics International Corporation, an IDEX Material Processing Group. She is responsible for performing proof of concept, process optimization and scale up tests. Kelley McCabe received her Bachelors of Science degree in Chemical Engineering and Physics in May 2016 from Syracuse University.

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.

Heinz Mehlhorn

Prof. Dr. Heinz Mehlhorn, Düsseldorf, Germany. He has investigated the transmission pathways of human and animal parasites for over 40 years at German and international universities and he and his university spin-off company Alpha-Biocare have developed many antiparasitic medical products based on more than 20 patents – several in cooperation with big international companies. He has published 25 books, more than 250 original papers, and has served as Managing Editor of the journal Parasitology Research since 1981. A long list of renown international scientists did their PhD work in his laboratory and remain still today interconnected as a large group of lovers of parasitology.

Dora Mehn

Ph.D.

Dr. Mehn is scientific research project assistant at the Consumer Products Safety Unit of Health, Consumers and Reference Materials Directorate, Joint Research Centre, Ispra, Italy. She works on characterization of nanoparticles including nanomedicine applications using various techniques like FFF systems, DLS, MALS, CPS, AUC, HPLC, Raman spectroscopy. Dr. Mehn holds an MSc as a teacher of Chemistry and Biology and a PhD in Environmental Chemistry from the University of Szeged, Hungary. During her PhD she synthesised and characterised mesoporous silica materials. Later she worked on nanoparticle synthesis and characterisation at the University of Szeged, Hungary and for one year at the FUNDP in Namur, Belgium. She was researcher at Solvo Biotechnology in Szeged, later leading the Fee for Service Screening Laboratory of the company and performing *in vitro* drug - transporter protein interaction studies. She spent three years as grantholder at the Joint Research Centre, Ispra, Italy working on microfabrication, micropatterned cell cultures and stem cell based neuro-toxicity assays. She was employed at the Laboratory of Nanomedicine and Clinical Biophotonics (Labion) of Fondazione Don Carlo Gnocchi, Milano, Italy, developing Surface Enhanced Raman Spectroscopy based bioassays for leukaemia marker gene detection. Since 2014, she works again for the European Commission, JRC, Ispra, Italy.

RECENT PUBLICATIONS:

- Niosomal approach to brain delivery: Development, characterization and *in vitro* toxicological studies; C. Ingallina, F. Rinaldi, A. Bogni, J. Ponti, D. Passeri, M. Reggente, M. Rossi, A. Kinsner-Ovaskainen, D. Mehn, F. Rossi, B. Botta, M. Carafa, C. Marianecci; (2016) Int J Pharm. 511 (2) 969-82
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- Polymer Nanopillar–Gold Arrays as Surface-Enhanced Raman Spectroscopy Substrate for the Simultaneous Detection of Multiple Genes, S. Picciolini, D. Mehn, C. Morasso, R. Vanna, M. Bedoni, P. Pellacani, G. Marchesini, A. Valsesia, D. Prosperi, C. Tresoldi, F. Ciceri, F. Gramatica, (2014) ACS Nano, 8 (10), 10496–10506
- A P-gp vesicular transport inhibition assay - optimization and validation for drug-drug interaction testing; K. Herédi-Szabó, J.E.Palm, T.B. Andersson, Á. Pál, D. Méhn, Z. Fekete, E. Beéry, K.T. Jakab, M. Jani, P. Krajcsi, (2013) Eur J Pharm Sci. 49 (4) 773-81.

Sara Menina

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I graduated in June 2009 as a pharmacist from the Pharmacy Department at Batna University in Algeria and worked as a pharmacist until January 2013. In June 2013, I joined the Laboratory of Professor Claus-Michael Lehr at the Department of Biopharmacy and Pharmaceutical Technology, Saarland University as a Diploma student. The aim of my Diploma thesis was to formulate gentamicin-loaded liposomes and functionalize them with invasin to target β1 integrin-expressing cells of the gastrointestinal tract to treat intracellular bacteria. Those nanocarriers proved to be efficient against intracellular bacteria and able to reduce the infection load. The formulation used in my Diploma thesis was patented under the number "WO 2016/024008 A1".

From December 2014 until today, I am conducting my PhD in Helmholtz Institute for Pharmaceutical Research Saarland, Drug Delivery Department. My project focuses on the incorporating of anti-infective agents into lipid-based nanocarrier systems, which are surface functionalized with invasive moieties, in an effort to increase cellular internalization of the drug cargo, via oral administration. Beside my PhD project, I am also working on the development of suitable nanocarrier system to facilitate a targeted and enhanced glycolipid uptake by macrophages for the treatment of Leishmaniosis and Tuberculosis, as a part of a project funded by the German Center for Infection Research in collaboration with Bernhard Nocht Institute for Tropical Medicine.

During my prior studies, I took the opportunity to cultivate my interests in nanotechnology and drug delivery which allowed me to learn many techniques especially nanoparticles preparation and characterization, High-Performance Liquid Chromatography and Mass Spectrometry for drug quantification, Scanning electron microscopy and Confocal laser scanning microscopy for visualization, cell and bacterial cultures as well as molecular biology techniques.

I am a highly motivated person and a team player as well. I am flexible and fast learning. I have the ability to handle a variety of tasks effectively and solve problems. I enjoy my free time traveling, playing different types of sports such as badminton and basketball and I also enjoy reading books.

PUBLICATIONS

- Menina, S et al. RSV Adv. 2016, 6, 41622-41629.
- Labouta, H.I et al. J. Contr. Rel. 2015, 220, 414-424.

Olivia Merkel

Olivia Merkel, is a Professor of Drug Delivery in the Department of Pharmacy at LMU Munich in Germany. From 2011 until 2016 she was an Assistant Professor of Pharmaceutics and an Associate Faculty Member of Oncology at Wayne State University, Detroit, MI, USA, where she was also a Scientific Member of the Molecular Therapeutics Program and Faculty in the Cancer Biology Graduate Program at Barbara Ann Karmanos Cancer Institute in Detroit, MI. She became a Registered Pharmacist in 2005. In 2006, she received a MS in Pharmaceutics from Martin-Luther-Universität Halle-Wittenberg, and a PhD in Pharmaceutics from Philipps-Universität Marburg, Germany, in 2009. She received several awards, including an ERC Starting Grant, the Galenus Foundation Technology Award, the Young Investigator Award by the College of Pharmacy at Wayne State, the Young Pharmaceutical Investigator Award granted by the European Federation for Pharmaceutical Science, an invitation to the Lindau Nobel Laureates Meeting, the Carl-Wilhelm-Scheele-Award by the German Pharmaceutical Society (DPhG) and the award for the best PhD thesis at Philipps-Universität Marburg. Currently Prof. Merkel's research focuses on targeted siRNA delivery in cancer and inflammatory diseases.

Sarah L.J. Michel

Sarah L.J. Michel received her B.A. degree in Chemistry from Cornell University in 1995. She subsequently completed her M.S. and PhD in Inorganic Chemistry at Northwestern University and then undertook postdoctoral training in Biophysics and Biophysical Chemistry at the John Hopkins University School of Medicine where she was NRSA National Institutes of Health Postdoctoral Fellow. Dr. Michel began her independent career in 2004 in the Department of Pharmaceutical Sciences at the University of Maryland Baltimore School of Pharmacy. She rose through the ranks, receiving tenure and promotion to Associate Professor in 2010 and promotion to Full Professor in 2016. She was appointed the Graduate Program Director in 2013. Dr. Michel's research is focused on the area of metal ions in biology and medicine. In one area, she has identified new zinc proteins involved in inflammation and neuronal development, as well as zinc and iron proteins that are targeted by the human influenza virus. In a second area, Dr. Michel is developing novel bioanalaytical assays based upon Inductively Coupled Plasma Mass Spectrometry to measure metal ion distribution in biological samples. Her current efforts in this area are focused on using a LC-ICP-MS approach to understand how iron gluconate nanoparticle drug products release iron in blood plasma as part of an FDA funded clinical trial with colleagues at Maryland.

Moien Moghimi

(moien.moghimi@sund.ku.dk)

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

Moein's research activities are focused on pharmaceutical nanoscience, nanomedicine and nanosafety, and he has been the recipient of numerous awards in recognition of his work in these areas. He has over 200 peer-reviewed publications/patents in nanomedicine and nanosafety to his credit. He currently functions as the Editor-in-Chief of Current Bionanotechnology (Bentham) and features on the editorial board of several journals to include Advanced Drug Delivery Reviews, Nanomedicine-UK (Future Medicine), Journal of Controlled Release (Elsevier) and Scientific Report (Springer-Nature).

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

Stefan Mühlebach

Stefan Mühlebach, PhD, Prof. is a pharmacist by training. He chairs since 2010 the Non-Biological Complex Drugs (NBCDs) Working Group c/o Lygature, a non for profit, private-public partnership in the Netherlands (http://lygature.org/nbcd). He is professor for pharmacology and hospital pharmacy at the University of Basel in Switzerland, a member of the Clinical Pharmacy & Epidemiology Unit in the Dept. of Pharmaceutical Sciences (https://pharma.unibas.ch/home/) and a member of the Medical Faculty. His research and teaching activities are within pharmacology, clinical nutrition, hospital pharmacy, and regulatory sciences. He authored more than 100 peer-reviewed papers, over 90 indexed in Pubmed/EMBASE, and several book chapters. He is a member of several (inter-)national professional associations and a board member of the Swiss Acad-

emy of Pharmaceutical Sciences (http://www.saphw.ch/en). From 1980-2005 he served as a Chief Hospital Pharmacist and head of department in Switzerland. From 2005 to 2008 he worked as Head of the Pharmacopoeia at Swissmedic, the Swiss Agency for Therapeutic Products, and of the Swiss Delegation at EDQM in Strasbourg. In 2008 he joined Vifor Pharma Switzerland as Chief Scientific Officer and holds actually a role as Regulatory Science Lead Non Biological Complex Drugs at the Vifor Pharma Headquarter in Switzerland (http://www.viforpharma.ch/en/index.php).

Dev Mukhopadhyay

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

Dev Mukhopadhyay: Professor of Biochemistry and Molecular Biology, Mayo Clinic, Mayo Clinic College of Medicine and Sciences, has a joint appointment with the Department of Physiology and Biomedical Engineering and Florida Department of Health Cancer Research Chair. He has a broad background in tumor microenvironment, with specific training and expertise in key research areas including Cancer, Cardiovascular Diseases, and Diabetes. As a postdoctoral fellow, later as an independent investigator followed by as an Associate Professor at Harvard Medical School, Boston, he carried out angiogenesis and tumor microenvironment related research. After moving to Mayo Clinic as a Professor and also as Directors of both Tumor Microenvironment program and Translational Nanomedicine Center, he has been supervising additional research areas including stellate cell biology, new drug delivery systems and trained more than 50 young investigators and several of them are now independent faculties in different institutions throughout the world. He has been serving as reviewer in federal and also international funding agencies and participating as editorial board members of several distinguish journals. He has published more than 190 peer-reviewed publications including Nature, Nature Medicine, Cancer Cell, Cancer Research, Circulation Research, Journal of Clinical Investigation and several other reputed journals. He is also involved several translational research and clinical trials in both cancer and cardiovascular diseases.

Bert Müller

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

Sesha Neervannen

PhD
Sr. Vice President, Pharmaceutical Development

Dr. Sesha Neervannan is currently Sr. Vice President of Pharmaceutical Development at Allergan. In his current role, he is responsible for Biologics and Small Molecule Drug Product Development from Discovery to Commercialization. Prior to joining Allergan in 2007, Sesha held senior positions at Bristol-Myers Squibb where he helped establish Topical Dermal and Transdermal Delivery groups and advanced various internal molecules to clinical dev via transdermal delivery approach, when oral route was not feasible. He then took on an exciting leadership role at Amgen, where, as part of Pharmaceutics R&D organization, he helped to start, build and grow Small Molecule Pharmaceutics functions as well as contributed to several Biologics products. At Allergan, he oversaw and contributed to several global product filings and approvals in the last few years **(Ozurdex™, Latisse™, Acuvail™, Lumigan 0.01%™, Zymaxid™, Trivaris™, Restasis® Multi-Dose, Rhofade™, Aczone® Gel 7.5%, etc.)** as well as development, validation and launch of several OTC artificial tears products.
Sesha has several research publications and patents and is a well recognized invited speaker at several national and international conferences. He serves on several Advisory Boards including the Scientific Advisory Board for CHDI, a non-profit organization, Customer Advisory Board for NineSigma, an open innovation company, Board member of International Consortium of Innovation and Quality (IQ), Steering Committee member for Non-Biologics Complex Drugs (NBCD) Working Group, and the Editorial Advisory Boards for Expert Opinion on Drug Delivery and Life Sciences Leader, as well as Scientific Advisor to Editors of JPharmSci.
He received his Ph.D. in Pharmaceutical Chemistry at The University of Kansas.

André Nel

Distinguished Professor of Medicine,
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CAREER HISTORY:

André Nel obtained his medical (M.B.,Ch.B), Internal Medicine board specialization and Doctorate degrees at Stellenbosch University in Cape Town, South Africa. He he is a practicing Immunologist at UCLA and received peer selection as one of the Best Doctors of America for 20 years. His interest in allergic airway disease and air pollution led to the establishment of major air pollution centers in Los Angeles to study the effect of particulate matter (including ultrafine particles) in the causation of asthma. Under Dr. Nel's direction, UCLA has successfully established large federally funded nanotechnology research programs in the California Nanosystems Institute, including the UC Center for the Environmental Implications of Nanotechnology (UC CEIN), as well as the UCLA Pancreas Nano Cancer Center. Professor Nel is a recipient of the Harry Truman Award and received the 2013 California Governor's Economic Leadership Award (on behalf of CEIN). He plays an active role in the governance, ethics, regulation, safe implementation and sustainable nanotechnology development in the US and internationally, and served as panel member for nanotechnology on President Obama's Counsil of Advisors (PCAST). Dr Nel and his collaborators have filed a large number of U.S. patents and he is actively engaged in entrepreneurial and commercial startup activities as Associate Director of Research at the CNSI. He is Associate Editor for ACS Nano.

SUMMARY OF PRESENT WORK:

Dr. Nel's current research focuses on nano cancer and nanosafety. His interest in nanocancer is focused around pancreatic cancer, with a view to develop an engineered approach by targeting the cancer stroma, accessing stromal vascular mechanisms and generating a systemic immune response to the cancer. He is the co-inventor of the silicasome platform, comprised of lipid bilayer coated mesoporous silica nanoparticles, which can be adapted for high drug loading of a large number of chemotherapeutics, including synergistic drug delivery, and the successful integration of chemo- with immunotherapy for the cancer treatment. His research in nanosafety is focused on developing high throughput screening approaches to assess nanomaterial behavior at the nano/bio interface for the purposes of toxicological profiling of broad and specific nanomaterial categories, tiered risk assessment approaches, safer by design strategies and utility of non-vertebrate, alternative test strategies for regulatory decision-making.

MAJOR PUBLICATIONS:

1. Nel, A.E. Atmosphere. Air pollution-related illness: Biomolecular effects of particles. Science 308: 804 (2005)
2. Nel, A.E., Xia, T., Maëdler, L., and Li, N. Toxic potential of materials at the nanolevel? Science. 311:622-627 (2006)
3. Nel, A.E., Maedler, L., Velegol, D., Xia, T., Hoek, E.M., Somasundaran, P., Klaessig, F., Castranova, V., and Thompson, M. Understanding biophysicochemical interactions at the nano–bio interface. Nature Materials. 8:543-57 (2009).
4. Liong, M., Lu, J., Kovochich, M., Xia, T., Ruehm, S.G., Nel, A.E., Tamanoi, F., and Zink, J.I. Multifunctional Inorganic Nanoparticles for Imaging, Targeting, and Drug Delivery. ACS Nano. 2(5): 889-896 (2008).
5. Nel, A.E. and Malloy, T.F. Alternative test strategies for the safety assessment of new chemical substances. Science. (2017) In Press.

Inge Nelissen

Inge Nelissen joined VITO (Flemish Institute for Technological Research, Mol, Belgium) in 2004 where she started as a researcher in the development and validation of *in vitro* cell-based assays for chemical and nanomaterials safety assessment. In 2011 she became project manager leading the research programme on nano-bio interactions in the Health Department, with a focus on technological solutions for nanodiagnostics. She is partner in several European projects in the field of nanosafety and nanodiagnostics, and expert member of the OECD Working Party on Manufactured Nanomaterials, EU NanoSafetyCluster, European Technology Platform on Nanomedicine, and International Society of Extracellular Vesicles. She is co-author of more than 45 peer-reviewed international scientific papers. Inge Nelissen is trained as Bio-engineer in Biotechnology (1998) and obtained her PhD degree in Medical Sciences in 2003 from the Catholic University of Leuven (Belgium).

PUBLICATIONS

Deville S. et al. 2017. Transient loading of CD34+ hematopoietic progenitor cells with polystyrene nanoparticles. Int J Nanomed 12: 459-472.

Deville S. et al. 2016. Interaction of gold nanoparticles and nickel(II) sulfate affects dendritic cell maturation. Nanotoxicology 10(10): 1395-1403.

Deville S. et al. 2015. Intracellular dynamics and fate of polystyrene nanoparticles in A549 Lung epithelial cells monitored by image (cross-)correlation spectroscopy and single particle tracking. Biochim Biophys Acta 1853: 2411-9.

Guangjun Nie

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

His most recent research activities generated a group of interdisciplinary works in nanobiology, nanomedicine and blood physiology fields, including over 108 papers published in Acc Chem Res, Adv Mater, Angew Chem, Adv Funct Mater, Blood, Biomaterials, Br J Haematol, JACS, JBC, Molecular Cancer Therapeutics and Small. He has filled over 34 patents on novel nanomedicines and 16 of them have been granted, with two patents on antitumor drug development have been transferred to a biotechnology firm for pre-clinical investigation. He is an experienced supervisor of postgraduate students and collaborates widely both within China and internationally. Now he is leading a multidisciplinary team with over 30 people working toward better and safer antitumor nanomedicine for pancreatic cancer and liver cancer. He is also the Affiliated Professor of Northeast University, Shenyang, China and East China University of Science and Technology, Shanghai. He is also an Affiliated Senior Member of Houston Methodist Research Institute, Houston, US.

Eugénia Nogueira

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Biochemistry, PhD

Eugénia Nogueira born in 1985 in Barcelos, Portugal. She has the Degree in Biochemistry in 2008 and Master in 2009, both from University of Porto (Portugal). She received her PhD in Molecular and Environment Biology from University of Minho (Portugal) in 2015. She participated in NANOFOL FP7 European project, developing folate-targeted liposomes to activated macrophages for chronic inflammatory diseases. Actually is researcher in FOLSMART H2020 European project, aiming bring to phase I clinical trials the folate-targeted liposomes to rheumatoid arthritis therapy. She has published over 12 papers in peer reviewed journals, in several areas such as nanoparticles and rheumatoid arthritis, 2 patents and a chapter of book.

Andrew Owen

PhD FRSB FBPhS

Andrew Owen is Professor of Molecular and Clinical Pharmacology at the University of Liverpool, UK. He is also affiliated to the MRC Centre for Drug Safety Science and the Wolfson Centre for Personalised Medicine. He is Chair of the British Society for Nanomedicine, a fellow of the Royal Society of Biology, a fellow of the British Pharmacological Society and a member of the steering committee for the APS Nanomedicines Focus Group. His clinical and basic research focuses on understanding mechanisms that underpin inter-patient variability in pharmacokinetics and pharmacodynamics. In recent years a major emphasis has been to employ knowledge of these mechanisms to accelerate the translation of nanomedicine candidates to clinical applications. Work is supported by the US Agency for International Development, US National Institutes for Health, UK Medical Research Council, European Commission, and UK Engineering and Physical Sciences Research Council. Professor Owen also has strong links with the Clinton Health Access Initiative and Medicine Patent Pool. He has published over 160 original publications, is co-inventor of patents relating to the application of nanotechnology to drug delivery and a co-founder of Tandem Nano Ltd and PKTK. He is a Founder and Editor in Chief for the Journal of Interdisciplinary Nanomedicine.

Marisa Papaluca Amati

Internal Medicine specialist, Marisa joined the EMA in late 1994 and occupied scientific and managerial positions in the EMA Unit for Human Medicines Development and Evaluation. Deputy Head of Quality up to 2002 and of the Efficacy and Safety Sectors up to 2009, Marisa is currently Head of the Scientific Support office providing scientific support to the Agency core activities in transversal and multidisciplinary areas such as clinical trials statistical methodology, raw data analysis, non-clinical drug development, pharmacogenomics and nanotechnology. The office is also in charge of the EMA the Innovation Task Force, reference group at EU and international level for innovative pharmaceuticals developments with current increasing activities on novel clinical trials designs, genomic biomarkers, combined products, synthetic biology and nanomedicines. Marisa has been appointed in March 2015 Senior Scientific Advisor attached to the Division for R&D support. Since September 2016 she moved as Senior Scientific Advisor in to the Scientific Committees Regulatory Science Strategy Division. The two main tasks of the Division are the coordination of the scientific committees board and the establishment and running of the regulatory science observatory.

Wolfgang Parak

Since 2007: Full Professor (chair) for Experimental Physics at the Philipps-University of Marburg, Germany
Since 2013: in addition head of the Biofunctional Nanomaterials Unit at CIC biomaGUNE, San Sebastian, Spain
2009: "Nanoscience" – award 2008 from the Association of Nanotechnology-Centres Germany (AGenNT)
Since 2010: Associate Editor for ACS Nano from the American Chemical Society
2011: ranked #59 in Top Materials Scientists of the past decade by Essential Science Indicators (http://science.thomsonreuters.com/products/esi/)
2012: Awarded Chinese Academy of Sciences Visiting Professorship for Senior International Scientists
2014: highly cited in the category materials sciences (http://highlycited.com/)
2014: listed in "The World's Most Influential Scientific Minds: 2014" (http://www.sciencewatch.com)
2015: highly cited in the category materials sciences (http://highlycited.com/)
2015: listed in "The World's Most Influential Scientific Minds: 2015" (http://www.sciencewatch.com)
2017: Visiting Professor Australian Research Council Centre of Excellence in Convergent Bio-Nano Science & Technology (CBNS) present h-index: 63 WebOfScience; 73 GoogleScholar
ResearcherID: M-3998-2014; ORCID: orcid.org/0000-0003-1672-6650
Wolfgang Parak is/was Associate Editor of ACS Nano **(2010-)**, and Nanotoxicology **(2009-2010)**. He is/was in the advisory board of the following journals: Chemistry of Materials **(2015-)**, Angewandte Chemie **(2014-)**, Theragnostics **(2014-)**, Nanomaterials **(2014-)**, ChemNanoMat **(2014-)**, Colloids and Interface Science Communications **(2014-)**, Particle & Particle Systems Characterization **(2013-)**, Nanotoxicology **(2010-)**, Journal of Colloid and Interface Science **(2009-)**, The All Results Journal **(2008-)**, Journal of Nanobiotechnology **(2011-)**, Recent Patents on Nanotechnology **(2007-2010)**, Journal of Nanobiosensors in Disease Diagnosis **(2011-2013)**. Wolfgang Parak is/was member of the following steering comiittees: National Research Programme NRP 64 "Opportunities and Risks of Nanomaterials" of the Swiss National Foundation **(2010-, Switzerland)**, Andalucian Initiative for Advanced Therapies **(IATA, 2012-, Spain)**, Insitute for medical/pharmaceutical exams (IMPP, Institut für medizinische und pharmazeutische Prüfungsfrage, **2012-**, Germany), Minerva Center for Bio-hybrid Complex Systems at the Hebrew University Jerusalem (Chairperson of the Center's Beirat, **2013-**, Israel), CIBER-BBN (**2013-**, Spain), committee of external evaluators of the Italian Institute of Technology (IIT, **2013-**, Italy). Wolfgang Parak is co-organizer of the following conference series: "Colloidal Nanoparticles for Biomedical Applications" of the SPIE Photonics West meeting **(2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016)**, NANAX **(2003, 2008, 2012, 2016)**, Amercial Chemical Society ACS Spring/Fall meeting **(s2014, s2015, f2015, s2016, f2016)**.

Gianfranco Pasut

Gianfranco Pasut is Associate Professor of "Pharmaceutical Technology" at the Pharmaceutical and Pharmacological Sciences Department, University of Padova. He received a M.S. in Pharmaceutical Chemistry and Technology in 1999 and the PhD degree in Pharmaceutical Sciences in 2003 from the University of Padova. He is an expert in polymer conjugation and drug delivery, in particular in the application of PEGylation to biotech drugs. His main research interests are in chemical and enzymatic method of polymer conjugation to bioactive molecules, targeted polymer-anticancer drug conjugates, stealth liposomes, ADC. In the field of drug delivery of small drugs, he investigated targeted conjugates and conjugates for combination therapy for the treatment of cancer.
He has published about 80 articles, 12 book chapters and he is the inventor of 8 patents.

Anil Patri

Dr. Anil K. Patri serves as the Director of Nanocore, National Center for Toxicological Research & Chairs the Nanotechnology Task Force in the Office of the Commissioner at the U.S. Food and Drug Administration (FDA). His laboratory is very active in Nanotechnology research relevant to FDA regulated products. He coordinates research, training, funding, and standards development activities for the agency. Dr. Patri also serves on the National Nanotechnology Initiative (NNI) as a member from FDA in the Nanoscale Science, Engineering and Technology (NSET) Subcommittee and the Nanotechnology Environmental and Health Implications (NEHI) working group and serves as the Co-chair of the US-EU Communities of Research on Characterization. He is a member of ISO TC229 and ASTM E56 and engages stakeholders on standards development activities relevant to the agency. He serves on the International Pharmaceutical Regulators Forum Nano working group.
Prior to joining FDA in August 2014, Dr. Patri served as the Deputy Director of the Nanotechnology Characterization laboratory (NCL) at the Frederick National Laboratory for Cancer Research. In a decade long tenure at NCL, he assisted collaborators from industry and academia towards clinical translation of nanomedicines, many currently in clinical trials. He led a collaborative multi-disciplinary team of scientists at NCL and oversaw 85 projects through preclinical assessment that included proposal review and guidance, material characterization, *in vitro* and *in vivo* studies on different nanomaterial platforms intended for drug delivery, gene delivery and imaging. From 2006-2014, he served as a guest scientist at NIST and helped co-develop the first Nanosized gold reference material standards. Dr. Patri served at the University of Michigan Medical School, Center for Biologic Nanotechnology, and developed targeted drug delivery and imaging agents until 2005. He is a co-author of over 65 publications, serves on editorial boards of Molecular Pharmaceutics, Nanomedicine & Nanobiotechnology, and organized many meetings and conferences.
Dr. Patri earned a Ph.D. degree in Chemistry from the University of South Florida followed by a post-doctoral training at the University of Michigan. He worked at Astra Zeneca and as a lecturer in Chemistry prior to graduate school.

Dan Peer

Dr. Peer is a principal investigator and Professor who leads an NIH- and ERC-funded laboratory at Tel Aviv University, Israel. Prof Peer completed his education in biochemistry and biophysics from Tel Aviv University, Israel (TAU) with internships both in Cambridge University, UK (Cesar Milstein Lab) and MIT, Cambridge, MA, USA (Robert Langer lab). From 2005 to 2008, he worked at Harvard Medical School (M. Shimaoka and T. Springer). He then joined the Department of Cell Research and Immunology, in the Faculty of life sciences at TAU to establish the laboratory of Precision Nanomedicine. Prof. Peer's research was one of the first to demonstrate systemic delivery of RNA interference (RNAi) molecules using targeted nanocarriers to the immune system, and the first to utilize RNAi for *in vivo* validation of new drug targets within the immune system. Prof. Peer's work was pioneering also in the field of gut inflammation and he was involved in the development of antibodies that block lymphocytes homing into the gut during inflammatory bowel disease. He is the Director of the Leona M. and Harry B. Helmsley Nanotechnology Research Fund and the Director of the Focal Technology Area on Nanomedicines for Personalized Theranostics. He has received numerous awards; among them, he was recognized by the Kenneth Rainin Foundation by their Innovator (2010) and Breakthrough (2011 – 2013) Awards for his pioneering work in inflammatory bowel diseases and by the AAAS for this development of the Gagomers platform technology for targeted drug delivery for immuno-oncology.
Dr. Peer is the Chair of Tel Aviv University Cancer Biology Research Center, which includes 17 affiliated hospitals surrounded Tel Aviv and he foresee the science and activity of preclinical and clinical work in this Center.
Dr. Peer holds around 50 pending and granted patents. He co-founded three companies based on his work aiming to bring the area of nanomedicine into clinical practice.
Dr. Peer is a member of the Israel Young Academy of Science.

Jai Prakash

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

FIVE RECENT PUBLICATIONS:

1. Bansal R, Nagorniewicz B, Prakash J. (2016) Clinical advancements in targeted therapies against liver fibrosis. Mediators of Inflammation doi:10.1155/2016/7629724.
2. Van der Berg P*, Bansal R*, Daudi K, Steenbergen W, Prakash J. (2016) Preclinical detection of liver fibrosis using dual modality photoacoustic/ultrasound imaging. Biomedical Optics Express

14;7(12):5081-5091.
3. Priwitaningrum D, Blonde JP, van Baarlen J, Hennink WE, Storm G, Le Gac S, Prakash J. (2016) Tumor Stroma-containing 3D Spheroid Arrays: A Tool to Study Nanoparticle Penetration. J Control Release. 28;244(Pt B):257-268
4. Binnemars-Postma KA, ten Hoppen H, Storm G, Prakash J. (2016) Differential uptake of nanoparticles by human polarized macrophages. Nanomedicine (Lond);11(22):2889-2902.
5. Kuninty PR, Bojmar L, Tjomsland V, Larsson M, Storm G, Ostman A, Sandström P, and Prakash J. (2016) MicroRNA-199a and -214 as potential therapeutic targets in pancreatic stellate cells in pancreatic tumor. Oncotarget Feb 24. doi: 10.18632/oncotarg

Adriele Prina-Mello

Ussher Assistant Professor in Translational Nanomedicine
LBCAM Director
AMBER and CRANN Principal Investigator
Chair of Characterization and Toxicology group at ETP Nanomedicine

Dr. Prina-Mello scientific interests are focused on advanced translation research in NanoMedicine (*in vitro*/*in vivo* diagnostic, imaging and therapeutics), microfluidic, biomedical devices and tissue engineering applications of nanotechnology and nanomaterials. The continuous exploration of the dynamic interaction between nano-developed-products and biologically relevant models constitute the basic ground for Dr Prina-Mello's multidisciplinary scientific work within and outside The Trinity Translational Medicine Institute (TTMI), the AMBER (Advanced Materials and BioEngineering Research as Science Foundation Ireland funded centre), CRANN nanoscience institute and the School of Medicine.Dr. Prina-Mello is part of the Executive Board of the European Technology Platform of Nanomedicine, as Chair of the Characterization and Toxicology working group. At European level, he has been involved in several EC-H2020 and FP7 projects among these EU-NCL, NoCanTher, AMCARE, MULTIFUN, NAMDIATREAM and others.Among these, he is the principal investigator behind the TCD participation to the European Nanomedicine Characterization Laboratory infrastructure project (H2020-Infra-1). Dr Prina-Mello is also a lecturer in Nanomedicine and Translational Nanomedicine at the TTMI.

PUBLICATIONS AND PROJECT DETAILS AVAILABLE AT:
Google Scholars – search Prina-Mello A
Research Gate – search Prina-Mello A
Website info: http://www.tcd.ie/IMM/lbcam/
Website info: http://ambercentre.ie/people/dr-adriele-prina-mello

Francesca Re

School of Medicine and Surgery
Laboratory of Biochemistry and Nanomedicine
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Research Associate – Ricercatore a Tempo determinato, lettera (a) Macrosettore O5/E1; SSD BIO/10 Biochimica

Francesca Re graduated in Medical Biotechnology from the University of Milano-Bicocca (UNIMIB) in 2005 and in 2008 completed a PhD degree in Neuroscience at UNIMIB with research conducted at the Laboratory of Biochemistry, School of Medicine and Surgery (Monza) under the supervision of Prof. Massimo Masserini (UNIMIB). From 2009, she worked with Prof. Massimo Masserini, a world-leading laboratory in Neuro-Nanomedicine, for a 6-year post-doctoral period. Starting 2015, she is creating her independent line of research at the UNIMIB. During her scientific career, F.R. has published 39 articles in top international peer-reviewed journals (including high IF journals, i.e. ACSNano, IF 12.88; Biomaterials, IF 8.55; NanoResearch, IF 7.01 and J. Neurosci., IF 6.34) and she has currently 3 papers in revision process; Her h-index is 14 and has 549 citations (Scopus). From her 4 PhD papers, she appears as 1st author position in 2 articles revealing that the pH, the lipid composition and the membrane curvature affect the protein structure and enzymatic activity of GPI-anchored proteins. In addition, F.R. made important advances in the field of the search for ligands to target and overcome the blood-brain barrier (BBB) in order to design nanoparticles carrying drugs for CNS disorders, i.e. Alzheimer's disease, during her post-doctoral stay at the UNIMIB, as part of a research project funded by the European Community (NAD "Nanoparticles for therapy and diagnosis of Alzheimer's disease" from 2008 to 2013 FP7-NMP-2007-LARGE-1). This research resulted in 18 articles published in collaboration with different EU partners. Of note, F.R. had 3 career breaks between 2009 and 2016 due to maternity leave and was absent from research during approximately 15 months. Throughout her career, F.R. has been mainly focused in understanding the mechanisms to cross the BBB by macromolecules and nanoparticles and, most recently, to study new strategies to boost the amyloid-β peptide clearance from the brain across the BBB, as potential therapy for Alzheimer's disease. She has also acquired skills is the study of nanoparticles protein corona composition and its implication in the BBB crossing. F.R. has been able to attract funding from prestigious European entities, being a recipient of a H2020 Project funded by EU Joint Programme (JPND) as a project coordinator in 2015, where will be developed a novel *in vitro* model of Alzheimer's disease-like BBB; She has been a recipient of a Project founded by the European Center of Nanomedicine (CEN) as PI of the UNIMIB Unit in 2014, with the aim to design nanoparticles for human glioblastoma imaging and treatment. In 2013, she was a coordinator of a Privately funded Project (Fondazione Banca del Monte di Lombardia) with the aim to study the toxicity of nanoparticles designed for the therapy and diagnosis of neurodegenerative diseases. In 2013, she was awarded of "63rd Lindau Nobel Laureate Meeting – Chemistry". During her trajectory, F.R. has been able to establish close collaborations with outstanding researchers from very diverse scientific backgrounds. She has been invited to give Ad Hoc seminars; her research work was presented in major conferences and contributed for attracting competitive funding from international agencies. In addition, F.R. has knowledge in drug discovery as she is co-inventor in 2 families of international patents and is Chief Operating Officer (COO)/Head of R&D of AmypoPharma S.r.l. (spin-off of UNIMIB). She has also teaching experience as lecturer in Biochemistry and Nanomedicine of graduate students and as tutor for students degree at UNIMIB. In summary, F.R. track record demonstrates an extended research experience and technical maturity (also proven by #5 articles where she appears as last author position) that allows her to conduct future research with leadership and independent-thinking abilities (also proven by #5 articles where she appears without her PhD supervisor).

Andreas Reisch

Maître de conferences
(Assistant professor) in biophysics
Université de Strasbourg –
Faculté de Pharmacie
Laboratoire de Biophotonique et Pharmacologie (UMR 7213)
Equipe Nanochimie et Bioimagerie – Nanochemistry and Bioimaging group
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My research focuses on the design of functional polymeric materials for biomedical applications. I am combining polymer synthesis

and physical chemistry to assemble materials with precisely defined organization, functionality, and responsiveness. A major aim of my work is to control the interactions between polymeric materials and biological systems. Most recently I am working on ultrabright fluorescent polymer nanoparticles and their use in bioimaging.1-3 In this context I coordinate the French young researcher grant (ANR JC/JC) "supertrack" since end of 2016.
After studies of chemistry with specialization in polymer chemistry and physical-chemistry at the universities of Stuttgart and Dresden and at the École Européenne de Chimie, Polymères et Matériaux (ECPM) in Strasbourg, I completed a Ph.D. thesis on biomimetic polymer surfaces under the supervision of Prof. Schaaf at the Institut Charles Sadron, Strasbourg in 2009. During my postdoctoral studies at the Florida State University, Tallahassee, in the group of Prof. Schlenoff I then developed saloplastic polyelectrolyte complexes.5 A subject I continued working on during a postdoc at the University of Strasbourg alongside the development of mechano-responsive materials,4 before joining the faculty in 2012.

SOME RECENT PUBLICATIONS

- A. Reisch, A. S. Klymchenko Small 2016, 12, 1968.
- A. Reisch, A. Runser, Y. Arntz, Y. Mély, A. S. Klymchenko, ACS Nano 2015, 9, 5104.
- A. Reisch, P. Didier, L. Richert, S. Oncul, Y. Arntz, Y. Mély, A. S. Klymchenko, Nat. Commun. 2014, 5.
- A. Reisch, E. Roger, T. Phoeung, C. Antheaume, C. Orthlieb, F. Boulmedais, P. Lavalle, J. B. Schlenoff, B. Frisch, P. Schaaf, Adv. Mater. 2014, 26, 2547.
- R. F. Shamoun, A. Reisch, J. B. Schlenoff, Adv. Funct. Mater. 2012, 22, 1923.

Daniel Ricklin

Daniel Ricklin studied Pharmaceutical Sciences at the Swiss Federal Institute of Technology (ETH) in Zurich. After graduating in 1999, and gaining experience in bioanalytical techniques during an internship at the Proteome Center in Rostock, Germany, he conducted a Ph.D. thesis in the group of Prof. Beat Ernst at the University of Basel, which sparked his interest in drug design and immune-modulatory therapies. In 2006, Daniel Ricklin moved to the United States to join the laboratory of Prof. John D. Lambris at the University of Pennsylvania in Philadelphia as a postdoctoral researcher. Initially focusing on molecular aspects of the human complement system, a central pillar of innate immunity, in health and disease, his research activities gradually shifted to therapeutic approaches of treating clinical disorders and complications mediated by complement activation. In 2010, Daniel Ricklin was appointed Research Assistant Professor at the Perelman School of Medicine of the University of Pennsylvania and was promoted to Research Associate Professor in 2015. Shortly after, he was offered a professorship at the Department of Pharmaceutical Sciences of the University of Basel, and serves as head of the Molecular Pharmacy unit since January 2017.
The emphasis of Daniel Ricklin's current research is on the therapeutic modulation of host defense pathways, including the complement, innate immune, and coagulation systems. Taking inspiration from microbial immune evasion strategies, and utilizing techniques such as peptide synthesis, protein engineering and medicinal chemistry, he aims to design novel therapeutic concepts with potential use in a broad range of clinical conditions, including immune, inflammatory, age-related, and biomaterial/transplant-induced disorders. Among the therapeutic concepts, in the discovery and development of which he was involved, are soluble peptidic complement inhibitors with broad activity, engineered complement regulators with unique targeting properties toward diseased host cells under complement distress, and protective coatings that prevent complement activation on biomaterial or cell surfaces by recruiting physiological complement regulators. He participated in several biomaterial-related studies to explore the benefits of complement-modulatory treatments during clinical complications arising from hemodialysis filters, surgical implants or drug delivery vehicles.
Daniel Ricklin is author of more than 80 articles and reviews on the topic of complement in health, disease and therapy, edited books/journals and co-organized several conferences on complement therapeutics and complement-biosurface interactions, and is co-inventor of patents describing complement-modulator strategies. In 2012, he has awarded the Young Investigator Award by the International Complement Society and currently serves as treasurer and member of the board of the society.

Bernd Riebesehl

Dr. Bernd Riebesehl is Senior Technical Project Leader in the Pharmaceutical Development Parenteral, Topical, Ophthalmic 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.

Eder Lilia Romero

Eder Lilia Romero was educated at the National University of La Plata, Faculty of Exact Sciences Buenos Aires, Argentina where she obtained her MD in Biochemistry and PhD in Exact Sciences (1997). She performed her posdoctoral research at the Groningen University (Netherlands). She is currently a member of the scientific career at the National Scientific and Technical Research Council (CONICET), full professor of Chemistry at the Science and Technology Department and Director of the Nanomedicine Research Program-2 at the Universidad Nacional de Quilmes (UNQ). She has supervised 5, co-supervised 2 currently supervising 3 doctoral theses, published more than 50 articles in peer reviewed international journals, 6 book chapters, and given nearly 100 national and international conferences and invited lectures. She is responsible for Nanomedicine Schools in Latin America and is an Editorial Board Member of the European Journal of Nanomedicine (De Gruyter) between other peer reviewed journals. Her main research interests are:
New and unique materials from sustainable sources: Nanovesicles and solid lipid nanoparticles made of archaeolipids, from hyperhalophile archaea from Patagonia, Cuyo, center and north west Argentine salt ponds.
Polymeric legos: Design of tecto-dendrimers (commercial dendrimers as units) for delivery of antitumoral agents.
Organic-metal hybrids: Design of lipid nano-vesicles /metal oxide nanoparticles (ZnO) or metal nanoparticles (Au) as source of oxida-

tive stress triggered by UVA light, as vaccine adjuvants for topical route.
Focus on injectable avoidance: Design of nanostructures for drug delivery or adjuvancy to be administered by mucosal route (respiratory, oral) or skin: archaeosomes, pHsensitive archaeosomes, ultradeformable archaeosomes.
Design of anti-inflammatory, anti infective and anti atherosclerotic plaque therapeutic strategies

Barbara Rothen-Rutishauser

Prof. Dr. Barbara Rothen-Rutishauser has received her Ph.D. in 1996 in cell biology at the Swiss Federal Institute of Technology (ETH) in Zurich. From 1996 to 2000 she held a post-doctoral position in Biopharmacy at the Institute of Pharmaceutical Sciences at the ETH where she developed and characterised cell culture models for drug transport studies. In 2000 she joined Prof. Peter Gehr's research group at the Institute of Anatomy, University of Bern, Switzerland. During the period of her research B. Rothen-Rutishauser has become an expert in the field of cell-nanoparticle interactions in the lung, with a special focus on the development of alternative lung models. Since 2011 she is the new chair in BioNanomaterials at the Adolphe Merkle Institute, University of Fribourg, Switzerland, the position is shared equally with Prof. Alke Fink. She has published more than 190 peer-reviewed papers and is an associate editor of the Particle and Fibre Toxicology. In 2013 the Swiss National Science Foundation (SNSF) awarded a National Competence Centre of Research (NCCR) on Bio-Inspired Materials to the UNIFR, which includes Prof. Rothen-Rutishauser, as a module and project leader. Prof. Rothen-Rutishauser also serves as a Faculty Delegate for Women and Young Researchers in this NCCR.

Kumiko Sakai-Kato

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

Anna Salvati

Groningen Research Institute of Pharmacy, University of Groningen, A. Deusinglaan 1, 9713 AV, Groningen, The Netherlands
a.salvati@rug.nl
Dr Anna Salvati (assistant professor) is a physical chemist with background in biology. After graduating in biology and a PhD in physical chemistry in 2007 from Florence University (Italy), in the last 10 years, she has pioneered studies of the interface between nanomaterials and biology, investigating the modifications that nanomaterials encounter when exposed to biological environment and the resulting interactions and behaviours on cells. In her previous employment in the Centre for BioNano Interactions, University College Dublin, Ireland she has also been involved in several European FP7 collaborative projects on nanosafety such as Neuronano, Nanotranskinetics and the Research Infrastructure QualityNano. Her work has resulted in 48 papers with more than 3500 citations (h index Scopus 25).
After being awarded a Rosalind Franklin Fellowship in 2014, she has established her group in Groningen University, Netherlands. She has recently been awarded an ERC Starting Grant (NanoPaths) where she will focus on the mechanisms nanomaterials use to enter cells for nanomedicine applications. She is also partner of the recently awarded Marie Curie COFUND "ALERT", where she will explore the use of nanomaterials for fighting antimicrobial resistance.

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 to the end of 2018). This project involves collaboration between 10 Norwegian research groups covering synthesis of nanoparticles, *in vitro* and *in vivo* biology studies, *in vivo* imaging and clinical studies. In addition, international collaboration is included. The Sandvig group is also involved in an INNO INDIGO granted project, which started April 2016. INNO INDIGO is an innovation-driven initiative for the development and integration of Indian and European research.We also characterize exosomes from prostate cancer cells and prostate cancer patients with the goal of detecting lipid and protein biomarkers. Our research spans all the way from basic to translational medicine, including innovation. We aim at providing a rational basis for diagnosis, treatment and prevention of disease. The group has extensive national and international collaboration.

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

SCIENTIFIC ACTIVITY:

Published more than 300 articles and supervised a large number of Ph.D. students and master students. Sandvig has been invited as plenary speaker at more than 100 international meetings, and the work is heavily cited, Hirsch index is 70.

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.

Ronit Satchi-Fainaro

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Prof. Ronit Satchi-Fainaro (Ph.D.) is Head of the Cancer Angiogenesis & Nanomedicine Laboratory; Chair of the Department of Physiology & Pharmacology, Sackler Faculty of Medicine and serves on the Preclinical Dekanat of the Faculty of Medicine. Prof. Satchi-Fainaro received her Bachelor of Pharmacy from the Hebrew University, Israel (1995) and her Ph.D. from the University of London, UK (1999). She then spent two years as postdoctoral fellow at Harvard University and Children's Hospital Boston working with Judah Folkman on novel angiogenesis-targeted nanomedicines. In 2003, she was appointed Instructor in Surgery at Boston Children's Hospital and Harvard Medical School and continues to have a Visiting Associate Professor position there to date. She joined Tel Aviv University in 2006.

Prof. Satchi-Fainaro is a leader in the field of nanomedicine and angiogenesis (cancer and vascular biology). She has major expertise in tumor biology, tumor dormancy, angiogenesis, molecular imaging, non-invasive intravital imaging of animal models, personalized nanomedicines for cancer theranostics (therapy and diagnostics). Throughout, she has maintained an interest in understanding the biological rationale for the design of nanomedicines suitable for transfer into clinical testing. Her multi-disciplinary research laboratory focuses on basic research elucidating the mechanisms underlying the switch from cancer dormancy leading to the discovery of new molecular targets interrupting host-tumor interactions. Her approach is followed by the design of highly-selective targeting molecules integrating biology, chemistry, protein engineering, molecular imaging, computational approaches, material sciences and nanotechnology to selectively guide drugs into pathological sites.

Prof. Satchi-Fainaro serves as advisor to several Israeli and International Biotechnology and Pharmaceutical companies, was President of the Israeli Society for Controlled Release, and is on the editorial boards of several biological and chemical journals. She has published more than 80 papermanuscripts, 12 book chapters, edited 2 books, is named inventor on 45 patents, and was awarded numerous prestigious grants and prizes, among them Fulbright, Rothschild, Wingate, Alon, Young Investigator Award of the European Association for Cancer Research, JULUDAN Prize for the Advancement of Technology in Medicine, and the 2013 Teva Pharmaceutical Industries Founders Award for the Discovery of new molecular mechanisms and targets that would lead to new therapeutic approaches. Her scientific achievements were acknowledged numerous times by inclusion in honorary lists by leading magazines such as "the 50 Most powerful and influential women" list of the Forbes journal, Israel (2014, #13/50), "50 Most influential women of 2011 and 2013" lists of the Globes journal, Israel, "50 Most promising women" list of the Calcalist journal, Israel (2009) and "40 people under the age of 40" list of the TheMarker journal, Israel (2008). Recently, she received the European Research Council (ERC) Consolidator Award, the Saban Family Foundation-Melanoma Research Alliance (MRA) Team Science Award and was selected to represent Israel at the Biennale in Venice in 2016.

Raymond Schiffelers

I obtained my PhD degree from Erasmus University Medical Center Rotterdam, The Netherlands. My thesis focused on liposomal targeting of antimicrobial agents. From 2000–2011, I worked at the dept Pharmaceutics of Utrecht University working on polymers, micelles and liposomes, loaded with small molecular weight drugs as well as biologicals, targeting a variety of diseases. Within this period, I spent 2002–2003 working for Intradigm Co. (USA) on formulations for *in vivo* targeting of siRNA. In 2007, I obtained a Vidi grant from NWO to start my own research line, in 2009 I received the Galenus Research Award for my drug delivery work and in 2010 was awarded an ERC Starting Grant (consolidator phase) to investigate extracellular vesicles for drug delivery. In 2011, I moved to University Medical Center Utrecht where I complement my research lines on drug targeting with investigations on nanoparticles in diagnostic applications.

Currently I am coordinating the H2020 project B-SMART on targeting RNA to the brain and national HighTech Systems Materials project (Targeting of multiple myeloma)/KWF-STW Technology for Oncology (Targeting glioblastoma)/NWO-CW Launchpad for Innovative Future Technologies (Innovative diagnostics for glioblastoma). In addition, I participate in several EU and national projects on nanomedicine.

Ruth Schmid-Baumberger

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

EDUCATION

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

EXPERIENCE

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

2011: Senior Research Scientist at SINTEF Materials and Chemistry, Department of Synthesis and Properties, Research Team Polymer Particles and Surface Chemistry
2011–Present: Vice President Marketing at SINTEF Materials and Chemistry

MAIN FIELDS OF COMPETENCE

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

PROFESSIONAL MEMBERSHIPS

- Member of the American Chemical Society
- Member of the Controlled Release Society (Board of Directors 2009 – present, Secretary 2012-2013, Treasurer-Elect 2013-2014, Treasurer 2014-2015)
- Member of the European Technology Platform in Nanomedicine
- Member of the EARTO WG Emerging Technologies for Healthcare
- 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

43 scientific publications, 18 patent and patent applications, 60 oral presentations, 24 poster presentations, 16 webinar, mass media and popular science publications.

SELECTED PUBLICATIONS

- S.E. Borgos, A. Brunsvik, A. Kristiansen, F. Männle, Y. Mørch, R. Schmid, K. Vernstad & K. Zahlsen, 41st Annual Meeting of the Controlled Release Society, 13.-16.7.14, Chicago, IL, USA. "Mass Spectrometry for Comprehensive Characterization of Controlled Release Systems".
- Y.A. Mørch, S. Snipstad, A. Åslund, E. Sulheim, H. Baghirov, C. De Lange Davies, S. Berg, R. Hansen, & R. Schmid, Nordic Polymer Days, 1.-3.6.2015, Copenhagen, Denmark. "Ultrasound-enhanced drug delivery using nanoparticle-stabilized microbubbles."
- Y. Mørch, R. Hansen, S. Berg, A.K.O. Åslund, W.R. Glomm, S. Eggen, R. Schmid, H. Johnsen, S. Kubowicz, S. Snipstad, E. Sulheim, S. Hak, G. Singh, B.H. McDonagh, H. Blom, C. de Lange Davies, P.M. Stenstad, Contrast Media and Molecular Imaging 10 (5), 356-366 (2015). "Nanoparticle-Stabilized Microbubbles for Multimodal Imaging and Drug Delivery."
- R. Schmid, XI Spanish-Portuguese Conference on Controlled Drug Delivery, 21.-23.1.2016, Granada, Spain. "Ultrasound-enhanced drug delivery using nanoparticle-stabilized microbubbles." (invited plenary speaker).
- A.K.O Åslund, E. Sulheim, S. Snipstad, E. von Haartman, H. Baghirov, N. Starr, M. Kvåle Løvmo, S. Lelú, D. Scurr, C. de Lange Davies, R. Schmid & Y. Mørch, Mol. Pharmaceutics; doi: 10.1021/acs.olpharmaceut.6b01085 (2017). "Quantification and Qualitative Effects of Different PEGylations on Poly(butyl cyanoacrylate) Nanoparticles."

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 since October 2012. Dr. Schroeder conducted his Postdoctoral studies at the Massachusetts Institute of Technology, and his PhD in the Hebrew and Ben Gurion Universities.
Avi is the recipient of more than 20 awards, he is a Kavli Fellow, a Horev Fellow – Leaders in Science and Technology, and an Alon Fellow; he is the recipient of the Intel Nanotechnology-, TEVA Pharmaceuticals-, and the Wolf Foundation Krill Awards, as well as a scientific entrepreneur, involved in translation of these discoveries to the clinic. Avi is the author on 32 papers and 12 patents.

Simó Schwartz

Director of CIBBIM-Nanomedicine and Team leader of the "drug delivery and targeting group" focused on new biomedical nanotechnology-based applications. Member of the Science Advisory Board of the Vall d'Hebron Research Institute (VHIR) and Science Advisor of the European Nanotechnology Characterization Laboratory (EU-NCL). He is also Science Advisor of SOM BIOTECH and CELGENE, member of the Advisory Board of NANOCAN, Southern Denmark University, and has been recently appointed as President of the European Society of Nanomedicine and Executive Board member of the International Society of Nanomedicine. He helds 13 patents, most transfered to leading companies of the biotech and pharma sectors and coauthors more than 80 papers in high impact factor journals. Dr Schwartz Jr was also appointed as Deputy Director and technology transfer coordinator of "CIBER de Bioingeniería, Biomateriales y Nanomedicina" (CIBER-BBN) of the Spanish Health Institute CarlosIII (ISCIII) which gathers a total of 45 research groups of national excellence in the field of nanotechnology and nanomedicine. Dr Schwartz was also Co-founder and Science Advisor of ARGON Pharma SL and is also member of the editorial Board of the journals Nanomedicine-NBM and the Eur. J. Nanomedicine. He is President of the European Socitey for Nanomedicine (ESNAM).

Giacinto Scoles

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

PERSONAL STATEMENT

GIACINTO SCOLES' scientific career has spanned an unusually long length of time and an equally unusually broad range of subjects. The general philosophy was to exploit new physical ideas and novel instrumentation to solve outstanding problems in chemistry and

materials science before, and biology and medicine now. Much before the coming of age of Nanotechnology and Nanoscience, particular emphasis was given to the behavior of nano systems and materials

POSITIONS AND LEADERSHIP

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

HONORS AND AWARDS

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

RESEARCH SUPPORT

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

Oksana Sergeeva

Oksana was born in Moscow, but ended up growing up in sunny San Diego, California. She attended Harvey Mudd College in Claremont, California to obtain her BS in chemical biology. Having done many summer undergraduate research fellowships, she set her sights on graduate school in biology. She attended MIT for her PhD where she did biochemistry research with Jonathan King. After her PhD, she moved to Europe where she is currently pursuing a post-doc in the lab of Gisou van der Goot, studying cell biology. She is currently supported by an EPFL Fellowship co-sponsored by Marie Curie. **She has published various papers on her graduate work:**

- Darrow MC, Sergeeva OA, Isas JM, Galaz-Montoya JG, King JA, Langen R, Schmid MF, Chiu W. Structural Mechanisms of Mutant Huntingtin Aggregation Suppression by the Synthetic Chaperonin-like CCT5 Complex Explained by Cryoelectron Tomography. J. Biol. Chem. 2015, 290:17451-17461.
- Sergeeva OA, Tran MT, Haase-Pettingell C, King JA. Biochemical Characterization of Mutants in Chaperonin Proteins CCT4 and CCT5 Associated with Hereditary Sensory Neuropathy. J. Biol. Chem. 2014, 289:27470-80.
- Sergeeva OA, Yang J, King JA, Knee KM. Group II Archaeal Chaperonin Recognizes Various Human γD-Crystallin Mutants. Protein Sci. 2014, 23: 693-702.
- Sergeeva OA, Chen B, Haase-Pettingell C, Chiu W, King JA. Human CCT4 and CCT5 chaperonin subunits expressed in E. coli form biologically active homo-oligomers. J. Biol. Chem. 2013, 288:17734-44.
- Knee KM, Sergeeva OA, King JA. Human TRiC complex purified from HeLa cells contains all eight CCT subunits and is active *in vitro*. Cell Stress and Chaperones 2013, 18:137-144.

Hripsime Shahbazian

Mrs. Hripsime Shahbazian holds a MSc. in Medical Physics and a BSc in Molecular Physics. She joined Health Canada in 1988 as a Technology Assessor at the Medical Devices Bureau (MDB) and from 1991 to 1998 she acted in different managerial roles within the Bureau. In 1998 Mrs. Shahbazian joined the Office of Science within the Therapeutic Products Directorate (TPD) as an Associate Manager. She is currently a Senior Science Advisor in the Office of Science. Her duties include management of the activities of Scientific/Expert Advisory Committees and Panels that are established to obtain medical/technical/scientific advice and recommendations on regulatory issues for drugs and medical devices in specific therapeutic areas/classes or on specific drug and medical device issues. She is responsible for reviewing Opportunity to be Heard Requests and Second Level of Appeals for medical devices and making recommendations for resolution of outstanding issues.
Mrs. Shahbazian is one of the key members working on nanotechnology related activities at Health Canada. She is a founding member of the Health Protection Branch (HPFB) Nanotechnology WG and an active participant of Nanotechnology related international

activities for regulated health products for the Branch. She is a member of the Health Portfolio Nanotechnology Working Group composed of key officials across the department, coordinating departmental approach to science, policy and research needs for nanotechnology. She is also a member of the Ad Hoc Interdepartmental Discussion Group on Nanotechnology that was established to provide an overview of departmental roles and interests in nanotechnology and update on departmental activities.
Mrs. Shahbazian represented HC on the International Regulators on Nanotechnology Working Group since its inception in summer of 2009 to discuss nanotechnology related issues relevant to regulated products that may contain nanoscale materials. In 2014 the International Pharmaceutical Regulators Forum (IPRF) proposed to have an inclusive Nanomedicines WG within IPRF for the exchange of non-confidential information. In 2015 the original members of the International Regulators on Nanotechnology Working Group formed the Nanomedicine WG under IPRF to share non-confidential information. Hripsime Shahbazian is representing HC on this WG. Starting 2017 HC assumed the role of the chair of this WG.

Amotz Shemi

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

Marco Siccardi

Dr Marco Siccardi obtained his PhD at the University of Liverpool (UoL), 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 integrating experimental data to investigate the pharmacokinetics of drugs and nanomedicine in virtual patients. He was appointed as a Lecturer in Nanomedicine across the faculties of Health & Life Sciences and Science & Engineering at UoL and promoted to Senior Lecturer in Pharmacology in 2016. He has authored 81 peer reviewed publications, he is currently supervising 7 PhD students and teaching in 5 undergraduate modules. He is principal investigator of 4 grants for a total of >£1.1 million and has been co-applicant for a total of 8 additional grants funded by UNITAID, NIH, EPSRC, Clinton Health Access Initiative for a total of >£8 million. In the recent past he has worked in collaboration with different international research centers in USA, Italy, Switzerland, Spain, Germany, UK, Chile, Brazil, Argentina, Nigeria and Uganda. His research interests focus on the optimisation of novel nanomedicine and traditional formulations for drug delivery based on experimental pharmacological data from in silico, *in vitro* and *in vivo* models, aiming to improve pharmacokinetics, efficacy and side effects. Additionally, he is interested in the clarification of the ADME processes involved in drug disposition and the identification of nanoformulation characteristics influencing drug exposure.

Dmitri Simberg

Dr. Simberg received his Ph.D. in Biochemistry from the Hebrew University of Jerusalem, Israel. His thesis in the laboratory of Professor Chezy Barenholz was on biochemical and biophysical mechanisms of lipofection using cationic lipids *in vitro* and *in vivo*. After receiving the PhD he did a 2-year postdoctoral study on amplified tumor targeting of iron oxide nanoparticles in the laboratory of Prof. Erkki Ruoslahti at the Burnham Institute, La Jolla. This was followed by a 1-year postdoctoral research at the Department of Radiology, University of California San Diego. Dr. Simberg was a project scientist at the Center for Cancer Nanotechnology Excellence at UCSD, where he developed his research program in nano-bio interface and immune recognition of nanomaterials. In 2013, Dr. Simberg joined the faculty of the Skaggs School of Pharmacy, University of Colorado Dr. Simberg is the corresponding author or coauthor of over 40 research papers, reviews, opinion articles, perspectives and book chapters, and a recipient of funding totaling over $6M. His current research interests are focused on the development of iron oxide nanoparticles and red blood cells for drug delivery and imaging, on mechanisms of complement activation by nanomedicines, and isolation of cancer biomarkers from blood using nanotechnology.

Tore Skotland

Tore Skotland is a biochemist by training and received his PhD from the University of Bergen, Norway in 1980. After 11 years at the university studying protein chemistry and enzymology, he moved to pharmaceutical R&D (Nycomed AS, Oslo, Norway) in 1983. He stayed within the same field of research for 26 years in one of the world leading companies developing contrast agents for medical imaging; Nycomed was bought by Amersham in 1997 and Amersham was bought by GE Healthcare in 2003. During the last 20 years in pharmaceutical R&D he was heading work to describe the biodistribution, metabolism and excretion of all types of contrast agents (water soluble as well as particle based) for CT, MRI, ultrasound, SPECT, PET and optical imaging. He has been involved in bringing 5 products to the marked (including 2 particle-based) and another 5 products into clinical trials (also including 2 particle-based). Skotland is the first or last author of publications related to all these 10 products. He is co-author of approx.110 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, endocytosis and intracellular transport of protein toxins and nanoparticles. This group is heading a 5-year national competence building project in Norway going to the end of 2018. The project title is “Biodegradable nanoparticles for cancer diagnosis and therapy”. Skotland is co-ordinating the in vivo studies in this project, which has members from academia, university hospitals, research institutes and pharmaceutical industry. The 10 groups involved have expertise in nanoparticle syntheses and characterization, in vitro studies of cellular uptake and intracellular transport, immunology studies, and studies using small animals with xenograft models, including use of different in vivo imaging modalities such as MRI, PET/CT and fluorescence. Clinicians are also involved. Our group is also partner

in an INNO INDIGO granted project which started summer 2016. INNO INDIGO is an innovation-driven initiative for the development and integration of Indian and European research.

Most important publications in the field of nanoparticle research:

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

Per Spindler

Dr. Per Spindler, DVM, Executive MBA, MSc (Applied Toxicology), ERT, Fellow DIA, is Director of Biopeople, Denmark's Life Science Cluster at University of Copenhagen. Dr. Spindler has professional international experience within safety sciences, regulatory sciences and affairs, project management R&D and leadership in medicinal products, health & life sciences. Experiences include Novo Nordisk, H. Lundbeck, the scientific committees of the European Medicines Agency, ICH, and others. Dr. Spindler currently leads Denmark's Life Science Cluster, Biopeople, which was the first Life Science cluster in Europe that was awarded the Gold Label of the European Cluster Management Excellence in recognition of excellent management performance. Dr. Spindler is among others member of the Scientific Advisory Board of the Copenhagen Centre for Regulatory Science (CORS) and Chair of the European Cluster Excellence Expert Group (CEEG) of the European Secretariat for Cluster Analysis (ESCA). Dr. Spindler has been active in several IMI education and training project such as EUPATI (training of patients), SafeSciMET (training of the next generation of safety scientists) and PharmaTrain (Pharmaceutical Medicine and Regulatory Affairs).

Stephanie Stanford

I received my Ph.D. in Genetic, Molecular and Cellular Biology from the University of Southern California, and underwent postdoctoral training at the La Jolla Institute for Allergy and Immunology (LJI). My research training was heavily focused on regulation of signal transduction in immune cells. During my postdoc, I worked with several rheumatologists on projects to study anomalous signal transduction pathways in rheumatoid arthritis, particularly in fibroblast-like synoviocytes. I was recruited to the University of California, San Diego Clinical and Translational Research Institute as an Assistant Professor in June 2016. I am applying my background in cell signaling and biochemistry to research focused on understanding the pathogenesis of rheumatoid arthritis.

POSITIONS

08/05 – 05/10: Graduate Student Research Assistant, University of Southern California Institute for Genetic Medicine
05/10 – 07/14: Postdoctoral Fellow, La Jolla Institute for Allergy and Immunology Division of Cellular Biology
07/14 – 06/16: Instructor, La Jolla Institute for Allergy and Immunology Division of Cellular Biology
06/16 – present: Assistant Professor, University of California, San Diego Clinical and Translational Research Institute

HONORS

07/07 – 06/09: Recipient of National Institutes of Health-Cellular, Biochemical and Molecular Sciences Predoctoral Fellowship
09/08: Recipient of BioSymposia Young Investigator Award
05/12 – 03/15: Recipient of Juvenile Diabetes Research Foundation Postdoctoral Fellowship
10/16: Co-author manuscript received Arthritis Foundation Lee C. Howley Sr. Prize

PROFESSIONAL MEMBERSHIPS

07/15 – present: Member, American Heart Association
12/14 – present: Member, American Diabetes Association
06/13 – present: Member, American College of Rheumatologists

LIST OF PUBLISHED WORK

http://www.ncbi.nlm.nih.gov/sites/myncbi/1HqLeDMQfhxQl/bibliography/48608500/public/?sort=date&direction=ascending

Scott Steele

Ph.D.
Scott SteelePh.D., serves as the Director of Regulatory Science Programs, in the Clinical and Translational Science Institute at the University of Rochester Medical Center (NY). He is actively involved in developing and leading regulatory science educational programs, serving as Program Director for a Certificate in Regulatory Science and Core Director of the Regulatory Science to Advance Precision Medicine initiative. Dr. Steele also coordinates national Clinical and Translational Science Award (CTSA) affiliated initiatives, including co-leading the development of a set of Regulatory Science competencies to guide training and education in this area. Dr. Steele was recently selected as a member of the U.S. FDA Science Board and also serves as a Senior Editor for the Journal of Clinical and Translational Science. He is an associate professor in the Department of Public Health Sciences, where his academic interests are focused on a range of science and technology policy issues including translational research and regulatory science, public health preparedness, and national security. Additionally, he serves as the Deputy Director of the Goergen Institute for Data Science at the University of Rochester. Prior to joining the University of Rochester, Dr. Steele served in the U.S. White House Office of Science and Technology Policy (OSTP), initially as a policy analyst and later as the Executive Director of the President's Council of Advisors on Science and Technology (PCAST). Dr. Steele coordinated PCAST studies addressing issues in personalized medicine, nanotechnology, information technology, energy technologies, and approaches to enhance university-private sector research partnerships. At OSTP, he also led several programs related to medical countermeasures development, biotechnology, and science education. Dr. Steele received his BS with Honors in Biology from Union College in Schenectady, NY. Following this, he performed research at the General Electric Center for Research and Development (NY), was a fellow at the National Institutes of Health (Bethesda, MD) and performed research at the University of Geneva (Switzerland). Dr. Steele completed his MA and PhD in Molecular Biology at Princeton University.

Francesco Stellacci

Prof. Francesco Stellacci got his degree in Materials Engineering at the Politecnico di Milano in 1998 with Prof. Zerbi. He then moved as a post-doc with Prof. J.W. Perry in the Department of Chemistry at the University of Arizona. In 2002 he became as assistant professor in the Department of Materials Science and Engineering at MIT (Cambridge, USA). There he became associate professor with tenure in 2009. In 2010, he moved as a full professor to EPFL where he holds the Constellium chair. Stellacci has published more than 130 papers and has more than 15 patent applications. He has won numerous awards, among the the Technology Review TR35 'top innovator under 35', the Popular Science Magazine 'Brilliant 10', and the EMRS EU40.

Gert Storm

Gert Storm, professor Targeted Drug Delivery at the Department of Pharmaceutics (80% employment), obtained his Ph.D. degree in 1987 at the Utrecht University. 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 became 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. He is honorary professor (Biomacromolecular Drug Delivery) at the University of Copenhagen. From 2012 on, he is also professor (Targeted Therapeutics) at the MIRA institute of the University of Twente (20% employment). Furthermore, he keeps a position (seconded) at the University Medical Center Utrecht (UMCU) (Division Imaging).

Research

The design, characterization and (pre)clinical testing of targeted nanomedicine formulations is the core activity. Additionally, the implementation of imaging-guided drug delivery protocols (using e.g. SPECT, MRI, PET) is a major research objective. Over the last decade, clinical translation of academic results has become a vital element of Prof. Storm's ambition, as exemplified by the clinical development of liposomal corticosteroids (by the creation of the spin-off company Enceladus Pharmaceuticals BV in 2005), the clinical breast cancer study at the UMCU evaluating image-guided targeted doxorubicin delivery with hyperthermia (follow-up of the HIFU-CHEM project), his involvement in public-private partnership projects, i.e. EC (coordinator Meditrans (FP6), partnering in 4 FP7 projects and 2 Horizon2020 projects), CTMM (PI HIFU-CHEM), NanoNextNL (director Program Drug Delivery), TI-Pharma and AgentschapNL. Over the years, he also obtained significant funding for applied research from STW and several large industries (e.g. Astellas, Astra Zeneca, Novartis). He is on the Board of the CLINAM (European Foundation for Clinical Nanomedicine) organization in Basel. He is included in the 2014, 2015 and 2016 lists of The World's Most Influential Scientific Minds of Thomson Reuters (Highly Cited Researchers).

SELECTED PUBLICATIONS

- Lammers T, Kiessling F, Ashford M, Hennink WE, Crommelin DJA and Storm G. Cancer nanomedicine: Is targeting our target? Nature Review Materials 1, 16069, 2016
- Crielaard BJ, Rijcken CJF, Quan L, van der Wal S, Altintas I, van der Pot M, Kruijtzer JAW, Liskamp RMJ, Schiffelers RM, van Nostrum CF, Hennink WE, Wang D, Lammers T, and Storm G. Glucocorticoid-loaded core-crosslinked polymeric micelles with tailorable release kinetics for targeted therapy of rheumatoid arthritis. Angewandte Chemie 51, 7254-7258, 2012
- T. Lammers, S. Aime, W.E. Hennink, G. Storm*, F. Kiessling*, Theranostic nanomedicine, Accounts of Chemical Research, 44, 1029-38, 2011 (* shared senior authorship)

SELECTED GRANTS

- 2016–2020: ITN OcuTher, Ocular delivery of drugs (ITN program) (with Prof. Hennink) 550 K€
- 2016–2020: PhD Grant Singapore National Eye Centre, Targeting eye inflammation (with Prof. Wong)
- 2016–2020: KWF-STW, Drug delivery by sonoporation in childhood brain cancer (with UMCU and AMC) 568 K€
- 2016–2020: KWF, Image-guided targeted doxorubicin delivery with hyperthermia (at UMCU) 500 K€
- 2014–2018: ITN NABBA, Nanomedicines to overcome biological barriers (with RWTH Aachen University) 275 K€

Yung Doug Suh

PhD

Director, Research Center for Convergence NanoRaman Technology (RC2NT)
Head, Laboratory for Advanced Molecular Probing (LAMP),
Korea Research Institute of Chemical Technology (KRICT), Yuseong P.O. Box 107, DaeJeon 305-600, Korea & 2 School of Chemical Engineering, Sungkyunkwan University (SKKU), Suwon 440-746, Korea.
E-mail: ydsuh@krict.re.kr, ydsuh@skku.edu

Prof. Dr. Yung Doug Suh studied at Seoul National University for his BS(1991), MS(1993), and PhD(1999) under the guidance of Prof. Seong Keun Kim in Chemistry Department, Prof. Young Kuk in Physics Department, and Dr. Dongho Kim in Korea Research Inst. of Standards and Science (KRISS) researching gas phase molecular reaction dynamics, surface physics with UHV-STM, and laser spectroscopies, respectively. After finishing his Postdoctoral research in ETH Zurich working with Prof. Renato Zenobi in 1999-2000,where he co-invented TERS(Tip-enhanced Raman Scattering), he worked at the Pacific Northwest Nat'l Laboratory (PNNL), USA, in 2001-2002 doing single molecule spectroscopy. He accepted a recruited principal research scientist position in 2003, to form his own research group: Laboratory for Advanced Molecular Probing (LAMP) at the Korea Research Institute of Chemical Technology (KRICT) in DaeJeon, South Korea. He is currently a director of Research Center for Convergence NanoRaman Technology (RC2NT), KRICT, and also serves as an adjunct professor of Chemical Engineering Department, SungKyunKwan Univerisity (SKKU), Korea since March 2013.

Hulda Shaidi Swai

Nanoscientist
Director of African Centre of Excellence
School of Life Science and Bio-engineering
The Nelson Mandela African Institution of Science and Technology (NM-AIST)
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Extraordinary Professor; University of Pretoria

Prof. Hulda Swai, a Nanotechnology scientist, holds a PhD in Biomaterials, from Queen Mary's College, University of London, UK. where she also worked for 9 years as a Researcher.
In 2013 Prof Swai was appointed as an Extra ordinary Professor in University of Pretoria.
Prior to that she was senior Principal Researcher at the Council for Scientific and Industrial Research (CSIR), in South Africa, where she led the Encapsulation and Delivery Research Group. She has a proven ability to independently formulate and execute research projects and implement scientific research programmes, both in public and private sectors. Furthermore Prof. Swai instituted and headed up the Department of Science and Technology-(DST)/CSIR Pan-African Centre of Excellence in Applied Nanomedicine Research and Training, with a focus on infectious diseases of poverty worth R 60 million (about US $ 7 million) in research and training grants.
She has recently joined The Nelson Mandela African Institution of Science and Technology (NM-AIST) in Tanzania as a Professor in School of Life Science and Bio-engineering. After joining NM-AIST in July 2016 she was tasked to lead her group in applying for African Center of excellence funded by the World Bank; (ACEII) call. NM-AIST was awarded the African Center of Excellence worth USD 6 million. Prof Swai is the Director of the newly established Eastern and Southern African Centre for Research advancement in Agriculture, Teaching Excellence and Sustainability in Food and Nutritional Security (CREATES). This is a post graduate (PhD, MSc Post Doc) capacity building grant.

Janos Szebeni

Janos Szebeni, M.D., Ph.D., D.Sc., Med. Habil., immunologist, director of the Nanomedicine Research and Education Center at Semmelweis University, Hungary. He is also founder and CEO of a contract research SME "SeroScience", and full professor of (immune) biology at Miskolc University. He has held various guest professor and scientific positions in Hungary and abroad, mostly in the USA where he lived for 22 years. His research on various themes in hematology, membrane biology and immunology resulted ≈150 publications including peer-reviewed papers, book chapters, patents, etc. (citations: ≈6000, H index: 39 i10 index: 73), and a book entitled "The Complement System: Novel Roles in Health and Disease" (Kluwer, 2004). Three fields stand out where he has been most active: artificial blood, liposomes and the complement system. His original works led to the "CARPA" concept, i.e., that complement activation underlies numerous drug-induced (pseudo)allergic (infusion) reactions.

Ennio Tasciotti

Dr. Ennio Tasciotti graduated in Molecular Biology at Scuola Normale Superiore in Pisa in 2000, and earned a PhD in Molecular Medicine in 2005 at the International Center for Genetic Engineering and Biotechnology.
In 2006 he accepted a Postdoctoral position at the Department of Biomedical Engineering at the University of Texas' Health Science Center. In 2008 he published his first research on 'multistage nanodelivery systems', which was selected by Nature Medicine as one of the "Top 5 breakthroughs in Nanomedicine".
In 2009 he became an Assistant Professor at the first Department of Nanomedicine of a Medical School and in 2010 was recruited by the Houston Methodist Research Institute as Chair of the Department of Nanomedicine. He is a Professor of Nanomedicine at the Institute of Academic Medicine and he holds affiliated and honorary positions in 6 universities in USA, Europe and China.
From 2012 he is the Director of the Surgical Advanced Technology Lab of the Department of Surgery. In 2015 he founded and still directs the Center for Biomimetic Medicine. In 2017 he became the director of the Center for Musculoskeletal Regeneration of the department of Orthopedics and Sports Medicine.
Between 2009 and today, he received over $15M by DoD and DARPA, to create bioactive nanomaterials to regenerate musculoskeletal tissues and over $5M from NIH, NSF and CPRIT to develop nanoparticles for drug delivery. He coordinates a multidisciplinary research group of 25 people, has published more than 115 research articles, 10 books and presented at over 150 international conferences.
Dr. Tasciotti has filed 10 international patents on nano- and biomaterials for biomedical use and he is currently engaged in translating to the clinic two technologies for targeted drug delivery and musculoskeletal tissue regeneration.

James Taylor

James is the CEO and co-founder of Precision NanoSystems, Inc. (PNI), a commercial stage biotechnology company at the convergence of nanotechnology, genomics, and personalized medicine. James has a B.A.Sc. in engineering physics from UBC and a Ph.D. in genetics from the Institute for Systems Biology in Seattle, WA. James worked at the Seattle based Venture Capital firm, the Accelerator Corporation concurrent with his Ph.D. and has extensive experience in the science and commercialization of microfluidics, nanotechnology, and systems biology. James has been the leader of PNI since its inception.

Steliyan Tinkov

Steliyan Tinkov has graduated in pharmacy at the Medical University in Sofia and finished his PhD education in pharmaceutical technology at the LMU-Munich. Later on, Steliyan graduated MBA at the WHU-Otto Beisheim in Dusseldorf.
Steliyan has more than ten years of experience in the nanotechnology, seven of each in the pharmaceutical industry. During his time at Baxter, Steliyan was engaged in the establishing of a dedicated facility for contract manufacturing of parenteral nanomedicines for clinical and commercial use. Currently at Novartis, Steliyan is in charge for the management of clinical supplies including nanomedicines and biopharmaceutics.

Panagiotis N. Trohopoulos

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

Dr med Trohopoulos is the Founder (13 years ago, since 2004) and the Scientific / Exploitation / Strategic Coordinator of the CosmoPHOS-nano Project (GA 310337) which is a Large-scale EU FP7 NMP Funded Translational Nanomedicine R&D Project in Cardiovascular Diseases, and more specifically in Atherosclerotic Heart Disease. The Project co-funded by the European Union under the FP7 Programme / NMP Theme (Nanosciences, Nanotechnologies, Materials and New Production Technologies) with 8,5 Million Euros, and additionally co-funded by All Project Beneficiaries with 4,5 Million Euros, having a total project budget of 13 Million Euros. The EU FP7 NMP Funded Large-Scale CosmoPHOS-nano Project (GA 310337) is a Multidisciplinary Five-year R&D Project started on March 1, 2013 and will be concluded on February 28, 2018, and consists of 20 World-Class Participants, including 14 Universities and Research Foundations and 6 Companies, from 11 European Countries, Japan, and USA, with a wide variety of complementary and cutting-edge scientific, technological and manufacturing expertise and know-how. The EU FP7 NMP Funded Large-Scale CosmoPHOS-nano Project (GA 310337) is the World's Largest R&D Project of Nanomedicine in Cardiology aiming to develop a Radical Innovative Theranostic (Diagnostic and Therapeutic) "Smart" Nanomedicine Product, the CosmoPHOS System, to enable: a) Near-Infrared Fluorescence-based Molecular In Vivo Imaging (NIRF-based Molecular In Vivo Imaging), b) Targeted Near-Infrared nanoPhotodynamic Therapy (Targeted NIR nanoPDT), and c) Real-time and Follow-up Therapy Monitoring of Atherosclerotic Coronary Artery Disease (CAD) of the Heart, which is the number one cause of human death and morbidity in Europe and Worldwide. The CosmoPHOS-nano Project (GA 310337) is the First EU FP7 NMP Funded Large-scale R&D Project planning to apply Nanomedicine for Cardiac Patients. It foresees conducting during the final Project-year, a First-in-man Phase-I Clinical Trial in CAD Patients, to evaluate the safety and feasibility of the novel CosmoPHOS System for human use.

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

Dr med Trohopoulos is also a Platinum Member of the ETPN (European Technology Platform on Nanomedicine), which is an Initiative led by the Industry and set up together with the European Commission, addressing the application of nanotechnology to achieve breakthroughs in healthcare.

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

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

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Katherine Tyner

Dr. Katherine Tyner is the Associate Director of Science (acting) in the immediate office of the Office of Pharmaceutical Quality (OPQ), Center for Drug Evaluation and Research at the United States Food and Drug Administration (FDA). As Associate Director, Dr. Tyner leads the OPQ Science Staff in coordinating the intersection between science, review and policy in OPQ as well as facilitating interactions between other CDER offices and FDA Centers. She received her PhD in Chemistry from Cornell University and joined the Food and Drug Administration in 2007 as a chemist specializing in nanotechnology. While at the FDA, Dr. Tyner has investigated the quality, safety, and efficacy of drug products containing nanomaterials, and she currently leads the CDER nanotechnology working group and is active in other CDER and FDA nanotechnology initiatives. Dr. Tyner is the author of multiple book chapters and journal articles concerning the appropriate characterization and biological impact of nanoparticle therapeutics.

Gooitzen M. van Dam

Gooitzen M. van Dam, MD, PhD is a surgeon oncologist and Professor of Surgery He currently leads the Optical Molecular Imaging Groningen (www.omig.nl) research group and coordinates several multidisciplinary projects of optical imaging in oncology. In 2004, he was the initiator and director of the BioOptical Imaging Center Groningen. In 2007 the clinical program, in an ongoing close collaboration with prof Vasilis Ntziachristos of the Technical University of Munich, was initiated which lead to the first in-human use of targeted optical imaging in patients with ovarian cancer, awarded with the Erwin Schrodinger Prize in Germany and the Jorge Barrio Clinical Award by the WMIC in 2011. There is particular expertise at the UMCG in regards to the clinical translation and GMP production of optical agents for human use for both biological as non-biologicals. Especially the synthesis and regulation involved in such a process has brought us to a fast-track process of testing and production of antibody based agents, among others. The current main focus of the research group is on clinical studies using such agents in image-guided surgery, image-guided parthology and image-guided endoscopy.

RELEVANT PUBLICATIONS:

- van Dam GM, Themelis G, Crane LMA, Harlaar NJ, Pleijhuis RG, Kelder W, Sarantopoulos A, de Jong JS, Arts HJG, van der Zee AGJ, Bart J, Low PS, Ntziachristos V. Intraoperative tumor-specific fluorescent imaging in ovarian cancer by folate-receptor-alpha targeting: first in-human results. Nat Med 2011;18:1315-9.
- Terwisscha van Scheltinga AGT, van Dam GM, Nagengast W et al. Intra-operative near-infrared fluorescence tumor Imaging with VEGF and HER2 targeting antibodies. J Nucl Med 2011;52:1778-85.
- Scheuer W, van Dam GM, Schwaiger M, Ntziachristos V. Using drugs as optical imaging agents at microdosing amounts: a realistic gateway to fluorescence-guided surgery? Sci Transl Med 2012;16:134ps11

Hans van der Voorn

Hans van der Voorn is the CEO of Izon Science Ltd and now based in New Zealand. He originally trained as an engineer. Hans was one of the founders of Izon and became its fulltime CEO in 2007. He has been the inventor on several Izon patents and is involved in developing nano-measurement capabilities for nano-and nano-bio particles for biomedical uses.
His particular interests are in Tunable Resistive Pulse Sensing (TRPS) for nanomedicine development and in combined SEC column separation + TRPS for extracellular vesicle (EV) research, diagnostic development and exosome based therapeutics.

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 1984. 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.
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.

Viola Vogel

PLaboratory of Applied Mechanobiology, Institute of Translational Medicine, Department of Health Sciences and Technology, ETH Zurich, Switzerland

Professor Viola Vogel studied Physics and Biology in Frankfurt, at the Max-Planck Institute for Biophysical Chemistry in Göttingen and in Berkeley before starting her academic career in the Department of Bioengineering at the University of Washington (1991). In Seattle, she was the Founding Director of the Center for Nanotechnology at the University of Washington (1997–2003). She moved to Switzerland in 2004, where she initially joined the Department of Materials. She is now heading the Laboratory of Applied Mechanobiology in the Department of Health Sciences and Technology at the ETH Zurich, and the Founding Director of the new Institute of Translational Medicine.
Viola Vogel pioneered the rapidly growing field of mechanobiology and its medical applications as she discovered many structural mechanisms how mechanical forces can turn proteins into mechano-chemical switches. Such mechanisms are exploited by bacteria, as well as mammalian cells and tissues to sense and respond to mechanical forces, and if abnormal, can cause various diseases. Her research was recognized by major awards, including the Otto-Hahn Medal of the Max-Planck Society 1988, the “First Award” from the NIH Institute of General Medicine (1993–98), the Julius Springer Prize 2006 for Applied Physics, the ERC Advanced Grant (2008–13), the International Solvay Chair in Chemistry Brussels 2012, and an Honorary Degree Doctor of Philosophy from Tampere University, Finland 2012. She also serves on various international advisory boards in the fields of nanotechnology and bioenginerring, including on the White House panel that finalized the US National Nanotechnology Initiative under the Clinton administration (1999) and on the World Economic Forum Global Agenda Council in Nanotechnology (2014–2016).

Sandra Vranic

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

AWARDS AND HONORS

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

PUBLISHED ARTICLES

- S. Vranic, I. Gosens, R. Schins, N. R. Jacobson, B. Bokkers, A. Kermanizadeh, V. Stone, A. Baeza Squiban, F. R. Cassee, L. Tran, S. Boland - Impact of serum as a dispersion agent for *in vitro* and *in vivo* toxicological assessments of TiO2 nanoparticles, Arch Toxicol. 2016 Feb 12. doi:10.1007/s00204-016-1673-3.
- S. Vranic, I. George, S. Boland, Courtois A, A. Baeza Squiban - Comparison of different cellular models to study translocation of NPs *in vitro*, Toxicol In Vitro. 2014 Sep 6. pii: S0887-2333(14)00156-8. doi: 10.1016/j.tiv.2014.08.003.
- S. Vranic, N. Boggetto, S. Mornet, N. Reinhardt, F. Marano, A.

Baeza-Squiban, S. Boland - Deciphering the mechanisms of cellular uptake of engineered nanoparticles by accurate evaluation of internalization using imaging flow cytometry; Part Fibre Toxicol. 2013 Feb 6;10:2. doi: 10.1186/1743-8977-10-2.

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- I. George,, S. Vranic, S. Boland, MC Borot, F. Marano, A. Baeza-Squiban - Translocation of SiO2-NPs across *in vitro* human bronchial epithelial monolayer, Journal of Physics: Conference Series.

BOOK CHAPTERS

- A. Baeza-Squiban, S. Vranic, S. Boland - Fate and health impact of inorganic manufactured nanoparticles. In: Nanomaterials: a danger or a promise? A chemical and biological perspective. EDS. R. Brayner, F. Fiévet and T. Coradin, Springer 2013. pp 245-267.
- S. Boland, S. Vranic, T. Stoeger, R. Schins - Current *in vitro* models for nanomaterial testing: Pulmonary System, Nanotoxicology Second Edition.

Andreas Wagner

Dr Andreas Wagner is currently the Head, Liposome Technology at Polymun Scientific GmbH. He has significant expertise in incorporation and optimization of hydrophilic, lipophilic and amphipatic substances into liposomes and development for clinical use. He studied Biotechnology in Vienna, Austria and earned his Master and Ph.D. degrees in the field of biopharmaceutical technology/liposomology at the Institute of Applied Microbiology supervised by Prof. Hermann Katinger. Dr Andreas Wagner is listed as inventor on several patents, like the liposome technology and some product patents of liposomal formulations. Furthermore, he has published several peer reviewed articles dealing with liposomes, the technology, products thereof and their application in preclinical and clinical studies.

POLYMUN SCIENTIFIC GMBH

is a private Austrian company, located in Klosterneuburg, offering contract development and manufacturing of biopharmaceuticals as well as development and production of liposomal formulations. Its patented liposome technology allows efficient manufacturing of constantly high quality in small and large scale. Polymun is an EMEA- as well as FDA-certified manufacturer conducting several own R&D projects. For more information, please visit www.polymun.com

Kay Warner

Kay Warner is a Director within the Patients in Partnership team at GlaxoSmithKline Research and Development.

Kay moved to her current role as Patient Engagement Lead in January 2017 and is also responsible for leading an internal global programme entitled 'Focus on the Patient'. This programme helps our employees understand patient needs and inspires them to do more to help improve the lives of patients. This work has included interaction with many individual patients, caregivers, patient advocates, patient advocacy organisations and health care providers. With her passion to make a difference in research and development, Kay is working to strengthen the patient voice in medicines development and research at key decision time points. She also represents GSK in the EUPATI project. Kay is the industry lead for EUPATI-UK.
GlaxoSmithKline – we are a science-led global healthcare company with a mission: we want to help people to do more, feel better, live longer.

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).

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, a local network of researchers from different disciplines and SMEs, responsible for the development of the Münster region into a leading nanobioanalytic location at the European level. Between 2003 and 2008 he was the deputy-coordinator of the Nano2Life Network of Excellence and leader of the "ELSA" Board in this network. He co-managed the Nanomedicine Round Table and the EuroNanoBio projects and participated in the NANOMED2020 project (FP7 CSA projects). Since 2009 he is a member of the Executive Board of the ETP Nanomedicine leading the ELSA Advisory Group of this platform. Since March 2015 he is the chair of the German platform NanoBioMedicine. At the local level he is the Chief Scientific Officer of the Nano-Bioanalytik-Zentrum Münster (NBZ) and manages the Nano-Characterization-Lab Muenster (www.NCL-Muenster.de) interfacing 11 local companies, which develops new and certified methods for characterization of Nanomaterials in consumer products and biological systems. Currently he is partner in the EU-projects ENATRANS and EU-NCL.
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Marieluise Wippermann

CEO, TECOmedical AG, Sissach, Switzerland
E-mail: wippermann@tecomedical.com
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

Alon Wolf

Director, Biorobotics and Biomechanics Lab
Technion Israel Institute of Technology

Prof. Wolf earned all of his academic degrees from the faculty of Mechanical Engineering at the Technion Israel Institute of Technology in Israel, receiving his Ph.D. in 2002. Immediately after receiving his Ph.D., he joined the Mechanical Engineering department at Carnegie Mellon University (CMU) as a Post-Doctoral Research Associate. A year later, he joined the Robotics Institute of CMU as well as the Institute for Computer Assisted Orthopaedic Surgery (ICAOS) as a research faculty and the University of Pittsburgh School of Medicine as an Adjunct Assistant Professor of CT Surgery. In March of 2006 Prof. Wolf joined the faculty of Mechanical Engineering at the Technion Israel Institute of Technology. Here he founded a new research lab, the Biorobotics and Biomechanics Lab (BRML). The objective of the research in the BRML is to develop fundamental theories in biokinematics and biomechanics as well as to apply these theories to applications in medical robotics and biorobotics. Prof. Wolf's work was published in many leading journals, Book chapters, and conferences (some as key note and invited lectures). Prof. Wolf is a co-inventor and co-founder of Medrobotics and is an Associate Editor for the prestige journal of Clinical Biomechanics. He won numerous research awards and was elected to the 2016-2017 IEEE Engineering in Medicine & Biology Society Distinguished Lecturer Program. Prof. Wolf's urban search and rescue snake robot and his surgical snake robot were elected best technology of 2012 and 2014 respectively, by the prestige journal of popular science.

Joy Wolfram

Faculty, Mayo Clinic, Jacksonville, Florida (USA); Affiliate Faculty, Houston Methodist Research Institute, Houston, Texas (USA).

Dr. Joy Wolfram is a faculty member in the Department of Transplantation at Mayo Clinic in Florida (USA) and an affiliate faculty member in the Department of Nanomedicine at the Houston Methodist Research Institute in Texas (USA). She received her bachelor's and master's degrees in biology from the University of Helsinki in Finland. In 2016, Dr. Wolfram completed her Ph.D. in Nanoscience and Technology at the University of Chinese Academy of Sciences in Beijing, China. She has authored more than 30 scientific publications and received 25 scientific awards from seven different countries. In 2016, Dr. Wolfram was included in the Amgen Scholars Ten to Watch List, which highlights the best and brightest up-and-comers in science and medicine across 42 countries (http://www.amgenscholars.com/alumni/ten-to-watch). In 2017, she was selected together with Nobel Laureates, prominent authors, and professional athletes to represent one of 12 internationally accomplished Finns in the 2017 Inspired in Finland campaign to celebrate Finland's 100 years of independence (http://maailmallamenestyvat.fi/joy-wolfram/). The focus of Dr. Wolfram's research is on nanotherapeutics, extracellular vesicles, and modulation of innate immunity. Her ultimate goal is to bring new treatment strategies in nanomedicine to the clinic. Dr. Wolfram also aspires to generate fruitful inter-institutional research collaborations around the world and she is currently looking for outstanding individuals to join her research group.

Andrew Worth

MA, MSt, PhD

Dr Andrew Worth is a senior scientific officer at the European Commission's Joint Research Centre (JRC), where he leads the Predictive Toxicology Group within the Chemical Safety and Alternative Methods Unit of the JRC's Directorate for Health, Consumers and Reference Materials. The JRC provides independent scientific and technical support to the European Commission and other policy makers in the EU, and is actively involved in the international scientific community.
Dr Worth has degrees in Physiological Sciences and in Linguistics from Oxford University, and a PhD in Computational Toxicology from Liverpool John Moores University. He has over 180 publications in the area of predictive toxicology, and has a particular interest in the development and assessment of computational methods and their application in the regulatory assessment of chemical safety. Dr Worth is a member of the editorial boards of Alternatives to Laboratory Animals and Computational Toxicology.
JRC webpage:
https://eurl-ecvam.jrc.ec.europa.eu/laboratories-research/predictive_toxicology

Ada Yonath

Ada Yonath focuses on the process of protein biosynthesis, on antibiotics paralyzing this process and on the origin of life.
She graduated from Hebrew University, earned her PhD from Weizmann Institute, completed her postdoctoral studies at Mellon-Institute and MIT, USA and spent a sabbatical year at the University of Chicago and was a visiting scientist at Max Plank Institute for Molecular Genetics in Berlin.
In 1971 she established the first biological-crystallography laboratory in Israel, which was the only laboratory of this kind in the country for almost a decade. Since then, she has been a faculty member at the Weizmann Institute, were she is also the Director of Kimmelman Center for Biomolecular Structures. In parallel, in 1978 she spent a Sabbatical year in the University of Chicago, and during 1980-2004,

she headed the Max-Planck-Research-Unit for Ribosome Structure in Hamburg while collaborating with Max-Planck-Institute for Molecular Genetics in Berlin.
Among others, she is a member of the US-National-Academy-of-Sciences; Israel Academy of Sciences-and-Humanities; German Academy for Sciences: European Molecular Biology Organization; Pontifical (Vatican) Academy of Sciences; Korean Academy of Sciences and Technology. She holds honorary doctorates from over 20 universities worldwide, including USA, Latin America, Europe and the Far East.
Her awards include the Israel Prize; Linus Pauling Gold Medal; Albert Einstein World Award for excellence; UNESCO-L'Oréal Award for Woman in science; Wolf Prize; the Louisa Gross Horwitz Prize; Erice Peace Prize; Indian Prime-minister medal; Nobel Prize for Chemistry.

CLINAM

European Foundation for Clinical Nanomedicine

10/2017

Basel, May 7–10

ABSTRACTS SPEAKERS

SPIONS – THERANOSTIC TOOLS IN BIOMEDICAL RESEARCH

CHRISTOPH ALEXIOU, Department of Otorhinolaryngology, Head and Neck Surgery, Head Section of Experimental Oncology and Nanomedicine (SEON), Else Kröner-Fresenius-Stiftung-Professorship, University Hospital Erlangen, Glückstrasse 10a, 91054 Erlangen, Germany.

Cancer and cardiovascular diseases remain still the major causes of death, their precise diagnosis is of invaluable importance. Imaging is recognized as pivotal to the management of patients to generate early detections and manage live threatening disease related events. However, these requirements meet groundbreaking developments not only in MRI and radiology but also in many other fields in imaging techniques. Nanotechnology, which has taken an impressive development in the past decades even in partial aspects like magnetic nanoparticles, could contribute essentially to this issue. Nanoparticles have belonged to various fields of biomedical research for quite some time. A promising site-directed application in the therapeutic field of nanomedicine is drug targeting using magnetic nanoparticles which are directed at the target tissue by means of an external magnetic field. Materials most commonly used for magnetic drug delivery contain metal or metaloxide nanoparticles, such as super paramagnetic iron oxide nanoparticles (SPIONs). SPIONs consist of an iron oxide core, often coated with organic materials such as fatty acids, polysaccharides or polymers to improve colloidal stability and to prevent separation into particles and carrier medium. In general, magnetite and maghemite particles are those most commonly used in medicine and are, as a rule, well-tolerated. The magnetic properties of SPIONs allow the remote control of their accumulation by means of an external magnetic field and are the essential of imaging utilizing the intrinsic properties of SPIONs in MRI. The particles themselves can be functionalized with therapeutic agents without compromising their imaging properties. Imaging of tumors can be both viewed from the standpoint of basic research and from the perspective of more application-oriented research. Here, of course, apply different standards. Methods for magnetic nanoparticle based imaging of tumors which are already clinically approved or not far away from their clinical implementation should be able to rely on already established technical equipment. This is the reason why for the new approaches of SPIONs for MRI, a surpassing importance is attached. It is the only method which works on its own and needs no further imaging method in principle. The particles themselves can be functionalized with therapeutic agents without compromising their imaging properties ("Theranostics") and additionally quantified by a new imaging method called MPI (Magnetic Particle Imaging). The suitability to detect primary tumors and metastasis reliably has been already proven. Similar aspects apply for cardiovascular diseases, e.g. the state of the vascularization and identification of plaques.

PROPHYLACTIC EFFICACY OF ORALLY ADMINISTERED BOVINE LACTOFERRIN NANO-CARRIERS WITH TARGETED DELIVERY IN MURINE MALARIA

ANAND NAMRATA[a], Kaur Sukhbir[b], Sehgal Rakesh[a] and Kanwar R Jagat[c].

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INTRODUCTION

Lactoferrin is a milk protein known to have multiple therapeutic functions against diverse range of diseases. Bovine Lf (bLf) has been used as a dietary supplement and preclinical and subclinical studies have shown its role in maintaining intestinal microbial flora, iron metabolism in infants and pregnant women. However, its prophylactic properties have been rarely studied against parasitic infections.

AIM: Present study was aimed to examine the prophylactic properties of bLf and its nanoformulation against malarial parasite Plasmodium using both *in vitro* and *in vivo* model.

METHODS AND RESULTS: bLf was used at a concentration of 40µg/ml with various iron saturations namely mono (single Fe^{2+}), apo (without Fe^{2+}) and holo (two Fe^{2+}) forms. In vitro efficacy of bLf was studied by examining Giemsa stained smears of test and control groups. Confirmation of Giemsa results was further done by using flow cytometry assay (Figure 1.a.). FACS analysis was done to isolate RBCs having parasites (Figure 1.b) and reactive oxygen species (ROS) production (Figure 1.c) in various groups. Eight different populations were separated using two dyes from 2µl culture which had differentiated the efficacy of bLf apo, mono and holo form. Out of all these forms, mono form of the bLf showed best results with minimum infected RBCs (IRBCs), ROS production and therefore chosen for *in vivo* studies (Figure 1).

Figure 1. a.

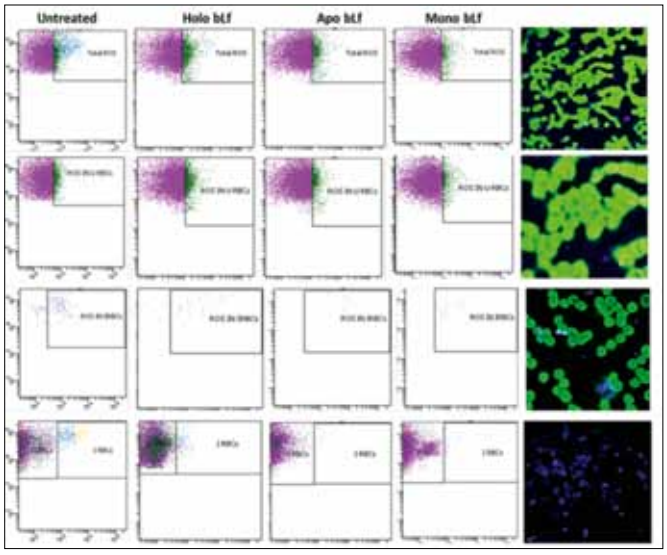


Figure 1. b

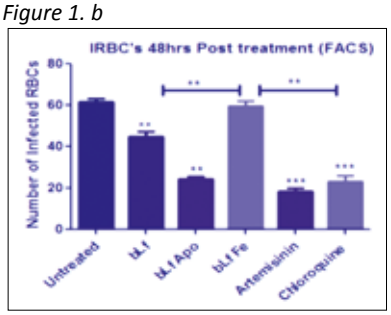
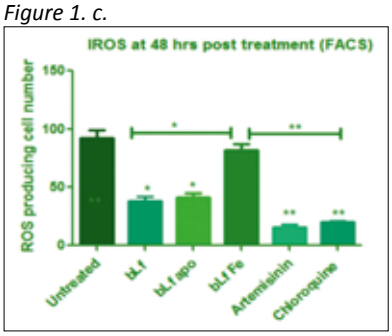
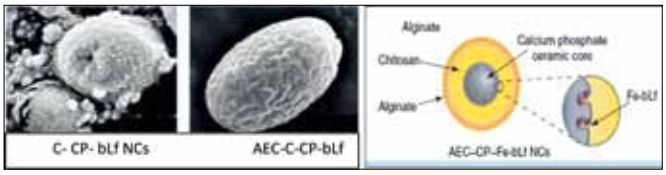


Figure 1. c.



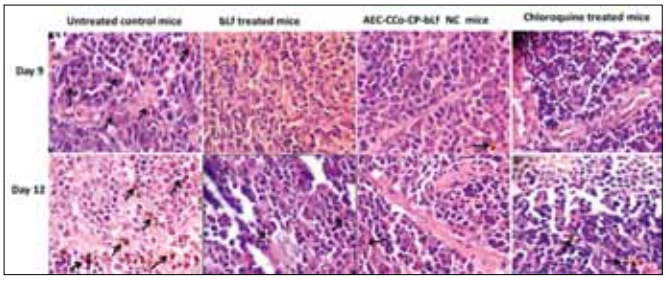
The *in vivo* studies were performed by developing mouse model (prophylactic study), treated with bLf and Alginate Chitosan coated Calcium phosphate bLf nanocarrier (AEC-C-CP-bLf NC) (Figure 2) for fifteen days prior to infection. Infection with P. berghei (NK65) was given on sixteenth day post treatment.

Figure 2.



Prophylactic efficacy of these nanocarriers (NC) against malarial mouse model was assessed by studying histopathology, parasite load, nitric oxide (NO), reactive oxygen species (ROS), immunohistochemistry (IHC) and cytokine levels. Spleen tissue pathology as well as Real Time PCR results (Figure 3) described high parasite load in untreated group followed by bLf, chloroquine and NC treatment groups

Figure 3



Histopathology of Spleen, black arrows showing pigmentation due to RBCs lysis and high parasite count.

NO, ROS levels were found to be elevated in NC treated group compared with other groups which showed protective effect of NC against malarial infection in mice. Bio distribution of bLf was done to assess the presence of bLf in various mouse tissues like, spleen, liver, brain, kidney, blood etc from bLf and NC treated group and showed 2-3 folds increase in availability in NC treated group (Figure 4.a). To identify the location of bLf released from NC at cellular level, IHC was performed on liver and spleen tissues. Positive IHC (development of brown color) was found in Kupffer cells of liver and red pulp of spleen which belongs to the macrophage region of both the organs (Figure 4.b). Immune response studies showed that the levels of Th1 cytokines IL-2,IL-5, IL-12,TNF-α, IFN-γ and IL-17 raised in comparison to Th2 specific cytokines IL-4 and IL-10 levels in NC treated group. Whereas, in the untreated control group the levels of Th2 cytokines like i.e., IL-4, IL-10 were increased 2 fold. This protective response of NC would have helped mice survive till day 35 post infection as compared to untreated group animals who died on day 15 (Figure 4.c)

Figure 4. a.

a. Concentration of bLf in various tissues showing from bLF and NC group. b. IHC of liver and spleen sections showing brown color development showing positivity of bLf in Kupffer and red pulp of liver and spleen respectively. c. Survival curve of various groups.

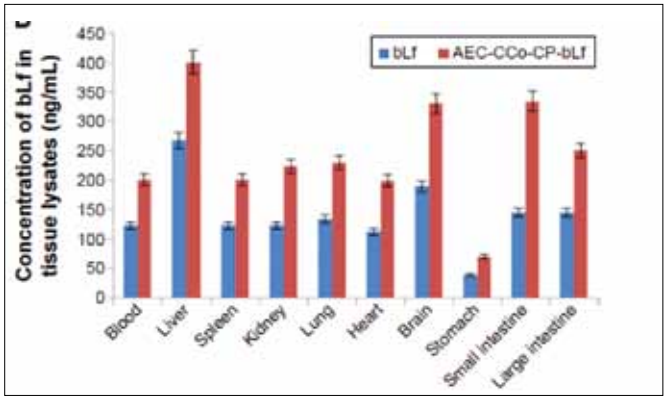
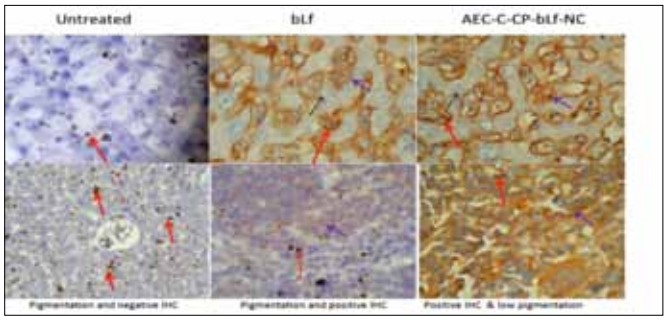


Figure 4. b



DISCUSSION

The present study has highlighted the importance of Lf protein present in the bovine milk by investigating its prophylactic and antiparasitic property against malarial parasite. This is the first ever study which has used nanoformulated bLf as a prophylactic drug to treat murine malaria model with oral administration. To obtain this model, we fed BALB/c mice with the native bLf and NC for fifteen days prior to challenge with P. berghei (rodent parasite) which mimics the human falciparum malaria. Our results had shown that as a prophylactic drug, bLf NC helped in delaying the appearance of infection in blood and visceral organs which was demonstrated through various molecular studies.The above study therefore demonstrates the prophylacticefficacy of bLf NC when used alone or in combination with standard drugs to combat resistance and toxicity. These results will further help us to explore the use of bLf and its nanoformulation as a prophylactic drug against malaria parasite and its use in humans.

DESIGN CRITERIA FOR ENGINEERING PEPTIDE SEQUENCES WITH HIGH AFFINITY FOR INORGANIC SURFACES

PRIYA ANAND, Monika Borkowska-Panek Wolfgang Wenzel, Institute of Nanotechnology, Karlsruhe Institute of Technology, Karlsruhe, Germany

Functionalization of inorganic surfaces with biomolecules especially peptides have gained a lot of interest in the last few years. Coating the surfaces with biomolecules can change the characteristics of surfaces such as hydrophobicity or adhesion properties. This modification increases their application in biotechnology and nanotechnology. At present, the de novo design of a peptide sequences with predictable binding affinities for multiple materials is not an easy task. The project aims to development a method to predict peptides with high degree of affinity for different inorganic materials. The atomistic representation of the inorganic surfaces like gold/silica/iron oxide/titanium oxide are however not easy as surface properties depends on the various parameters like surface preparation and preconditions like pH and temperature etc. We therefore developed EISM (simplistic implicit surface model) arguably the most straightforward, fastest and simplest model for

peptide surface interactions. EISM which can be parametrized by either experimental data or all atom explicit-solvent molecular dynamics simulations for individual amino acids in small peptides, which permits a rapid and accurate computation of peptide interaction profiles with inorganic surfaces. We demonstrate this approach by parametrizing the EISM using existing data for gold (Au {100} & {111}), silver (Ag{111}) and titanium dioxide surfaces, which is subsequently validated for larger peptides with both metadynamics and umbrella sampling simulations against experimental and computational investigations. We also present results on the adhesion of amino acids and small peptides on silica as a function of pH, calibrating EISM using a recently developed force-field for silica surfaces. Availability of the EISM model will open novel opportunities for rapid screening of the interaction profile of peptides to inorganic surfaces. With the availability of accurate surface models, EISM can be easily extended to other surfaces, such as polymers or hybrid materials.

PUBLIC INITIATIVES: THE CHALLENGE OF REGIONAL NETWORKING IN NORTHERN EUROPE

ULF G ANDERSSON, Chairman of NanoMed North and CEO Medeon Science Park & Incubator

NanoMed North is a nanomedicine consortium with more than 140 members. It is a platform for its members and an entry point for international collaborations.
The presentation will include a short description of NanoMed North, including purposes, activities and brief presentations of some of the members' core activities.
Furthermore, the major challenges in setting up and operating a regional network specifically within nanomedicine will be highlighted.

UPDATE ON THE DEVELOPMENT OF AZD2811, AN AURORA KINASE B INHIBITOR, INCORPORATED INTO NANOPARTICLES FOR USE IN HAEMATOLOGICAL AND SOLID CANCERS

MARIANNE ASHFORD, Pharmaceutical Sciences, Innovative Medicines, AstraZeneca, Silk Road Business Park, Charter Way, Macclesfield, Cheshire, SK10 2NA.

One of the major challenges to innovative medicines development remains a lack of therapeutic index[1]. Nanomedicines can enable new drug products by changing a candidate drug's distribution and increasing the drug concentration at tumour sites relative to healthy tissue. This biodistribution change in combination with careful selection of drug release rate from a nanoparticle has the potential to improve both efficacy and safety, thus enabling promising treatments otherwise limited by narrow therapeutic index.

Aurora B kinase plays a pivotal role in cell cycle progression and inhibiting Aurora B kinase results in mitotic catastrophe and cellular apoptosis. AZD2811 is a potent and specific small-molecule Aurora B kinase inhibitor. Its water-soluble dihydrogen phosphate prodrug, AZD1152, Barasertib, has been tested in clinical trials in various tumours. In acute myeloid leukaemia (AML), Barasertib delivered as a seven day infusion showed a significant improvement in complete response rate when compared to standard of care in a randomised phase 2 trial[2]. In solid tumours, however, aurora kinase inhibitors, have shown disappointing activity at tolerable doses[3]. The requirement for continuous intravenous infusion in AML and the toxicity profile in solid tumour indications have limited broader application in the clinic to date.

This presentation will describe the development of AZD2811 encapsulated into ACCURIN® polymeric nanoparticles.

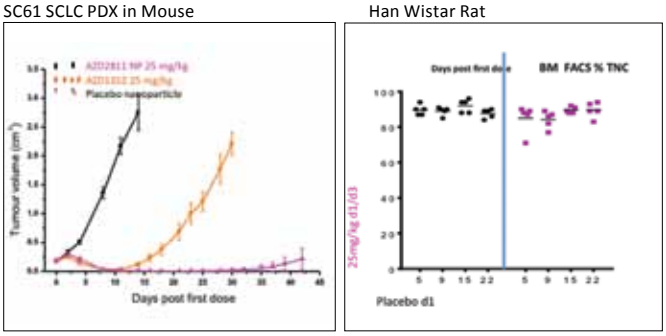
ACCURINS® are composed of block copolymers of poly-D,L-lactide (PLA) and poly(ethylene glycol) (PEG). A proof of principle formulation of AZD2811 in ACCURIN® nanoparticles demonstrated that anti-tumour activity and an improved therapeutic index could be achieved in nude rats bearing SW620 xenograft tumours. In addition the release rate of AZD2811 from the nanoparticles was critical to improving therapeutic index[4].

The clinical formulation of AZD2811 nanoparticles was optimised for drug loading, release rate and encapsulation efficiency using an ion pairing approach[5].

This clinical formulation has been used in different preclinical models to optimise drug delivery for use in both solid tumour settings and haematological disease such as acute myeloid leukaemia. Anti-tumour activity in solid tumours was achieved at doses where bone marrow toxicity is reduced (Figure 1). In contrast for haematological tumours increasing the dose of AZD2811 enables high and prolonged exposure in the bone marrow. In solid tumours, the nanoparticle accumulates in the tumour tissue and releases the drug preferentially in the tumour tissue. This tissue accumulation has been demonstrated using Mass Spec imaging across a number of tumour types. In haematological tumours, the nanoparticle provides an extended systemic pharmacokinetic profile to provide efficacious levels of drug in the bone marrow and overcoming the need, in the case of Barasertib (the pro-drug of AZD2811) to administer drug by continuous infusion for several days. These data demonstrate that drug delivery using nanoparticles is able to resolve therapeutic index challenges, and is able to do so across different disease types.

AZD2811 nanoparticles are currently in Phase 1 clinical trial (NCT02579226).

Figure 1: AZD2811 Nanoparticle has Tumour Efficacy at Doses with only Minimal Impact on Bone Marrow Pre-Clinically



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TAILOR-MADE NANO LIPID GEL ENCAPSULATING MICONAZOLE NITRATE-LOADED NANOPARTICLES IMPROVED ITS ANTIMYCOTIC ACTIVITY

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EXECUTIVE SUMMARY

Phospholipid-modified softisan-based nano lipid gels encapsulating miconazole nitrate (MN) were developed and evaluated for improved treatment of oropharyngeal candidiasis (OPC). Solid lipid nanoparticles (SLNs) formulated by high shear homogenization and incorporating MN were used to prepare mucoadhesive nano lipid gels. Drug release from the nanogels in simulated salivary fluid (SSF, pH 6.8) and anti-candidal activity against oral thrush swab (OTS) of Candida albicans were carried out. The SLNs were monodispersed, with nanometer-sized particles and had good physicochemical properties. MN-loaded phospholipid-modified softisan-based nano lipid gels exhibited better properties than marketed formulation (Daktarin® oral gel) ($p < 0.05$). This study showed that phospholipid-modified softisan-based mucoadhesive nano lipid gels could be employed to prolong localized oromucosal delivery of MN for effective treatment of OPC.

INTRODUCTION

Oropharyngeal candidiasis (OPC) is one of the common fungal infections affecting the oral mucosa. Current imidazole anti-fungals, including miconazole nitrate (MN) used for localized treatment of OPC is limited by low drug bioavailability and frequent administration[1, 2]. Nano lipid gel comprising of SLNs incorporated into a gel base[3], could be used to target imidazole antifungals to the oromucosal layers, for enhanced lethality against resistant C. albicans. The nano lipid gel developed in this study is a novel approach for the delivery of imidazole antifungals, with the specific aim of prolonging the localized oromucosal delivery of MN for effective treatment of OPC.

EXPERIMENTAL MATERIALS

Miconazole nitrate USP (Gutic Biosciences Limited, India), Phospholipon® 90G (P90G) (Phospholipid GmbH, Köln, Germany), Daktarin® oral gel (McNeil Products Ltd., Maidenhead, Berkshire, SL6 3UG, UK), Sabouraud Dextrose Agar (SDA) (United Technology Trade Corp, USA), Polycarbophil (Noveon®) (Lubrizol Corporation, Ohio, USA), Softisan® 154 (Cremer Oleo GmbH, Hamburg, Germany) and distilled water (Lion water, University of Nigeria, Nsukka, Nigeria) were used in the study. C. albicans was a clinical specimen. All other reagents were analytical grade.

PREPARATION OF THE LIPID MATRICES AND SOLID LIPID NANOPARTICLES (SLNS)

The lipid matrices (LMs) composed of combinations of Phospholipon® 90G and Softisan® 154 were prepared by fusion in paraffin oil bath and the thermal properties determined by differential scanning calorimetry (DSC). The SLNs were prepared using MN [0, 0.25, 0.5, 1.0 %w/w ((S0 – S3)], LMs (5.0 %w/w), Polysorbate® 80 (Tween® 80) (2.0 %w/w), sorbitol (4.0 %w/w) and distilled water (q.s. to 100.0 %w/w) by the high shear hot homogenization and thereafter evaluated using encapsulation efficiency (EE%), drug loading capacity (LC), average particle size (z-average), polydispersity indices (PDI), zeta potential (ZP) and morphology.

Preparation and characterization of nano lipid gels

The nano lipid gels were prepared using the mucoadhesive gelling agent (Polycarbophil®, PCP) (1.0 %w/w) SLNs from each batch [to yield 0.05, 0.10, 0.20 %w/w MN (S1-Gel, S2-Gel and S3-Gel)] and glycerol (3.0 %w/w), sorbic acid (0.02 %w/w) and distilled water (q.s. to 100 %w/w) and the pH adjusted to 6.8 using 0.5 M NaOH. The rheological properties, drug content, mucoadhesiveness, drug dissolution in simulated salivary fluid (SSF, pH 6.8), anti-candidal activity against OTS of C. albicans and stability were determined. The results were analysed statistically using analysis of variance and inter- and intra-group variability were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

DSC thermograms confirmed the amorphous nature of the LM and MN in the LM. The SLNs were monodisperse (Figure 1) (PDI = 0.336 – 0.530) and had z-average, ZP, EE% and LC values in the range of 133.9 to 393.2 nm, -30.0 to -39.5 mV, 51.96 to 67.20 % and 19.05 to 24.93 %, respectively. Continuous shear viscometry and dynamic (oscillatory) rheometry indicated that the nano lipid gels were viscoelastic systems. In vitro dissolution profiles (Figure 2) showed that MN-loaded softisan-based nano lipid gels exhibited better prolonged release properties than marketed oral formulation (Daktarin® oral gel). Similarly, MN-loaded softisan-based nano lipid gels gave better anti-candidal properties than marketed oral formulation (Daktarin® oral gel) owing to the higher inhibition zone diameters obtained (Figure 3a). The formulations were also stable and possessed suitable mucoadhesive strengths (Figure 3b) for prolonged delivery of MN in the treatment of OPC.

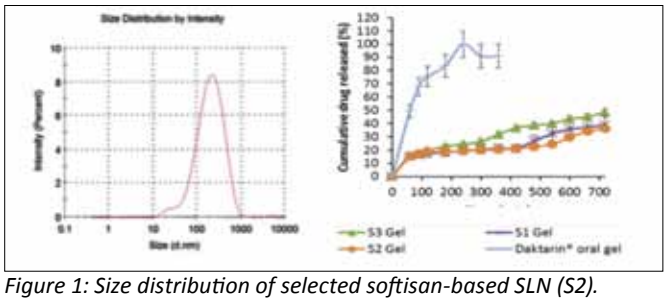
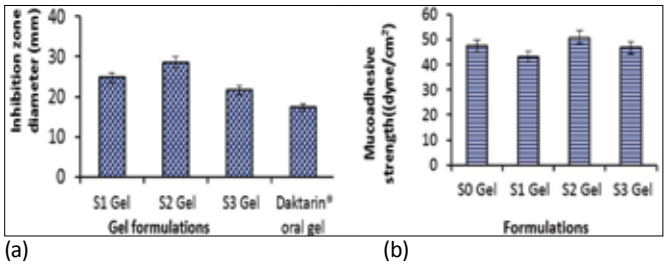


Figure 1: Size distribution of selected softisan-based SLN (S2).
Figure 2: Dissolution profiles of softisan-based nano lipid gels in SSF (pH 6.8).



(a) (b)

Figure3: Anticandidal efficacy (a) and mucoadhesive property (b) of softisan-based nano lipid gels

CONCLUSION

This study has shown that phospholipid-modified softisan-based mucoadhesive nano lipid gels could be employed to prolong localized oromucosal delivery of MN for effective treatment of OPC.

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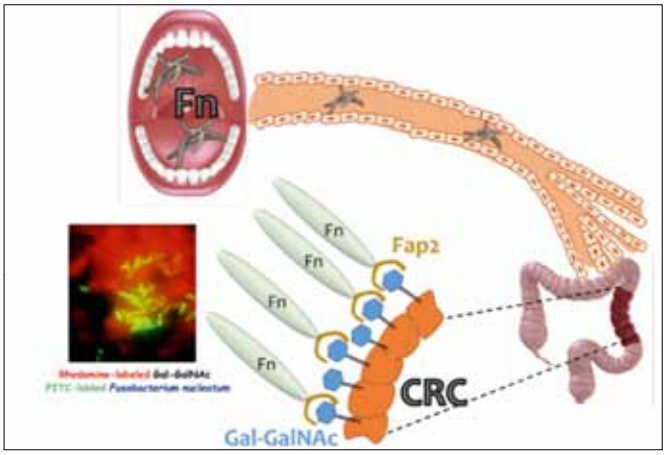
HOMING OF FUSOBACTERIUM NUCLEATUM TO COLON CANCER

GILAD BACHRACH, Jawad Abed, Lishay Prahi, Naseem Maalouf, Chamutal Gur, Ofer Mandelboim

Fusobacterium nucleatum is an oral anaerobic bacterium that is associated with the development of periodontal disease.
Surprisingly, F. nucleatum was recently found to be overabundant in colorectal carcinoma (CRC). In CRC, fusobacteria are hypothesized to accelerate tumor progression by promoting cellular proliferation, inducing the silencing of tumor suppression genes and by suppressing antitumor immunity.
Bleeding is frequent during periodontitis and provides an easy access for the oral bacteria to the circulatory system. We therefore hypothesized that oral fusobacteria might translocate to colon cancers via the hematogenous route.
We show here that F. nucleatum intravascularly injected to CRC-bearing mice, rapidly colonizes the colon tumors. This colon colonization is tumor dependent as hematogenous fusobacteria were not found in the colons of mice without CRC. It is also bacterium specific as Porphyromonas gingivalis (the major pathogen associated with periodontitis) used as control, was not found in the colon tumors. CRC-specific colonization by F. nucleatum is mediated by attachment of the Fap2 fusobacterial Gal-GalNAc- specific- lectin to Gal-GalNAc overexpressed in mouse and human CRC. In the tumor, Fap2 activates the human TIGIT inhibitory receptor and inhibits killing of cancer cells by human Natural Killer (NK) cells, and by Tumor Infiltrating Lymphocytes (TILs).



Hematogenous translocation of F. nucleatum from its oral reservoir to colon cancer. Blood-borne oral F. nucleatum bind Gal-GalNAc overexpressed in colon cancer using the Fap2 Gal-GalNAC – specific fusobacterial lectin.

HOW TO ACCESS AND USE TRANS NATIONAL ACCESS?

SIMON BACONNIER

The use of nanotechnologies in healthcare promises to tackle major medical challenges. However, the manufacturing process of nanomedicines is potentially complex and inconsistencies must be carefully assessed before clinical applications can be considered. Furthermore, safety concerns related to the use of nanomaterials must be addressed as early as possible during product development.
The European Nanomedicine Characterization laboratory (EU-NCL) will address these issues by providing the critical infrastructure and characterization services required to analyze physical and chemical attributes, *in vitro* biological properties, and *in vivo* characteristics of nanomedicines under development.
With this objective, EU-NCL will have to integrate de high diversity of materials to be analyzed. Actually, the current nanomedicines can be organic, inorganic, metal based, combined or loaded with active compound. Thus, EU-NCL will have to adapt and optimize its characterization capacities and strategies to the complexity of the product to be characterized as well as their different uses (Diagnosis, Treatment, targeted or not). On top of that, there is a strong need for high quality data, produced with standardized methods in line with regulatory requirements and standardization needs supported by robust and established standards and controls. This rigorous frame is the only way toward quality level that may serve the users of EU-NCL in their future clinical development.

PUBLISH OR PERISH IN NANOMEDICINE: THE JOURNEY FROM EXPERIMENT TO PUBLICATION

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Executive Editor, Manuscript Clinic

Getting published is crucial for academicians and researchers especially nowadays, when we are generating knowledge faster than ever before. Publications are ever more important for scholars and publishers, although for quite different reasons. Authors increasingly feel the pressure to 'publish or perish' and publishing companies are placing more and more emphasis on business aspects and relentlessly looking for new ways to generate more revenue. In nanomedicine, only about 1/3rd of authors are native speakers of the English language. For everyone else, especially in Asia, it is a challenge to write scientific manuscripts. Junior authors also often learn by the "sink or swim" method, and lack insight. Although all journals instruct them what to do, they won't tell you how to do it. Due to technological and social advances, scientific publishing is undergoing dynamic changes, even though the essential general question remains the same: how can value of research be determined before and/or after making it public, and how to monetize this value. The discussion about science publications has now expanded to the whole society and a number of articles have recently appeared in well-known journals (Science, Nature, The Economist, etc.) questioning the value and methods of scientific research itself. In the first part of this talk, the speaker will summarize major changes in publishing business, describe the use and abuse of "impact", and introduces the latest scientific methods to objectively estimate the prestige of journals. He will talk about publicly available scientific tools and web sites that should be used to evaluate publications of journals, individuals, institutions, and countries in different fields.
In the second part, Dr. Balogh will reveal recent trends in nanomedicine publications, and compares their output and impact. Finally he will share a few principles to write successful scientific publications. A question-and-answer opportunity will also be provided.

NANO-MUPIROCIN INJECTION: PARENTERALLY ACTIVE MUPIROCIN

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BACKGROUND

Using computational approaches we have recently discovered that the antibiotic mupirocin is an excellent candidate for being remotely[1–3] and stably[4] loaded into pegylated nano-liposomes[1]. Mupirocin is an antibiotic with a unique mechanism of action (inhibition of isoleucyl-tRNA synthetase) which is not shared by any other antibiotic. Its rapid systemic degradation and elimination prevent its parenteral use and limit its therapeutic application to topical use only. We proved the utility of our computation modeling by applying it to optimize a formulation of liposome based Nano-mupirocin. The optimized formulation is ~80nm pegylated nano-liposomes which resemble Doxil in its lipid composition and size distribution. Mupirocin which is an amphiphatic weak acid was remotely loaded using a transmembrane gradient of calcium acetate.[1–4] The rate of drug release in plasma *in vitro* and *in vivo* was slowed down and controlled by hydroxypropyl-beta-cyclodextrin (HPCD) present in the intraliposomal aqueous phase[1]. Nano-mupirocin is aimed to enable mupirocin to become a therapeutically efficacious parenteral antibiotic by protecting it in the circulation while passively targeting it to the infected tissue due to the enhanced permeability and retention (EPR) effect which occurs in bacterial infections. An ongoing stability study demonstrates an excellent chemical and physical stability for >1.0 year.

One of the major appeals of Nano-mupirocin as an antibiotic is its potential to exhibit excellent activity against "serious and urgent threats" including methicillin-resistant S. aureus (MRSA), S. pneumoniae and N. gonorrhoeae. This stems from mupirocin's low MIC values that were observed against these bacteria.

METHODS

The comparison of efficacies of Nano-mupirocin vs free mupirocin injection was tested in three clinical relevant animal models5: 1) mice necrotizing fasciitis caused by M14 group A streptococci 2) rabbit endocarditis due to MRSA 3) mice osteomyelitis caused by S. aureus. The pharmacokinetic (PK) profiles were

Passive targeting to infected tissues was demonstrated from the distribution of mupirocin in the wounds in necrotizing fasciitis and in the tibia in the osteomyelitis model.

RESULTS

In the rabbit endocarditis model, animals treated with Nano-mupirocin at 25 mg/kg, I.V, daily, for three days showed 57% survival vs no survival by the free mupirocin and untreated control groups. In the mice necrotizing fasciitis model, Nano-mupirocin administered either prophylactically at 15, 25 and 50 mg/kg or 5 h after the bacterial challenge (at 50 mg/kg) resulted in 100% survival vs 44% and 38% mortality in the control and free mupirocin groups, respectively[5]. Nano-mupirocin in the osteomyelitis model showed increase in body weight vs decrease in the free and blank liposomes groups and serum IL-6 levels resembled uninfected mice compared with high levels in the free and blank liposomes groups.

The PK profile of Nano-mupirocin showed much higher exposure and longer half-life compared to the free drug. Consistent with the PK data, Nano-mupirocin was detected in the wounds as well as in the tibia while no mupirocin was detected in the free mupirocin group.

CONCLUSIONS

We demonstrate that parenteral Nano-mupirocin is therapeutically efficacious. The prolonged plasma PK profile enables mupirocin accumulation in the infection site to exert its therapeutic activity. Our studies indicate that Nano-mupirocin may be a novel parenteral antibiotic.

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WHEN EDUCATION MEETS NEW LEARNING TECHNOLOGIES AND SOCIAL MEDIA

JACK BAROKAS

The emerging new technologies are changing our daily environment, at home, at work, on vacation - almost everywhere. To keep up with these changes, we need to learn new things every day and eventually we are all becoming lifelong learners. The growing need for lifelong learning and rapidly developing technologies introduce a new challenge to contemporary formal education – but before we talk about this challenge, let's go through the short version of the "history of education and training".

Even the short version of education goes back, as early as the Stone Age or even before, to when people just started living together. Imitation was probably the first learning/teaching mode in those times. Later on, observing, imitating and producing some meaningful sounds evolved to language and paintings on cave walls, evolved to writing. The use of language and writing enabled us to instruct and train each other in more efficient ways than just observing and imitating.

By those times there was no efficient means to distribute and preserve knowledge.The only way knowledge could be preserved and accumulated was by adults teaching and educating the youngsters, using the knowledge which they inherited from when they were youngsters.

After the usage of language and writing for communication, the next meaningful event in the evolution of education was the invention of print. At first, books were very rare and were possessed by small limited numbers of people. After books were printed in large quantities, knowledge started spreading rapidly and widely, weakening the monopoly of knowledge and information held by the very few who had access to hand-written books.

Following print, new means of mass communication like newspapers and electronic communications (such as radio and television) start emerging - but the introduction of the internet to the general public was the most important breakthrough of all. This breakthrough brought very radical changes regarding the way we now communicate and consume information.

The internet burst out of its very narrow founder's neighborhood and became utilized widely by the public in general, starting with only few contributors providing information to many users (web 1.0). Later come web 2.0, with many users making two-way utilization of the web by uploading information, and now we are talking about web 3.0 - "The semantic executing web". This refers to bridging the communication gap between human web users and computerized applications, where not only human users are sharing information but machines are asking each other meaningful questions and processing the data for different use cases.

Now, maybe in a way, we can look to the contemporary internet, as a huge communication network, connecting incredibly large amount of computers and people together. As an analogy, it can be compared to a huge artificial brain where computers with large computing capacity and memory join forces with human user's intelligence, working in synergy. The internet with the means of fast communication, grid of computers with almost limitless computation power and cloud services might bring us to new frontiers which were beyond the horizon until not many years ago.

This wide availability of information and the need for lifelong learn-

ing in the recent decade has brought about dramatic changes in accepted learning/teaching paradigms, mainly in higher education but not only. In most higher education and continuing education frameworks, the teacher no longer holds the monopoly on knowledge and, in some cases, students in class might even have more knowledge than their teachers on specific topics.
In the new student-centered learning paradigm, the teacher's role becomes more like being a mentor, providing scaffolding for students to build knowledge. Knowledge is an individual process and can't be transferred 'as is' to the learner. Learning processes can be very diverse among learners; there are many different learning profiles: Some learners need to be with other people while learning, for example in a class room and well-defined time framework, others prefer to be alone. Some can stay in focus for a two - hour long lecture; others might lose focus after 10 minutes. In the traditional education/training framework, it has not been possible to address this diversity of learning skills and thinking/learning styles in order to individually optimize the learning process.
The very same technology which brings about the large need for education and training and represents a major challenge to the education system, can also introduce solutions to this challenge. We need to think of the technology as the solution, not only as the problem.
The new learning/teaching technological tools, developed by the academia (mostly based on open source solutions) and some commercial companies, now enable learners to learn at their own pace, using widely available computers and hand held devices such as tablets and smart phones. Advantages of technology- assisted learning, in addition to offering anywhere, anytime learning, include the ability to choose the way of learning which best fits the individual learner's learning skills. Technology-assisted learning can be fully online, independent learning (as in MOOCS), but it can also be hybrid learning as in the flipped classroom concept. Online learning, whether fully online or hybrid, requires self-discipline, and is therefore more suitable to higher education and industry, where learners are more aware of the benefits learning can bring and how learning can contribute to their careers.
One of the most popular new technological tools is the learning management's systems (LMS). The LMS provides learners with an environment which aggregates all their learning resources in one online space, and also helps the teachers in monitoring and coordinating the learning/teaching processes. Future learning/teaching systems will probably be able to individually monitor each learner's learning profile, including which learning resources such as video, slides, text, simulations etc. can provide the learner with individually optimized (adaptive learning) learning paths.
Along with various new technologies developed for formal education, social media tools like: Facebook, Twitter, Instagram, YouTube, Snapchat are also slowly becoming part of teaching/learning scenarios. The very extensive use of social media probably will bring to mind the first learning/teaching tool - Imitation - which I mentioned at the beginning of "The short story of education". Now that social media easily enable everyone to send a post on what they are doing, what they are wearing, what their opinions are, anyone can be a model for imitation to many followers, sometimes even to many millions (especially when the posts are made by celebs).
Wide use of social media introduce questions like: whether it can be considered a type of educational environment? Can it be manipulated? Can it be an efficient tool for education? These all are open questions but due the very extensive use of the social media, especially among the young future generation, these tools should be more extensively explored and engaged in controlled ways in the teaching/learning and training process.
Hopefully the new emerging teaching/learning tools, together with new educational paradigms, can meet the growing need for learning/education/training and beyond. After all, it is not so difficult today to assume that in the near future, we will able to set up a virtual reality space, where learners will interact in real time, with teachers using telepresence technology and intelligent computer's software simultaneously to have the perfect learning experience tailored and adapted just for them.

A NANO-THERANOSTIC APPROACH FOR NEURODEGENERATIVE DISEASES: FROM INNOVATIVE BRAIN "NANO-BIOPSY" TO NANO PREVENTION.

FRANÇOIS BERGER

We are still dreaming of targeted therapies to slow down neurodegeneration that is the main bio-economic, societal and scientific challenge for European aging citizen. In oncology, direct access to fresh tissues has paved the way for molecular deciphering of oncogenesis providing dramatic therapeutic progress. Surgery or micro-invasive biopsies are key technologies for such improvement in cancer therapy. Neurodegenerative brain remains inaccessible, only post-mortem brain being available with inherent biases due to agonal and postmortem interval processes. Neurostimulation provides a unique non-lesional access to the neurodegenerative brain that is modulated by deep brain stimulation (DBS). AL Benabid in Grenoble demonstrated the therapeutic impact of neurostimulation in Parkinson's disease further extended to dystonia or psychiatric disorders.
Exploiting this unique non-lesional access to the human brain, we developed a bio-harvesting strategy to capture molecular information from brain cells and from the pathological microenvironment in contact with the DBS stylet. A specific kit was developed to address "sample prep" standardization in the surgery room, allowing mixed global DNA, transcriptome, epigenome and methylome analyses of the retrieved cell populations. Moreover, we developed a second-generation technology, involving nanoporous silicon providing a unique spatial in situ bioharvesting of brain tissues.
We are now developing all the regulatory and ethical prerequisites to translate this technology in a proof of concept clinical trial. Nano-electronic fabrication process needs to be implemented in the clean room. We anticipated the need for second generation nano-toxicology investigations, introducing in situ proteomic imaging and sequencing in non-human-primates.
Risk/benefit ratio in Parkinson disease is not risk benefit ratio in Glioblastoma (median survival 15 months). Is it ethical to start this kind of protocol directly in Parkinson disease? A strong questioning is also emerging from the society about the absence of research dealing with prevention and by the absence of effective European ban of pesticides. Moreover, nano-ferromagnetic contamination of the brain could be also an etiology for neurodegenerative diseases. Interestingly, nanotechnologies are also a unique opportunity to monitor and may be neutralize the nano-environmental risk.

BIOCOMPATIBILITY OF NANOMEDICINE APPROACHES FOR ARTHRITIS: FOCUS ON CARBON NANOTUBES

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Arthritis is a disease introducing irreversible changes in the articular tissues and skeletal muscles. One of its form, osteoarthritis causes change in the homeostasis of the cartilage region from cartilage-producing (anabolic) chondrocytes to cartilage-degrading (catabolic) chondrocytes leading to degradation of cartilage. Even

though significant research is ongoing in the field of osteoarthritis disease, and several intra-articular anti-inflammatory drugs are available in the market, their efficacy is short-lived. This is due to fast clearance by the resident immune cells and complexity of the cartilage region biological matrix formed of collagen fibers and proteoglycans[1]. Nanodrugs have shown multifunctional ability to efficiently deliver drugs, imaging payloads and as therapeutic agents. While recent studies have shown that poly (ethylene glycol) functionalized (pegylated) single-walled carbon nanotubes (PSNTs) can efficiently deliver therapeutic cargo to chondrocytes[2], concerns remain on the biocompatibility and the ultimate fate of the nanomedicines in the human body. Carbon based nanomaterials have shown significant promise in the field of nanomedicine applications both as nanovectors and nanotherapeutic agents[3]. Moreover, cells of the immune system were shown to mediate oxidative degradation of CNTs and we have demonstrated that PSNTs are also susceptible to biodegradation[4]. However, whether PSNTs undergo biodegradation in the knee-joint has not been evaluated before. Here, we studied the biodegradability of PSNTs *in vivo* in a mouse model of intra-articularly injected PSNTs using confocal Raman microscopy-spectroscopy. These studies were complemented with *in vitro* studies of biodegradation using relevant cell lines to study the underlying mechanism in more detail.

The PSNTs were functionalized with 2 kDa poly (ethylene glycol) chains and subjected to thorough physico-chemical characterization. To explore the *in vivo* fate of PSNTs, we studied the residence, trafficking, and biodegradation of intra-articularly (IA) injected PSNTs in the knee-joint of healthy mice at different time-points post-exposure following previously published procedures for injection[2]. To this end, a label-free detection method based on confocal mapping of the Raman intensity of the PSNTs asymmetric mode peak (D-band, ~1350 cm–1) and that of tangential C–C stretching modes (G-band, ~1598 cm–1), as well as the D:G-band intensity ratio was employed to track the PSNTs in the different regions of the knee-joint of healthy mice and to assess their degradation in situ. We found that the IA-injected PSNTs persisted for more than 2 weeks in healthy mice knee-joints and penetrated into the cartilage and the meniscus region, while egressing thereafter from the joint cavity through the synovium. Raman D:G band ratio analysis showed that oxidative degradation of the PSNTs on day 3 after IA administration. By 21 days most of the PSNTs were found to be biodegraded or trafficked out of the knee-joint. To further study the biodegradation mechanism, we exposed the murine chondrogenic ATDC-5 cell line activated with IL-1β and murine RAW 264.7 macrophages to PSNTs and analyzed the oxidative damage by Raman spectroscopy. Significant biodegradation of the PSNTs was evidenced in IL-1β stimulated chondrocytes and this was prevented when the cells were co-incubated with a pharmacological inhibitor of inducible nitric oxide synthase (iNOS). Biodegradation of PSNTs in RAW 264.7 macrophages was less pronounced, but increased when cells were exposed over a longer period of time to the PSNTs. The latter findings are in line with the recent demonstration of biodegradation of non-pegylated CNTs in macrophage differentiated THP.1 cells[5]. Taken together, the present study has demonstrated that PSNTs are cleared from the knee-joint through biodegradation in the cartilage region and digested *in vitro* in activated chondrocytes. Our study paves the way for the use of PSNTs as safe nanosystems in the treatment of osteoarthritis.

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WHY DOES EUROPE NEED EUNCL?

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With more than 500 SMEs and a strong research community with more than 1500 academic labs in nanomedicine, Europe offers a significant potential for developing nanomedicines… if the necessary conditions for moving efficiently the discoveries in labs into products validated in clinics are established. ETPN, The European technology Platform on Nanomedicine, highlighted in 2013, in its White Paper on Nanomedicine under Horizon 2020, the necessity to overpass the observed bottlenecks in nanomedicine development which are

- Nanomaterial fine characterisation, before the regulatory approval
- Scale up manufacturing of clinical batches for the early clinical trials
- Accompaniment of entrepreneurs on industrialization and strategic development

In close cooperation with the Directorate General for Research and Innovation of the European Commission, three complementary initiatives have been funded between 2013 and 2015 to set up the relevant infrastructures or services at the disposal of nanomedicine developers. They form together the ETPN Nanomedicine Translation Hub.

Part of ETPN Nanomed Translation Hub is EUNCL. EUNCL, the European Nanomedicine Characterisation Laboratory, aims at fostering innovation in nanomedicine by providing access to state of the art full characterisation of nanomaterials intended for medical applications, developed by public labs, spin –offs and innovative SMEs. The EUNCL also serves as a European knowledge base for researchers and industry ensuring that European knowledge is documented in Europe for the benefit of the European economy, healthcare systems and patients.

EUNCL's mission is to provide a trans-disciplinary testing infrastructure covering a comprehensive set of preclinical characterisation assays (physical, chemical, in-vitro and in-vivo biological testing) allowing researchers to fully comprehend the biodistribution, metabolism, pharmacokinetics, safety profiles and immunological effects of their medical nanoparticles. But EUNCL is also fostering the use and deployment of standard operating procedures (SOPs), benchmark materials, and quality management for the preclinical characterisation of medical nanoparticles (nanoparticles used for medical applications) to promote inter-sectorial and inter-disciplinary communication among key drivers of innovation, especially between developers and regulatory agencies.

To fulfill its mission EU-NCL aims to achieve 4 major objectives:

- **Objective 1:** To qualify a comprehensive portfolio of medical nanoparticles preclinical characterisation assays (more than 40 assays) within an efficient collaborative environment over the first year of EUNCL.
- **Objective 2:** To provide preclinical characterisation of 15 medical nanoparticles to researchers from academia and industry developing Med-NPs by opening trans-national access (TNA) the second year of EUNCL.
- **Objective 3:** To constantly refine and upgrade the assay portfolio

and processes of EU-NCL.
As nanomedicine is a fast evolving field of research, it is a key objective for EUNCL to constantly refine and adapt its assay portfolio and processes in order maintain the provision of state-of-the-art TNA to the scientific community. Therefore, we will progressively implement additional assays to increase our characterisation capacity, for instance in terms of medical application or route of administration.

- **Objective 3:** To disseminate the EU-NCL findings to the nanomedicine stakeholders in order to strengthen the innovation potential in that field.
  The emphasis of EUNCL is to serve as a nexus for trans-disciplinary research, development and clinical applications of nanotechnology. Therefore, lessons-learned, best practices, knowledge, tools and methods will be made available to the scientific community such as academic researchers, industry, regulatory bodies, metrology institutes and others. However, care will be taken to ensure that proprietary information and materials disclosed to the EU-NCL by the TNA users are protected.

EU-NCL provides a comprehensive set of characterisation tests (physical, chemical, *in vitro* and *in vivo* biological properties) allowing researchers and SMEs to better understand or predict the clinical *in vivo* effects of their medical nanomaterials. On top of that, a full characterisation is required by regulation agencies before approval of any tests on human beings. The knowledge base being developed by EUNCL will help the European Medicines Agency (EMA) or other relevant agencies (e.g. notified body) to adapt the current regulation and approval process to Nanomedicine products. Further links with Satellite Labs
The EU-NCL also has a strategic and political role in helping newcomers, like spin-offs or SMEs, in getting an easy access to nanocharacterisation and further to prepare their submission for product approval.
EUNCL is supported by 8 partners, connecting their 7 analytical platforms over Europe and USA. EUNCL is in operation since May 2015. Its Transnational Access is open to all developers of nanomedicines from August 2016.
More info at : www.euncl.eu

EUROPEAN AND AMERICAN PHARMACOPOEIAL EFFORTS TO DEFINE QUALITY AND FACTS OF NBCDS

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In the context of ensuring the quality and safety of medicines, pharmacopoeias, as standard references for pharmaceutical drug specifications in the form of monographs, play a pivotal role. Pharmacopoeias appeared as early as 50 AD (De Materia Media). In Great Britain, the national British Pharmacopoeia (BP) was published for the first time in 1864, the United States Pharmacopoeia (USP) already in 1820. In addition to such national pharmacopoeias, international forms do exist as well and may replace national ones. The foundation for the European Pharmacopoeia (Ph. Eur.) was laid in 1964 by a convention of the Council of Europe, and the first volume of the International Pharmacopoeia (Ph. Int.) was published by WHO in 1951. Having the common goal to assure access to good quality medicines, the organizational forms of these pharmacopoeias and the procedures followed to introduce new monographs differ largely.
Complex drugs of non-biological origin (NBCDs) share aspects of complex structure, potential immunogenicity and impossibility of full characterization by physicochemical methods alone with biological complex drugs. Examples for these complex drugs include "nanomedicines" such as glatiramoids (Copaxone®), liposomal formulations (Doxil®), and nanoparticles such as iron-carbohydrate particles (Venofer®). Their size and attributes at the molecular scale confer these systems certain properties to interact with their biological environment. For all three NBCDs monographs are in existence, or currently under discussion.
This presentation will give a brief overview on such issues, and analyze the level of awareness for NBCDs at the European and US pharmacopoeias.

NEW ANALYTICAL TECHNIQUES UNDER INVESTIGATION

SVEN EVEN BORGOS

The EU-NCL (European Nanomedicine Characterisation Laboratory) H2020 project was started to provide preclinical safety data on candidate nanomedicines, with the aim to bring more nanomedicines into clinical trials and eventually into the clinic.
One part of the EU-NCL project is to identify, assess and implement novel characterization techniques for nanomedicines, improved in terms of specific parameters like speed, cost, sensitivity, robustness – or that can provide new data types to improve the scientific foundation used to draw conclusions on nanomedicine safety (and, if possible, efficacy) in the preclinical setting.
The current lecture will describe the EU-NCL technology enlargement concept and current outcome.
A survey among expert scientists was performed to assemble a list of promising candidate technologies for improved characterization of nanomedicines. Ranking of these technologies was performed with a set of criteria, and three technologies are currently in EU-NCL laboratory trials, with the aim to improve and/or complement existing standard assays. These three are NMR spectroscopy for physicochemical parameters, WST8 cytotoxicity assay, and selected flow cytometry based assays for cell viability and toxicity.
Among the technologies under assessment for future improvements to the assay cascade are several advanced imaging technologies, including mass spectrometry imaging for biodistribution studies, as well as high-throughput/high-data-content techniques.

MULTIFUNCTIONAL NANOPARTICLES FOR OSTEOARTHRITIS THERAPY AND DIAGNOSIS

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Osteoarthritis (OA) is the most common form of arthritis, and is characterized by progressive degradation of articular cartilage, variable synovitis and structural changes of the subchondral bone. Over the course of years, it leads to pain, functional impairment and, ultimately, disability. The delivery of therapeutic, as well as diagnostic, small molecules and macromolecules into joint sub-compartments is ineffective through both systemic and local routes of administration. In polyarticular degenerative joint disorders, such as hand OA, a systemic route is required; however, it can lead to sub-pharmacologically effective doses in the target joints with substantial risk of systemic adverse effects. Alternatively, more accessible diarthrodial joints are treated through intra-articular (IA) administration, thus increasing drug amounts in the joint cavity while decreasing total dose, which limits off-target effects and the risk of systemic adverse effects. Although local administration is advantageous to increase drug bioavailability while reducing exposure to inappropriate sites, local use of small molecules and macromolecules to target joint sub-compartments is hampered by rapid clearance from synovial cavity. Consequently, frequent injections are often necessary to enable a pharmacologically relevant amount

of drugs to reach a specific site, with increased risk of patient discomfort and infections. Because of all these disadvantages related to the use of free drugs, multifunctional nanoparticles (MNPs) have been developed to extend drug joint residence time. The development of MNPs is a complex process that involves the tuning of their physico-chemical properties - the so called synthetic identity - in such a way that the biomacromolecules adsorbed onto nanoparticles will, as soon as they enter the joint milieu, confer a specific biologic identity defining, in large extent, MNP therapeutic and diagnostic efficacy. A global assessment of MNPs must also take into consideration the cost of generation, complexity, and potential regulatory hurdles. The few MNPs that have been approved by regulatory agencies and have been translated into clinical trials are mostly for cancer therapy and diagnosis. The clinical benefits of MNPs in cancer have recently driven academic research toward their application in OA. In this talk, I will describe the MNPs that my research group and others have developed to deliver therapeutic and diagnostic molecules to specific locations of OA joints. I will also discuss potential mechanisms driving the trafficking and toxicity profiles of MNPs in the joints.

THE POLITICS AND POLICIES OF IMPLEMENTING PERSONALIZED MEDICINE

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Rapid scientific advances in genomics such as in the light of epigenomics, microbiomics and systems biology supported by new ICT solutions and enabling technologies not only contribute to the understanding of disease mechanisms of common and rare diseases, but also provide the option of new promising applications in health management during the whole life-course of an individual. What was little time ago a vision for a new era of public health, in which advances from the 'omic sciences would be integrated into strategies at population and sub-population level, is now responding to the very pressing need for the development of effective personalised health and care going even beyond personalised medicine. So far, all stakeholders including policymakers and the private sector are struggling to translate the emerging knowledge into public health. Public Health Genomics (PHG) is the area of public health ensuring that scientific advances in 'omics ("from cell...") triggered by innovative technologies are timely, effectively and responsibly translated into health policies and practice for the benefit of population health ("...to society"). The implementation of personalised health and care requires global concerted action. Europe is highly committed to it. The European Commission (EC) has allocated large part of its research budget toward personalisation of medicine and health. The Coordination and Support Action (CSA) PerMed developed in 2015 the Strategic Research and Innovation Agenda (SRIA) for Personalised Medicine (PM) that elucidates the five big challenges Europe faces to bring forward the implementation of PM. With the launch of the International Consortium of Personalised Medicine (ICPerMed) in November 2016, European countries aim to coordinate health research policy to advance the implementation of PM. The initiative brings together the EC and health research funders and policy making organisations from 28 countries and five regions from Europe and Canada.

DISSEMINATION FOR LAY PUBLIC IN NANOMEDICINE: A GAME FOR NANOMEDICINE IN ATHEROSCLEROSIS

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To most lay people, nanotechnology is something to do with high tech. Fancy materials with strange particles, perhaps OK for paints or tennis rackets, but if it comes close to me, maybe it's a bit scary, certainly unfamiliar. The EC FP7 NanoAthero project is demonstrating the preliminary clinical feasibility of nanosystems for imaging and treating atherosclerotic plaques to reduce the likelihood of heart attacks and strokes. But how will people react, say, to magnetic nanoparticles in their body for an MRI scan of their heart? As nanomedicine becomes more established in clinical use, it is important that we build bridges to the general public and patients. There are many ways to disseminate, but perhaps the best is to engage with people, interactively, where they are.
In the NanoAthero project, one way we have tried to address this is to create a Democs* card game. The concept is a group discussion for 6-8 people, using cards, which can be played anywhere, something to do with your friends one evening. It needs no prior technical knowledge - the cards are the 'expert'. It uses three sets of cards case studies and factual cards to introduce the scientific topic in ways understandable to lay people, and then 'issue cards' to present ethical and social implications for discussion. It's not a game to play and win. The aim is to stimulate informed understanding and discussion in a group setting, where people learn together, benefitting from each other's different viewpoints to enrich, challenge and help to make individual and collective understanding. At the end, participants are invited to give written statements and opinions, and these qualitative outputs can then be analysed. We are alsp seeking in the NanoAthero project to draw upon the potential of social media to extend the Democs concept as an on-line group tool, using gaming platforms, with a global reach. In this talk we will present the concept, some of the first results from playing the game, and explore its potential for wider use.
* Democs (also known as Decide) was invented in 2001 by Perry Walker, then of the New Economics Foundation, and has been used successfully on subjects as diverse as climate change and tuberculosis. The NanoAthero game is available via www.nanoathero.eu and www.edinethics.co.uk/democs/nanoathero
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IMPACT OF DRUG RESISTANT PARASITES ON DISEASE CONTROL

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Neglected tropical diseases (NTDs) still represent a tremendous health burden to a significant part of the 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 many of these diseases, especially drugs which often lack efficacy and safety or require long and complicated application.
Good progress could be reached for the hemoflagellate disease human African trypanosomiasis or sleeping sickness. The Drugs for Neglected Diseases initiative (DNDi)1 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[2]. Hundreds of patients were treated and cured so far. The second one is the benzoxaborole SCYX-7158, another oral drug with excellent pharmacokinetic properties that allows for single dose treatment[2]. It passed safety in humans and will soon

be tested in patients. For malaria, Medicines for Malaria venture (MMV)[3] is the leading product-development-partnership with the most extensive drug pipeline ever. New chemical entities and new drug combinations will be brought to the patients during the next years. An additional benefit will be the extended efficacy of these new molecules against liver stages and gametocytes, thus addressing Plasmodium vivax and transmission blocking. Most pathogens tend to lose sensitivity to drugs after extensive use, this is especially the case for bacteria and protozoan parasites. In malaria, all drugs which came into use lost its efficacy within a few years. This was a dramatic development for chloroquine, sulfadoxine-pyrimethamine, mefloquine and in an early phase even for the artemisinins where the hope existed that drug resistance could fail to appear. For African trypanosomes we see a different picture: While there is massive drug resistance among animal pathogenic trypanosomes there are no real drug resistant human pathogenic parasites. The reason for this is the poor transmission and the special way human patients are treated which render development of resistant trypanosomes almost impossible. Several NTDs are ear-marked for world-wide elimination by the World Health Organization and the international community. According to the roadmap guinea worm disease[4], leprosy, lymphatic filariasis, blinding trachoma and African sleeping sickness[5] are targeted for elimination while for schistosomiasis, river blindness, Chagas disease and visceral leishmaniasis control is in the focus for the year 2020 to 20306. Effective drugs are the most important control measure for most of these diseases. By drug resistance drugs lose their efficacy and have to be constantly replaced by new ones, preferably drugs representing a new chemical class to avoid cross-resistance. If this cannot be realised then control and elimination of NTDs are endangered.

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VESICLES AS MEDIATOR OF MICROCHIMERISM AND SPLIT TOLERANCE

WILLIAM J. BURLINGHAM

Extracellular vesicles (EV) are the mediators of a 3-way alloactivation response that causes acute rejection of transplants. We recently found that EV also are the mediators of peripheral "split" tolerance caused by maternal microchimerism . Both types of alloantigen presentation require 1) secretion of exosome-sized nanovesicles by a relatively rare allogeneic cell, 2) uptake of these EV by relatively abundant host cells, 3) display of captured allo-major histocompatibility complex (MHC) proteins as an intact alloantigen to "effector" T cells, and 4) display of breakdown products thereof, as allo-peptide/self-MHC complexes, to "helper" T cells. Activation in the acute rejection pathway of organ transplants, and anergy in the case of maternal microchimerism, are the result of the co-stimulation context of the helper T cell component, whether positive(CD80/86), engaging CD28, or negative(PD-L1), engaging PD-1. In the case of microchimerism, direct protein transfer of PD-L1 from EV to host dendritic cell (DC) was ruled out, suggesting that the nucleic acid "cargo" of the maternal EV/exosomes may be the component that can modify expression of PD-L1 in host DC, inducing anergy in "helper" T cells. We are currently working on this possibility. Our imaging data suggests that the microdomains on the surface of modified DC where allo-peptide/self-MHCII complexes are presented to "helper" T cells become associated with PD-L1, while PD-L1 is excluded from those microdomains on the same DC surface arising from the acquired EV. This may account for the split nature of the anergy phenomenon.

Anergy in T helper cells and immunoregulation by T regulatory (Treg) cells are two opposite sides of same coin. Much new evidence suggests that Treg cells are prolific producers of EV, and that these EV contain not only CD39 and CD73, extracellular ATPase and ADPase, resulting in production of immunosuppressive adenosine, but also the miRNA let7d that blocks Th1 function. One of the Treg cytokines that we found associated with Treg EV, IL35, not only suppresses T effector cell function, but also sets them up for PD-L1 based anergy, by increasing their surface expression of PD1. IL35-coated EV are released from induced T regs after allo-specific stimulation in culture, and, when administered to mice at the time of a heart transplant, prolonged allograft survival.

CONCLUSION

Our work would suggest that the natural tolerance effects (anergy, immune regulation) of relatively rare cells are greatly amplified by EV pathways. The future of nanomedicine in clinical transplantation is therefore promising.

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THE ROLE OF EXOSOMES IN STRESS-INDUCED BYSTANDER EFFECT

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Treatment of cells with ionizing radiation causes DNA damage. Neighbouring cells that are not directly hit with radiation can nevertheless exhibit DNA damage as well, a curious phenomenon known as the bystander effect. Bystander effects can also lead to chromosomal/genomic instability within the progeny of bystander cells, similar to the progeny of directly irradiated cells. The factors that mediate this cellular communication have not been fully characterized. In this study we tested the hypothesis that the bystander effect mediator contains an RNA molecule that may be carried by extracellular vesicles (EVs). We show that irradiation of MCF7 cells with 2 Gy alters the protein and RNA content of EVs. Importantly, the EVs released by irradiated cells can cause increased DNA damage when placed onto recipient bystander cells. Treatment of EVs with RNase abrogated their ability to induce this observed bystander effect. The genomic damage induced by radiation-induced EVs was propagated for several generations, suggesting these EVs can induce long term genomic instability. Our subsequent studies confirm that other types of stress, including that induced by heat or cytotoxic agents, can also induce an EV-induced bystander effect. These results suggest that the bystander effect is at least in part mediated by EVs and that an RNA component is involved. The data also imply that EVs may induce a long-term program of genomic instability that may have an epigenetic basis. This work widens the repertoire of roles for EVs and has implications for cancer treatment.

SPECIFIC TOLERANCE AND IMMUNE STIMULATION

DR. WERNER CAUTREELS, CEO, Selecta Biosciences Watertown/Boston, MA (USA)

Selecta's Synthetic Vaccine Particles (SVP™) technology is a highly flexible platform that is capable of incorporating a wide range of antigens and immunomodulators, allowing for the development of products that either induce antigen-specific immune tolerance or antigen-specific immune stimulation. By varying the type of immunomodulator encapsulated in the SVP™, the technology is capable of either inducing a tolerogenic response, for example to mitigate the formation of anti-drug antibodies (ADAs) against a biologic drug, or a potent antigen-specific stimulatory response, such as an antibody response to a microbial antigen or a cytolytic T cell response to a tumor antigen. SVP™ are designed to remain intact after injection into the body and accumulate selectively in lymphoid organs, which include lymph nodes and the spleen, where significant immune responses are coordinated.
SVP™ are being applied to create differentiated therapies to effectively and safely treat rare diseases by mitigating the formation of anti-drug antibodies (ADAs) against life-sustaining biologic drugs. Tolerance inducing SVP™ products also have potential applications in the treatment of allergies and autoimmune diseases. SVP™ products that stimulate the immune system can potentially prevent or treat cancer, infections and other serious diseases.
Therapeutics utilizing uricase, an enzyme that metabolizes uric acid, have previously demonstrated the ability to significantly reduce uric acid levels and dissolve the uric acid deposits in refractory and tophaceous gout patients. Their efficacy and safety, however, have been adversely impacted by the formation of anti-drug antibodies (ADAs). Leveraging Selecta's proprietary immune tolerance SVP™ platform, SEL-212 (SVP-Rapamycin in combination with pegsiticase) is designed to be the first non-immunogenic version of uricase. Preclinical and clinical data for SEL-212 will be presented showing that SEL-212 prevents the formation of ADAs and preserves the clinical activity of the uricase enzyme. This clinical validation of the SVP™ technology to prevent immunogenicity against biologics has the potential to unlock a large field of therapeutic applications, such as gene therapy. Examples of the application of the technology in such additional therapeutic areas will be presented.

TRUE INNOVATION REQUIRES RISK TAKING

DR. WERNER CAUTREELS, CEO, Selecta Biosciences Watertown/Boston, MA (USA)

True innovation comes with the risk of failure, because innovation will address known and often unknown problems by applying new and unproven solutions. In the field of medicine, such innovation is required to tackle diseases with high medical need for which effective treatments are not yet available. But of course rewards will be commensurate with risks.
We all know that the discovery and the development of a new treatment is a very long road with a lot of challenges, a high risk of failure, and ever increasing costs. Engaging on this road requires from the start a high level of courage and perseverance because it is certain that setbacks will occur.
Risk taking and thus the acceptance of failure can be very different in different cultures or environments. In the field of biotech, such risk taking is most accepted in the US. This is much less the case in Europe, and failure may be looked upon as a crime in other cultures. The biotech ecosystem in Boston, for example, is ideal with academic inventors, entrepreneurs, business leaders, and investors all understanding a similar language of risk taking and willing to take financial or reputational risk in leaving their respective comfort zones. Differences exist also between the biotech and the large pharma world. Decision making is a good example of that difference, with multiple layers of committees and therefore more risk-adverse naysayers present in large pharma.
However, all the above stakeholders may see also a significant financial reward if successful.
Other important stakeholders, such as regulators and clinicians, may not have similar financial rewards or incentives and will work from the perspective of advancement of science and medicine and societal benefit. Together with the other stakeholders they will also preserve the safety of the patient.
The trick will be to bring all stakeholders together to bring innovation forward and this will be a very interesting topic for debate.

MICROBIOME AND NANOMEDICINE

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Nanoparticle based formulations for drug delivery is increasingly being introduced as a clinical therapeutic option to treat a variety of diseases. The importance of the human microbiome in health, including the ways that nanomaterials and other xenobiotic compounds affect the composition and functions of the oral, nasal, skin, urogenital and intestinal microbiota and thus contribute to disease, has recently been recognized as a major topic of research. The use and benefits of nanotechnology in medicine are significant; however, less is known about the impact of nanomaterials on the microbiome. Effective metagenomics, functional genomics technologies and bioinformatics tools that are now available and affordable have expanded our knowledge on microbial community composition, functions and metabolic activities to help understand microbiome-host interactions. Acute or chronic exposure of the microbiome to nanomaterials has the potential to disrupt the colonization barrier and alter important functions of the microbiota. Therefore, the microbiome has emerged as an important area to consider in nanotoxicology testing for human health risk assessments. This presentation will highlight an integrated systems biology approach for the safety evaluation and risk assessment of nanomaterials and their effect on the microbiome with the goal of insuring safety of the clinical application of nanocarrier –based drug delivery systems.

CELL-IN-SHELL STRUCTURES FOR PROTECTION OF LIVING CELLS AGAINST EXTERNAL STRESSES

INSUNG S. CHOI

Nature has developed a fascinating strategy of cryptobiosis for counteracting the stressful, and often lethal, environmental conditions. For example, certain bacteria sporulate to transform from a metabolically active, vegetative state to an ametabolic endospore state. The bacterial endospores, encased within tough biomolecular shells, withstand the extremes of harmful stressors, such as radiation, desiccation, and malnutrition, for extended periods of time and return to a vegetative state by breaking their protective shells apart when their environment becomes hospitable for living. Inspired by cryptobiosis found in nature, researchers have sought to chemically control and tailor the metabolic behaviors of non-spore-forming cells as well as enhancing their viability against adverse environmental conditions, by forming thin (< 100 nm), tough artificial shells. These living "cell-in-shell" structures, called artificial spores, enable chemical control of cell division, protection against physical and chemical stresses, and cell-surface functionalizability, armed with exogenous properties that are not innate to the cells but are introduced chemically. The field has further advanced to the stage of chemical sporulation and germination, where cytoprotective shells are formed on living cells and broken

apart on demand. The (degradable) cell-in-shell hybrids are anticipated to find their applications in various biomedical and bionanotechnological areas, such as cytotherapeutics, high-throughput screening, sensors, and biocatalysis, as well as providing a versatile research platform for single-cell biology. In this sense, the artificial spore can be considered as a micrometric Iron Man: what is important is not the shell but the cell inside the shell.

SQUALENE-BASED NANOASSEMBLIES AND NANO-MOFS: TWO EXAMPLES OF NEW CONCEPTS IN THE NANOMEDICINE FIELD

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The introduction of nanotechnology into pharmacology ("nanomedicine") has importantly influenced the drug delivery field, allowing the appearance of new targeted treatments, more specific and with improved efficacy. So far, current nanotechnologies have serious limitations due to:

(i) Poor drug loading which is usually < 10 % (weight % of the transported drug with respect to the carrier material). As a consequence, either the quantity of the drug administered is not sufficient to reach a pharmacologically active concentration in the body, or the amount of the carrier material to be administered is too high, engendering toxicity or side effects and

(ii) Uncontrolled rapid release (known as "burst release") of the fraction of the drug molecules when adsorbed at the surface of the nanocarrier.

This explains the limited number of marketed nanomedicines, despite the large number of publications in the field. There is, therefore, an urgent need for new ideas to revolutionize drug delivery.

Porous Metal-Organic Frameworks (MOF) nanoparticles (Figure 1) is a typical example of drug carriers allowing to increase the drug loading[1]. These MOF nanoparticles are typically built from the assembly of inorganic units (metals) such as Iron and organic linkers with coordinating groups such as carboxylates. They combine a high pore volume and a regular porosity, as well as the presence of organic groups easily tuneable within the framework. The nanoMOFs porous iron carboxylates have several advantages when used as drug nanocarriers. In terms of synthesis, they are obtained in aqueous solutions instead of using organic solvents, thus providing an example of what "green" technology can afford for biomedical applications. In this sense, they act as sponges, encapsulating hydrophilic drugs by immersion in their aqueous solutions. They are not toxic even when injected intravenously at 250mg/Kg[1]. In addition, the design of the porous hybrid solids, playing with the wide range of compositions and topologies, could allow adapting these porous hybrid matrices to the host molecule, according to its structure and its dosage requirements[2].

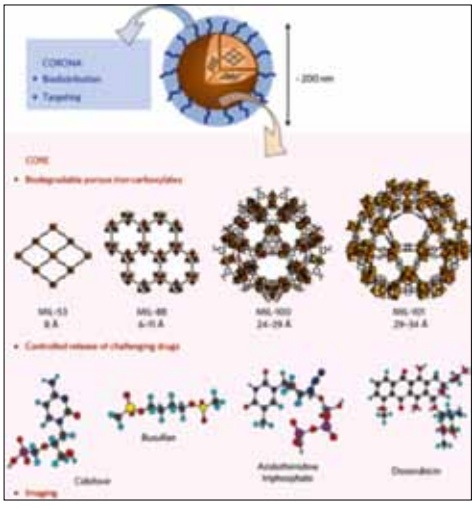

Figure 1 structure of Nano-MOFs and drugs to be encapsulated

The use of terpenoids is another example of an emerging nanomedicine concept with high drug loading and absence of burst release. Terpenes are natural compounds that are extraordinary diverse in chemistry, structure and function. Most of the natural terpenoids are flexible and biocompatible biopolymers, having physico-chemical characteristics able to adapt to a wide variety of biologically active compounds. Among them, squalene (SQ) is a natural triterpene which has the unique property of being transformed into the cyclic derivative lanosterol (a precursor of the cholesterol) by spontaneously passing through a highly coiled, compact molecular conformation. Surprisingly, although squalene is a natural and biocompatible lipid known for its dietary benefits, it was never used in the drug delivery and nanomedicine field.

Going beyond the commonly used "physical" drug encapsulation into nanoparticles, we shifted to the paradigm of the "chemical" encapsulation, which enables to dramatically increase the nanoparticle drug payload and to avoid the burst release thanks to the chemical linkage, thus overcoming the limitations of the others current nanotechnologies. Concretely, we have developed the concept of "squalenoylation/terpenoylation" which consists in the chemical linkage of anticancer or antimicrobial compounds to the squalene or to other terpenes. The resulting bioconjugates spontaneously self-assemble as nanoparticles in aqueous medium, displaying various supramolecular organizations, depending on the nature of the drug/terpene pair. Noteworthy, the nature of the polyterpenoid (ie. number of isoprenoid units) may be adapted to the hydrophilic/lipophilic character of the drug molecule to be transported, whereas the nature of the linkage (ester, amide, disulfide bonds etc.) may be selected according to the enzymatic content of the targeted diseased area. From the ratio between drug's and polyterpene's molecular weights, it is deduced that the drug loading may be dramatically improved as compared to the currently available nanomedicines. In other words, the pro-drug forms the nanomedicine by self-aggregation without the need of any other transporter material.

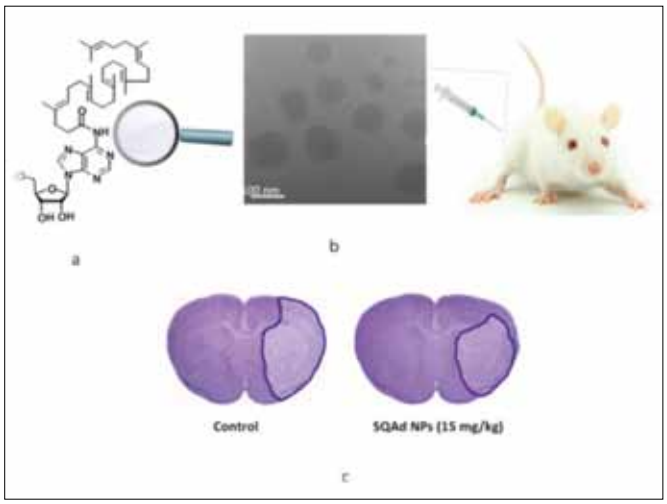

Figure 2 Adenosine-Squalene bioconjugate (a) spontaneously self-assemble in water as nanoparticles (SQAd NPs) of ca. 100 nm (b). When injected into mice subject to brain ischemia, nanoparticles induce reduction of ischemic zone (c)

The lecture will show how this breakthrough approach allows to design more efficient and less toxic nanomedicines for the treatment of cancer[3-5] and infectious diseases[6-7]. The application of the squalenoylation concept for the treatment of brain ischemia and spinal cord injury will be discussed too[8-10] (Figure 2). Special attention is given to the ability of those squalene-based nanomedicines to overcome mechanisms of resistance[11]. Noteworthy, by combining various drugs together[12] or a pharmacologically active compound with an imaging agent[13], it is possible to design multifunctional nanomedicines in a lego-like approach for theranostic applications. Similar approach allows also to design nanomedicines sensitive to an exogenous (ie. magnetic field, temperature, ultrasounds etc.) or an endogenous (pH, enzymes, etc.) stimulus[14].

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WHERE TO GO WITH NANO?

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Recently, the debate about the success of nanomedicine approaches, in particular in cancer therapy, has been revived. Articles with titles such as 'Analysis of nanoparticle delivery to tumors' and 'Why I'm holding onto hope for nano in oncology' or 'Cancer nanomedicine: is targeting our target? critically analyze the results obtained in the nanomedicine field until now and express views for the future.

The first challenge is the definition of 'nanomedicines'. The EMA has used as a working definition 'Purposely designed systems for clinical applications with at least one component at the nano-scale size'. The list of'(potential) product families in the nanomedicines arena include drug laden liposomes, albumin-based nanoparticles, polymeric micelles, and many others that have not been introduced on the market yet, e.g. fullerenes, gold nanoparticles, carbon nanotubes, dendrimers and nanodots.

Nanomedicines may have one or more of the following characteristics when compared with the 'free' drug: 1) targeted delivery of the drug or 2) – a less ambitious goal- changing the distribution of the (controlled release) drug in such a way that its therapeutic index improves compared to the free drug. Wilhelm et al. (2016) published a meta-analysis of the targeting potential of nanomedicines indicating that only 0.7% (median) of the administered nanoparticle dose is delivered to a solid tumor (animal experiments). Others (Lammers et al., 2016) argued that targeting is not a goal in itself, neither are the results from animal studies decisive. They also argued that the only outcome parameter that really counts is whether the patient benefits from the therapy or not and that (pre) selection of patients who may respond might improve the clinical performance of nanomedicines. A number of clinically successful nanomedicines have been introduced and there are more to come. A desired paradigm shift could be to investigate the use of carrier-dependent bioactive molecules instead of existing bioactives that were chosen on the basis of their inherent, free drug PK/PD profile. Interestingly, monoclonal antibodies -although fully falling under the nanomedicines definition - are always excluded from the discussion on the position or success-failure of nanomedicines. With an annual revenue (over 100 billion US$) many times the revenue of the marketed nanomedicines, monoclonal antibodies are much more therapeutically successful than 'recognized' nanomedicines. One should learn from their success.

Lit:Wilhelm et al., Analysis of nanoparticle delivery to tumours. Nature Reviews Materials,1, May 2016, 1 -12.

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STIMULUS RESPONSIVE SILICA MATERIALS FOR IN VIVO DELIVERY

LUISA DE COLA

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Porous silica materials and capsules have attracted a lot of interest since they combine their rigid and stable silica framework with an emptiness that allow the entrapment and release of desired molecules. They can therefore be used as a delivery system, as sequestering agents and as catalytic substrate. Our interest in these materials is mainly in the biomedical area and we have to face the problem related to the fate of the inorganic nanocontainers in *in vitro* and *in vivo* applications. Indeed the issue related to the use of materials for therapy and imaging in living organism, is their accumulation in vital organs that often prevent their use in clinical use. Recently, a new generation of breakable hybrid nanoparticles, able to response and degrade upon external stimuli (e.g. reductive agents, pH, etc.), have been developed in our group[1,2]. The insertion of responsive linkers in the framework of these particles, results not only in the destruction and safe excretion of the nanoparticles from the cells, but also in a faster and better delivery of the payloads. The stimulus can be a change in pH, a redox reaction or an enzymatic reaction. The time of the degradation can tuned changing the amount of the breakable groups. Moreover, to expand the breakability properties of this material for other purpose, the possibility to entrap proteins into a breakable silica shell has also been realized in our laboratory[3]. It has been shown that the activity of different proteins remains intact after their delivery into cancer cells.

Efforts devoted to the control of size and morphology of the particles show that we can indeed produce breakable silica materials possessing disc-shape, hexagonal or donuts shape.

The *in vivo* experiments showed that the breakable materials can be used to address different organs and results of the targeting and treatment of hepato carcinoma will be shown.

Acknowledgements: This work has been supported by the Fondation ARC through the project "TheraHCC" (grant N° IHU201301187)

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PET IMAGING ALLOWS ACCURATE WHOLE BODY DETECTION AND QUANTIFICATION OF LIPOSOMAL NANOMEDICINES IN TUMOURS AND METASTATIC ORGANS

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INTRODUCTION

Liposomal nanocarriers are of high interest in nanomedicine. In this context, PET and its excellent quantification properties, could be used to improve their efficacy via patient stratification into different treatment regimes[1]. Methods to radiolabel liposomal drugs with metallic PET isotopes to date have relied on the introduction of chelators to the lipid bilayer or by co-encapsulation with the drug[1]. We believe these represent a barrier for clinical translation and a simpler, chelator-free method would facilitate the introduction of imaging-based stratification strategies into clinical nanomedicine.

HYPOTHESIS/AIM

We hypothesized that preformed liposomal drugs could be radiolabelled, without adding chelators, if the encapsulated drug has metal-chelating properties. Our aim was to demonstrate this method using a multiscale-multimodal preclinical imaging strategy for monitoring and quantifying drug biodistribution in murine cancer models (Figure 1)[2].

METHODS

Liposomes of known clinical/preclinical therapeutic activity (liposomal alendronate (PLA), liposomal alendronate/doxorubicin (PLAD) and liposomal doxorubicin (DOXIL/CAELYX) where labelled with ^{89}Zr ($t_{1/2}$ = 3.2 d), ^{64}Cu ($t_{1/2}$ = 13 h) and ^{52}Mn ($t_{1/2}$ = 6 d) using hydroxyquinoline ionophores. PET/SPECT-CT imaging was performed in two mouse models of cancer (with ^{89}Zr/^{64}Cu-PLA): (i) a metastatic breast cancer model (3E.Δ.NT/NSG) that stably expresses the hNIS reporter gene traceable using [^{99}mTcO$_4$]- and GFP/RFP fluorescence[3], and (ii) ovarian cancer (SKOV3). Ex vivo biodistribution studies and histology/autoradiography of tissue sections were performed at the end of the imaging studies.

RESULTS

High radiolabelling yields of >98% and specific activities (^{89}Zr: 154 ± 75 GBq/µmol encapsulated drug; 38 ± 17 GBq/µmol lipid; n = 3) were achieved. Empty liposomes, with the same phospholipid composition and hydrodynamic size, did not radiolabel. In vitro stabilities in human serum were >85-95% after 48/72 h at 37°C. ^{89}Zr/^{64}Cu-PLA were imaged in the murine tumour models of breast cancer and ovarian cancer for up to 7 d. (^{89}Zr-PLA) or 2 d. (^{64}Cu-PLA). Radioactivity at the end of the studies was mainly found in the spleen (169 ± 50 %ID/g), liver (12.1 ± 7.6 %ID/g), primary tumour (8.4 ± 1.6 %ID/g) and blood (7.8 ± 3.3%ID/g) (Figure 1). Interestingly, uptake in metastatic sentinel lymph nodes (SLNs), ascertained by reporter gene imaging, was significantly higher (16.3 ± 7.1 %ID/g) than in non-metastatic LNs (3.8 ± 2.3 %ID/g) (Figure 2). Histology studies of primary tumors and metastatic LNs show a high degree of vascularization corresponding to areas of liposome uptake.

CONCLUSIONS

A new, highly efficient and stable method to radiolabel preformed liposomes with PET radiometals - including, for the first time, ^{52}Mn - has been developed. Liposomes radiolabelled using this method can be tracked *in vivo* using PET imaging for several days allowing accurate quantification and biodistribution measurements of liposomal drugs. Our technology is GMP-compatible and we are working towards translating it for human use in conjunction with clinically approved liposomal anti-cancer drugs.

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Figure 1. Schematic representation of the radiolabelling method (left) and PET-CT images at 72h after injection showing uptake of ^{89}Zr-PLA in primary tumor and metastatic lymph nodes in the 3E.Δ.NT/NSG mouse model of metastatic breast cancer.

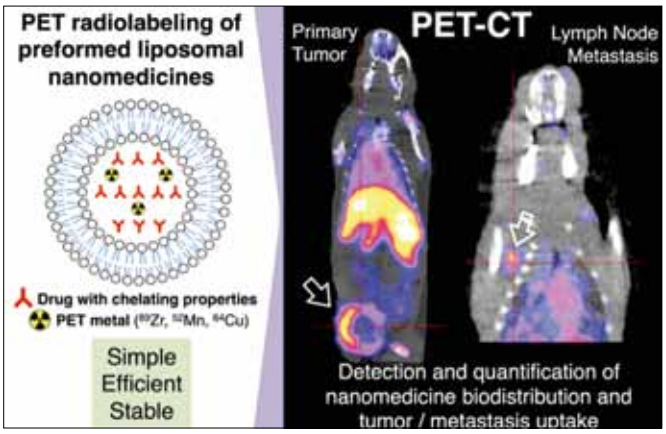
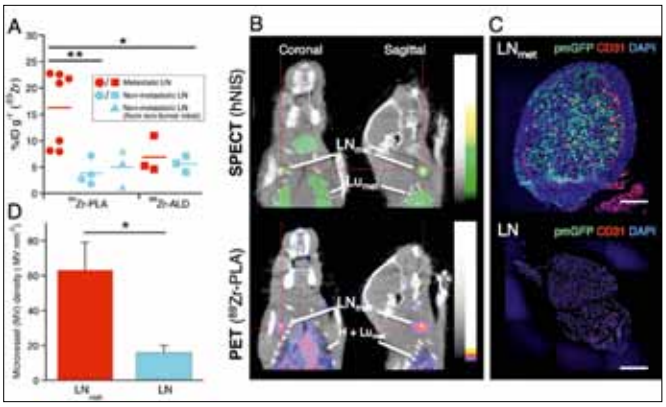


*Figure 2. Multimodal imaging data showing the accumulation of ^{89}Zr-PLA in metastatic lymph nodes (LNmet). (A) Ex vivo biodistribution data at t = 72 h showing the uptake of ^{89}Zr-PLA in: LNmet (red circles, 3E.Δ.NT/NSG mice, n = 7), non-metastatic lymph nodes (LN) (blue circles, 3E.Δ.NT/NSG mice, n = 4), LN (blue triangles, non-tumor bearing NSG mice, n = 3); or ^{89}Zr-ALD in: LNmet (red squares, 3E.Δ.NT/NSG mice, n = 3), LN (blue squares, 3E.Δ.NT/NSG mice, n = 3). ** P < 0.01, * P < 0.05, two-tailed t-test (unpaired). (B) Coronal and sagittal SPECT-CT (top) and PET-CT (bottom) images centred at the LNmet of same animal from at 72 h after injection of ^{89}Zr-PLA. (C) Fluorescence microscopy images of sections of LNmet (top) and LN (left brachial, bottom). A high degree of metastasis (green) and microvasculature density (red) is observed in LNmet, but not in LN. Scale bar is 250 µm long. (D) Quantification of microvasculature density in LNmet (black bar) and LN (grey bar) from fluorescence imaging, * P < 0.05; t-test, one-sided, unpaired, mean ± s.e.m.; n = 2).*



AN INJECTABLE NANOPARTICLE FORMULATION OF VALRUBICIN: INFLUENCE OF LIPID AND PROTEIN COMPOSITION, DRUG CONCENTRATION, STORAGE TEMPERATURE AND LYOPHILIZATION

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INTRODUCTION

Valrubicin [AD-32 (N-trifluoroacetyladriamycin-14-valerate)], a cancer therapeutic agent is used for treating Bacillus Calmette Guerin (BCG)-resistant bladder cancer[1,2]. Although valrubicin has been found to be less toxic to normal tissues[3,4] than doxorubicin during preclinical studies, its therapeutic applications has been restricted to nonsystemic administration due to its poor aqueous solubility. Its proven anti-neoplastic properties make it a promising candidate to serve as an effective chemotherapeutic agent for systemic use as a soluble nanoparticle formulation with injectable excipients. The current study was undertaken to formulate valrubicin as nanopar-

ticle formulation using our proprietary Quanticle Technology. Here we report the use of biocompatible and injectable phospholipid and protein to produce a stable nanoparticle formulation of valrubicin for systemic use.

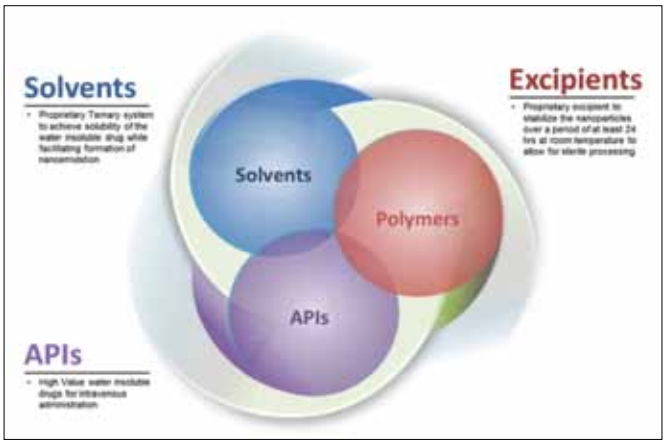

Fig. 1: Quanticles

METHODS

Nanoparticle synthesis was conducted using two methods; Method 1: proprietary Quanticles method using microfluidization and Method 2: thin film hydration (one pot method similar to Genexol-PM method). Briefly, in method 2, phospholipids and valrubicin were dissolved in ethanol and subjected to rotary evaporation until a thin film was formed and all the solvents were evaporated. The film was then hydrated using deionized (DI) water to produce paclitaxel loaded phospholipid nanoparticles. Nanoparticle size and zeta potential were measured using a Malvern ZS DLS system. The formulation was subjected to serial filtration using 1.2µm, 0.8µm, 0.45µm and 0.2µm syringe filters. The drug incorporation/loading in phospholipid nanoparticles was measured using UV-VIS method from a standard plot of absorption of valrubicin at 480 nm. We developed Quanticles manufacturing process which subjected the nanoparticle to a minimum number of fixed passes to avoid the deterioration of excipients by microfluidization process. This was possible through judicious combination of proprietary ternary solvent mixture and excipients (Figure 1). A Quality by Design (QbD) approach was utilized to design the experimental study and understand the effect of process variables on critical quality attributes (CQAs) during our proprietary medicinization of valrubicin. Drug loading, nanoparticle size (before and after filtration), particle size distribution, ease of filtration, and physical stability of particle during storage at room temperature were evaluated as CQA. The process variables being evaluated were aqueous to organic phase ratio, ratios of solvents constituting the total organic phase, amount of drug, microfluidizer pressure, number of passes, evaporation temperature.

RESULTS

A series of phospholipids, lyso-phospholipids, cholestryl ester and human serum alumin were tested for assembly of valrubicin nanoparticles. The short chain lipids of PC 10 (1,2-didecanoyl-sn-glycero-3-phosphocholine), Lyso PC 10 (1-decanoyl-2-hydroxy-sn-glycero-3-phosphocholine) and cholosteryl ester oleate did not produce smaller size particles in either methods although the formulations were easily filterable. However, the formulation with human serum albumin as excipients produced nanoparticles formulation of very small size of ~100 nm. The formulation was stable for 24 h at RT. This formulation was also lyophilizable and reconstitionable to its original size and the reconstituted formulation is stable for more than 24 RT. A typical formulation size and size distribution is shown in Figure 2.

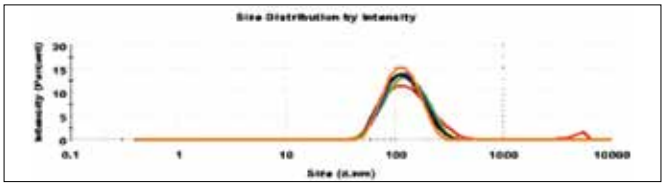

Figure 2: Valrubicin nanoparticle (LM-301) size and size distribution immediately after preparation

The formulation was reconstitionable in less than 3 minutes with 0.9% (w/v) saline and the final conc. of the formulation was ~2 mg/mL and the drug recovery was very high. Figure 3 shows the valrubicin nanoparticle size and size distribution before and after reconstitution indicating no change of the formulation.

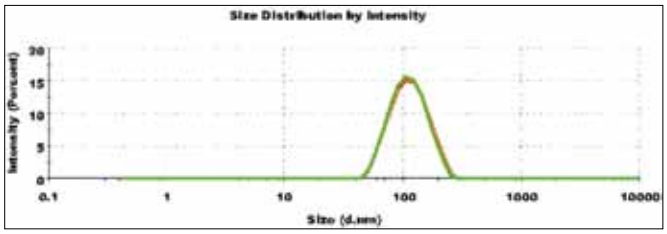

Figure 3: Valrubicin particle size and size distribution before and after lyophilization. The nanoparticle produced by our proprietary Quanticle method had zeta potential of ~-23mV (Fig. 4).

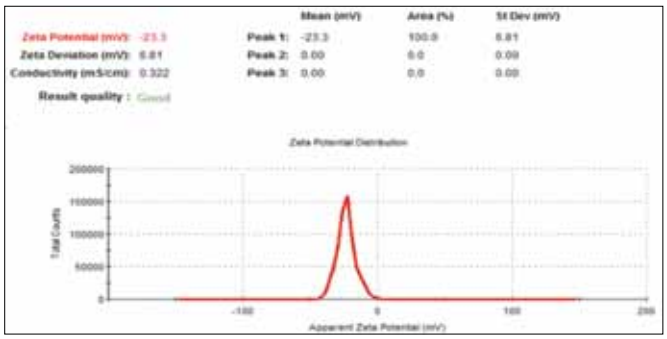

Figure 4: Zeta potential of LM-301 nanoparticle

CONCLUSIONS

A new nanoparticle valrubicin(LM-301) was formulated using injectable excipients. The formulation is sterile filterable, lyophilizable and stable at RT for 24h before and after lyophilization. We are planning for *in vitro* and *in vivo* application of this new LM-301 formulation in prostate (PC-3) and ovarian (SKOV-3) cancer cell lines.

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COMBINATORIAL NANOCONSTRUCTS FOR IMAGING AND TREATING CANCER

PAOLO DECUZZI, Laboratory of Nanotechnology for Precision Medicine Italian Institute of Technology – Genova
https://www.iit.it/research/lines/nanotechnology-for-precision-medicine

Multifunctional nanoconstructs are particle-based nano-scale systems designed for the 'smart' delivery of therapeutic and imaging agents. The Laboratory of Nanotechnology for Precision Medicine at IIT-GE synthesizes polymeric nanoconstructs with different sizes, ranging from a few tens of nanometers to a few microns; shapes, including spherical, cubical and discoidal; surface properties, with positive, negative, neutral coatings; and mechanical stiffness, varying from that of cells to rigid, inorganic materials, such as iron oxide. These are the 4S parameters – size, shape, surface, stiffness – which can be precisely tuned in the synthesis process enabling disease- and patient-specific designs of multifunctional nanoconstructs. In this lecture, the role of manipulating these 4S parameters over different temporal and length scales will be elucidated in the context of future nanomedicines.

NANO-ONCO: BIOPRAXIS ROADMAP TO NANOMEDICINE TRANSLATION FOR CANCER TREATMENT

ANGEL DEL POZO AND OIHANE IBARROLA

BIOPRAXIS faces drug development for rare cancers, including glioma and pancreatic cancer, using nanotechnology and biological molecules like proteins and peptides. Moreover, is also working in controlled release systems through nanoparticles (Solid Lipid Nanoparticles, Magnetic Nanoparticles, Lipid Nanovesicles), which improve therapeutic action of APIs and allow protection of biological molecules and targeting, imaging and future theranostic of different diseases including cancer. BIOPRAXIS and some European partners have existing working expertise in this area, which, more recently, it culminate into HEATDELIVER project: Heat and Drug Delivery nanosystem with active tumor targeting features (Eurotransbio project).
Through the development of the "nanocancer projects", Biopraxis has identified the main challenges about GMP scaling up and nanomedicine translation to patients, and has developed a technological road map to overcome those challenges and get products to the market.
Biopraxis developments are mainly based on "open innovation approaches". We will show how open innovation is crucial to succeed in this context, and will present two examples extracted from 2 European funded projects where Biopraxis, as industry, is enabling scale-up of nanomedicine at GMP level: Theraglio (FP7) and NoCanTher (H2020).
Assessment and implementation of the proposed production processes at GMP conditions will be the crucial role for Biopraxis, in order to get a formulation ready to be tested in a Phase I clinical trial.

NOVEL CLINICAL APPLICATIONS OF ABI-009, AN ALBUMIN-BOUND MTOR INHIBITOR

NEIL P. DESAI, PhD, CEO, Aadi Bioscience Inc, Pacific Palisades, California, USA

ABI-009 (nab-rapamycin) is a novel albumin-based nanoparticle version of sirolimus (rapamycin) with a mean particle size of approximately 100 nm, that can target various tissues based on mechanisms of albumin transport. This technology has previously achieved significant commercial success through marketing approval of albumin-bound paclitaxel for several oncology indications.
Based on its favorable pharmacological and pharmacokinetic profile relative to other known mTOR inhibitors, ABI-009 is being investigated in both cancer and non-cancer indications based on the recently demonstrated biological relevance of mTOR activation in these diseases. A phase 2 trial has been initiated in an extremely rare form of sarcoma called perivascular epithelioid cell tumor (PEComa). These tumors are almost exclusively driven through mutations or deletions of TSC2 in the mTOR pathway. Another phase 2 trial has been initiated using a combination of ABI-009 and gemcitabine in non-muscle invasive bladder cancer and a phase 1 study in combination with standard therapy has been initiated in the treatment of pulmonary hypertension. These and other clinical applications of ABI-009 will be discussed.

SIRNA AND GENE DELIVERY WITH NOVEL PEPTIDE NANOPARTICLES

NEIL P. DESAI AND GILLES DIVITA, Aadigen, LLC, Pacific Palisades, California (USA)

We have designed the ADGN-technology based on short amphipathic peptides that forms stable positively-charged nanoparticles with a large panel of nucleic acids, through non-covalent electrostatic and hydrophobic interactions. Self assembly of ADGN-peptide molecule around nucleic acids leads to nanoparticles associating several peptide/nucleic acid complexes, cemented together by a matrix of free peptides.
ADGN promotes efficient targeted-delivery of siRNA, mRNA, plasmid DNA or small oligonucleotides into a wide variety of cell lines in a variety of hard-to-transfect mammalian cell lines, including primary and T cells as well as *in vivo*. Cellular uptake mechanism of ADGN/nucleic acid nanoparticles bypass endosomal pathway and involves membrane dynamic.
When applied by systemic intravenous or subcutaneous injections, ADGN promotes the delivery of siRNAs in most of the tissues without triggering any nonspecific inflammatory response. The potency of ADGN-technology has been validated *in vivo* to target siRNA in the liver and we demonstrated than a single intravenous or subcutaneous administration of ADGN/siRNA nanoparticles allows a major siRNA based down regulation of Factor VII in a fully reversible behavior. ADGN-technology has also been validated for cancer treatment by targeting major cell cycle regulatory proteins in various mouse tumour models. We demonstrated that ADGN-mediated delivery of Cyclin B1 siRNA (0.1 mg/Kg) prevents tumour growth *in vivo* following systemic intravenous injections.
ADGN-technology mediated stable gene delivery into primary human T cells. ADGN:plasmid particles have been used to create anti-CD19-CAR-T cells as well as checkpoint resistant CAR-T Cells by combining with ADGN/siRNA particles containing siRNA targeting PD1
Given the robustness of the biological response achieved through this approach, ADGN-technology hold a strong promise for therapeutic administration of oligonucleotides.

HYPERSENSITIVITY TO IRON-CONTAINING NANOPARTICLES: CLINICAL INFORMATION AND MODELING IN PIGS

LÁSZLÓ DÉZSI[1], Harald Unterweger[2], Tamás Fülöp[1], Erik Őrfi[1], Rudolf Urbanics[3], Jan Zaloga[2], László Rosivall[4], Christoph Alexiou[2] and János Szebeni[1,2,3]

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2 Section of Experimental Oncology and Nanomedicine, University Hospital Erlangen, Germany
3 Seroscience Ltd., Budapest, Hungary
4 Institute of Pathophysiology, Semmelweis University, Budapest, Hungary

Superparamagnetic iron oxide nanoparticles (SPIONs) consisting of iron oxide cores and appropriate coatings, have been used in tissue engineering, drug delivery, hyperthermia, and enhanced resolution magnetic resonance imaging (MRI) techniques. A possible problem with the clinical application of these nanoparticles is their capability to cause hypersensitivity reactions (HSRs) called complement (C) activation-related pseudoallergy (CARPA)1. A few types of SPIONs (e.g. ferumoxides, ferucarbotran (Resovist®, Bayer Healthcare), ferumoxtran-10 (Sinerem®, Guerbet) and NC100150) had been designed and clinically tested as MRI contrast agents. In addition, ferumoxytol (Feraheme® or Rienso®, Takeda Ltd.) has been approved for the treatment of iron deficiency in adult chronic kidney disease patients, and was also used as MRI contrast agent. However, both Resovist and Rienso were withdrawn from the market, the latter because of hypersensitivity reactions, some of which were fatal. In the case of Rienso 35 deaths were reported worldwide.

For these reasons, there is increasing interest in the prediction and prevention of CARPA caused by iron oxide-based nanoparticles, using laboratory assays and animal models. Currently, in our laboratory the porcine model is well established to study CARPA. The major advantage of this model is its high sensitivity and human predictability. The mechanism of CARPA is rather complex. In addition to C activation, it involves anaphylatoxin-induction of mast cells and macrophages, and the subsequent release of vasoactive and inflammatory mediators. The symptoms of CARPA include hemodynamic and cardiopulmonary changes, among which pulmonary arterial pressure (PAP) elevation is most characteristic in pigs. In addition, hematological changes like leucopenia or leucocytosis, thrombocytopenia, skin reactions (flush and rash), and elevated TXB2 levels are also important markers of CARPA.

MATERIALS AND METHODS

Pigs: Domestic male Yorkshire pigs (20-25 kg) were sedated with Calypsol/Xilazine (10 and 2 mg/kg respectively) and anesthetized by isoflurane (2-3% in O2). Animals were breathing spontaneously. PAP was measured using a Swan-Ganz catheter introduced into the pulmonary artery via the right external jugular vein, while systemic arterial pressure (SAP) and heart rate (HR) were measured in the femoral artery. The left femoral vein was cannulated for blood sampling. Test agents were injected in bolus (< 10 sec) or slow bolus (2 to 5 min) via the left external jugular vein (n=2-3/group). Hemodynamic changes were continuously monitored using an AD Instruments (ADI) PowerLab System. Mean PAP, SAP and HR data were evaluated by the ADI LabChart software.

Blood sampling: Blood samples of 2 ml, each were collected from the pigs before (time 0), and at pre-determined time points (1-3-5-10-30 min) after the injection. Samples were collected into Hirudin or K2-EDTA Blood Tubes, of which samples for TXB2 analysis were containing Indomethacin. Aliquots of 100 µl blood were drawn into tubes with K2-EDTA for haematological analysis. Blood was centrifuged at 1500 rpm for 10 min at 4°C, and plasma was stored at -80°C until analysis.

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 was utilized for direct complement activation. Lauric acid/bovine serum albumin (LA/BSA)-coated SPIONs2, as well as dextran-coated SEON Dex-SPION (80 nm)3 and smaller diameter SEON Dex-USPIO (30 nm) were provided by the Section of Experimental Oncology and Nanomedicine (SEON), University Hospital Erlangen. Feraheme and ferucarbotran served as positive control for SPIONs (data not shown).

RESULTS

Cardiopulmonary effects of repetitive bolus administration of 1 mg/kg i.v. lauric acid/bovine serum albumin (LA/BSA)-coated SPIONs are shown in Fig. 1. Initial bolus was leading to a 4-fold increase in PAP with marginal changes in SAP or HR. Repeated dose caused similar, but smaller PAP elevation showing a partial tachyphylaxis. At the end of the experiment CARPA - similar to the first SPION bolus - was induced by 0.5 mg/kg i.v. zymosan (not shown).

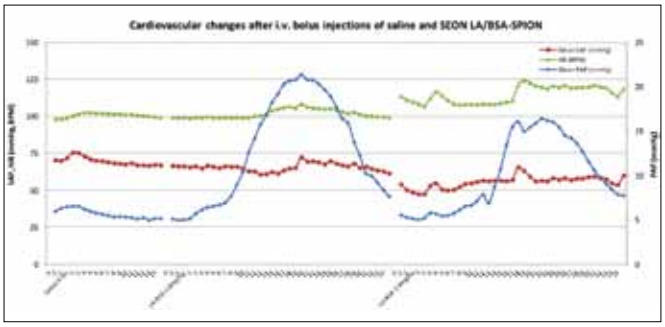

Figure 1: Time course of cardiovascular (PAP, SAP and HR) changes caused by repeated LA/BSA SPION injection in a pig. Doses are given in mg iron/kg. Curves were constructed pre-injection and from the 0 to 30 min readings after injection, evaluated at 1 min intervals.

Cardiopulmonary effects of consecutive bolus administration of 0.5 mg/kg and 5 mg/kg i.v. dextran-coated SPIONs with larger (80 nm) diameter (SEON Dex-SPION, upper panel) and smaller (30 nm) diameter (SEON Dex-USPIO, lower panel) are shown in Fig. 2. None of the boluses, small or high dose, led to any change in PAP or SAP, while HR has moderately risen towards the end of the experiments. At the end of the experiment in both series 0.1 mg/kg i.v. zymosan induced severe CARPA.

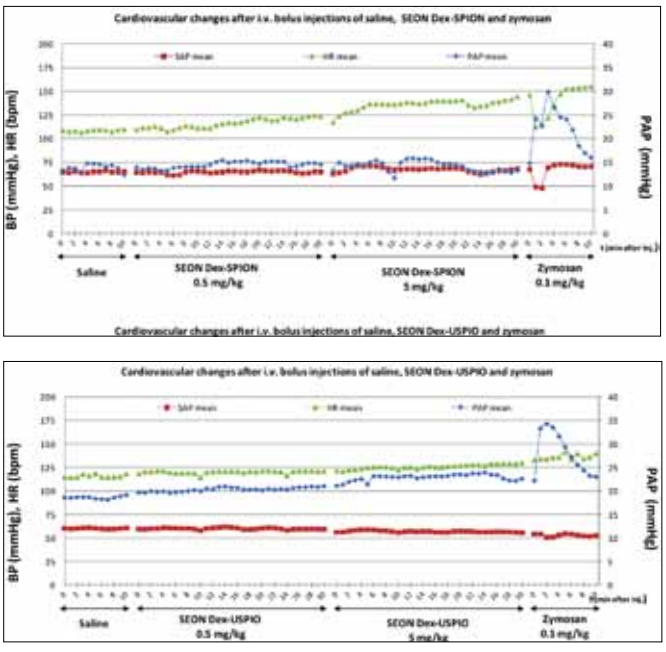

Figure 2: Time course of cardiovascular (PAP, SAP and HR) changes caused by consecutive SEON Dex-SPION (upper panel) or SEON Dex-USPIO (lower panel) injections of increasing doses in pigs. Doses are given in mg iron/kg. Mean values of 2 animals, each are depicted. Curves were constructed pre-injection and from the 0 to 30 min readings after injection, evaluated at 1 min intervals.

CONCLUSION

This study investigated the immune reactive properties of SPIONs in a highly sensitive model of CARPA in pigs. Our previous experience with other SPIONs and the results of the current studies with SEON LA/BSA-SPION indicated the possible rise of massive CARPA by such nanoparticles. However, by modifying the synthesis procedure and surface coating by dextran in a manner that had been done in SEON Dex-SPION or SEON Dex-USPIO the reaction-free particles were achieved, which implies significant advancement in this field and the potential for safe clinical use of these nanoparticles in the future.

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GENE EDITING WITH CRISPR/CAS9 USING PEPTIDE BASED NANOPARTICLES

GILLES DIVITA AND NEIL DESAI, Aadigen, LLC, Pacific Palisades, California (USA)

The recent discovery of CRISPR (clustered regularly interspaced short palindromic repeats)-Cas9 (CRISPR-associated protein 9) has provided a highly efficient tool for site-directed genome engineering in eukaryotic cells, opening new perspective for innovative gene therapy. However, the cellular delivery of the RNA-guided nuclease CAS9 and of the associated guide RNA (gRNA) remains a limitation to the overall potency and for the therapeutic application of the CRISPR-CAS9 system. Here we report a new delivery platform that can potently deliver active CAS9 mRNA/gRNA or CAS9/gRNA ribonucleoprotein complexes in a variety of hard-to-transfect mammalian cell lines, including primary and T cells as well as *in vivo*.

We have designed the ADGN-technology based on short amphipathic peptides that form stable neutral nanoparticles with CAS9mRNA:gRNA or CAS9/gRNA ribonucleoprotein complexes through non-covalent electrostatic and hydrophobic interactions. Self assembly of these peptide molecules around CAS9mRNA/gRNA or CAS9/gRNA leads to peptide/nucleic acid or peptide/protein/nucleic acid nanoparticles that remain stable over time in serum conditions.

ADGN-nanoparticles mediate functional targeted-delivery of CAS9 mRNA and CAS9:gRNA complexes into a wide variety of cell lines including primary cells and T cells, allowing simultaneous and multiple gene-editing via both Non-Homologous End Joining (NHEJ) and Homology Directed Repair (HDR) without inducing any significant toxicity. We also demonstrated that ADGN-nanoparticles promote *in vivo* delivery of CAS9 mRNA/gRNA, leading to the expression of active CAS9/gRNA in mice and a robust editing of a selected target gene in the liver.

Therefore, given the robustness of the biological response achieved through this approach and the absence of associated toxicity, we are further exploring the ADGN-technology for therapeutic applications of CRISPR-CAS9 based genome editing along with gene delivery which

TRANSLATION OF TARGETED AND THERANOSTIC NANOMEDICINES

DARYL DRUMMOND, Vice President, Discovery, Merrimack Pharmaceuticals, Cambridge, MA (USA)

delivery which

Antibody-Directed Nanotherapeutics (ADNs) represent a promising next generation format for targeted drug delivery. Highly stabilized liposomal formulations of small molecule cytotoxics have been developed to provide slow and sustained release of the drug specifically at the tumor site. Two of those drugs, Onivyde, a liposomal formulation of irinotecan, and MM-302, an ErbB2-targeted pegylated liposomal formulation of doxorubicin have already been evaluated in the clinic. A third drug (MM-310) is an EphA2-targeted liposomal formulation of a novel docetaxel prodrug. The reduced systemic exposure of this new drug has resulted in a dramatic alteration of the toxicity profile for docetaxel in preclinical models, including a dramatic reduction in the primary toxicity neutropenia. Engineering considerations and preliminary *in vivo* proof of concept data will be described. In addition, nano-sized imaging agents to potentially identify patients with tumors that more efficiently accumulate ADNs and respond to treatment with them.

DELIVERING NANOPARTICLE-ALLERGEN CONJUGATES TO HUMAN IMMUNE CELLS: IMPLICATIONS FOR ALLERGEN IMMUNOTHERAPY AND SAFETY

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BACKGROUND

Nanoparticles (NP) interact with biomolecules as soon as they get in contact, a process which has become known as protein corona formation[1, 2]. Allergens bound to NP are a specific type of corona that is investigated for delivery of allergens to phagocytic immune cells for Allergen-specific Immunotherapy (AIT). Numerous studies have pursued this concept, but mainly in mouse models[3, 4]. Besides medical treatment with NP-allergen conjugates, unintentional co-exposure to both compounds, and therefore also to allergens as part of a particle corona, can occur especially at the workplace, where increasingly, large amounts of NP may be handled and where (indoor) allergens are usually present as well. Inhalation of such substances may have consequences on the immune state, in particular in cases of allergic sensitization or during AIT.

OBJECTIVES

Our studies aimed at investigating the response of human immune cells towards NP decorated non-covalently with allergens. Exposed cells included primary basophils, to establish whether binding to NP changes the IgE-dependent response of effector cells compared to exposure towards free allergens. In order to investigate whether non-covalent binding is sufficient to deliver substantial amounts of allergens to human phagocytic cells, we coated NP with allergens or the surrogate protein GFP and monitored the amount of coating over time, combined with uptake into macrophage-like THP-1 cells. These concepts are essential to enable studies on allergen-NP-cell interaction, which determine the outcome of unintentional exposure as well as of AIT in humans.

EXPERIMENTAL PROCEDURES

We used allergens that are highly prevalent in Europe, including the major birch pollen allergen, Bet v 1, the timothy grass pol-

len allergen, Phl p 5, and the house dust mite allergen, Der p 1. NP coating, basophil testing and protease activity of Der p 1 on a model peptide and on a single layer of human alveolar epithelial derived A549 cells were determined as described[5].

For stability and uptake studies, a panel of differently sized TiO_2 and SiO_2 NP were produced in house according to established methods, whereby TiO_2 NP were produced in the gas phase and SiO_2 NP, mesoporous or non-porous, were produced in liquid phase. Particle behavior was determined by standard physico-chemical characterization techniques including transmission electron microscopy (TEM) and dynamic light scattering (DLS). Fluorescent model proteins such as green fluorescent protein (GFP) or fluorescence-labelled recombinant allergens were produced in house[6-8]. Detailed interaction studies were performed addressing binding capacity, selectivity for components of therapeutic allergen extracts, time-resolved replacement of the proteins of interest by r proteins from serum-containing cell culture media. Two well-established model cell lines, i.e. human lung epithelial A549 and macrophage-like THP-1 cells, were used in single as well as co-cultures. In order to mimic different pre-inflamed conditions, macrophage-like cells were polarized into type 1 (pro-inflammatory) and type 2 (anti-inflammatory) states. Cellular uptake was monitored in time-resolved manner using life cell imaging (LCI), confocal laser scanning microscopy (CLSM), and for quantitative purposes using fluorescence-activated cell sorting (FACS). Immune responses were determined by enzyme-linked immunosorbent assay (ELISA).

RESULTS

When bound to Au NP, Der p 1 elicited stronger IgE-dependent degranulation of basophils from all allergic donors tested. Der p 1 is a protease, and protease activity was found to be significantly enhanced by binding to NP. Modulation of allergen properties by binding to NP, including the enzymatic activities of some allergens has to be taken into account when considering the safety of therapeutics.

To investigate stability of allergen coronas in medium and upon uptake into human cells, coronas were formed using TiO_2 and SiO_2 NP. All components, NP as well as proteins of interest, were tested free of bacterial endotoxin (lipopolysaccharide, LPS). Replacement studies revealed some degree of time-dependent exchange with serum proteins derived from cell culture medium. However, the replacement of allergens or GFP attached to the NP surface with serum proteins was not complete at any time point and substantial amounts were reproducibly determined even after 24 h incubation. Therefore, the established setup proved suitable for quantitative monitoring of free vs. NP-associated protein uptake. Cellular uptake by A549 and THP-1 cells was observed and quantified at variable exposure conditions, including serum-free conditions. Using CLSM, intracellular uptake could be differentiated from cell surface association, which was observed using certain NP-protein conjugates even upon extended exposure periods. Figure 1 depicts an example of long-term uptake/cell surface association of GFP-coated TiO_2 NPs at A549 cells.

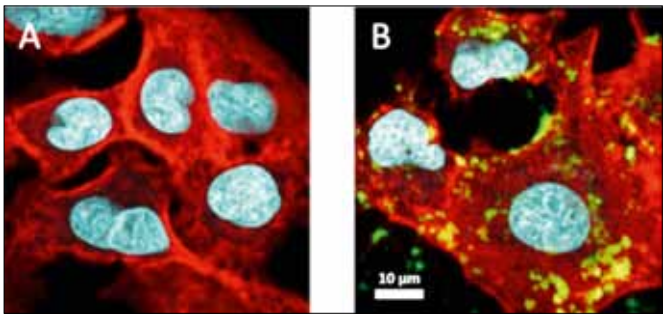

Figure 1. Accumulation of GFP-coated TiO_2 NPs by A549 cells. The cells were incubated without (A) or with 10 µg/mL GFP-coated TiO_2 NP (B) for 24 hours and uptake/cell surface association of NP were visualized in green by CLSM. The cytoskeleton was stained in red with rhodamine phalloidin and the nuclei were stained in blue with DAPI. The size bar in B applies to both panels.

CONCLUSIONS

Safety considerations of nanopharmaceuticals intended for delivering allergens to phagocytic immune cells – primarily dendritic cells – need to take into account which effects could be elicited before uptake into the target cells. Primary basophils are a suitable representative for the study of human effector cells. Of note, enzyme activities are not rare in allergens and proteases are particularly common. These activities have to be covered as well, since binding to NP can substantially modulate them. It is an attractive option to use non-covalent binding of allergens to NP: "Delivery-by-corona" would be cheap, does not require allergen modifications, and it could be possible to use allergen extracts that are presently the mainstay of immunotherapy. However, the time-dependent evolution of the protein corona implies that delivery of substantial amounts of allergens cannot be taken for granted. Quantitative data for time-resolved uptake of non-covalent protein-NP conjugates into human cells need to be carefully controlled for bio-corona stability under the conditions of investigation. Cellular uptake needs to be differentiated from cell surface association. The capacity of protein NP conjugates being retained extracellularly at the epithelium or in the tissue for extended periods may confer depot effects. Cell surface association may provide conditions required for efficient adjuvant action during SLIT.

KEYWORDS: Allergen immunotherapy, cellular uptake, nanoparticles, recombinant allergens, effector cells, target cells.

ACKNOWLEDGMENTS

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THE NEED TO UNDERSTAND THE DIFFERENCE BETWEEN RESEARCH (K€) AND DEVELOPMENT (M€)

MIKE EATON

The biggest barrier to the Development of Nanomedicines for patients remains the twin obstacles of:

1. A lack of knowledge by academics (and students!) of Development Sciences and market forces
2. Funders inappropriate (academic) peer review of Nanomedicine projects.

These two reciprocal problems can be overcome with major advantages for all stakeholders, but rien ne change without the political will and support of vested interests. Most projects are not developable at ideation and it is pointless to give them €Millions when there a certainty there will be no patient benefit. It has been argued that it is impossible for academics to adapt to market forces - however a few funding initiatives have shown this to be untrue with benefits for ALL stakeholders.

Many long-held mantras are incorrect such as the importance of getting to the clinic – much more important is having a USP. A successful clinical study does not mean there is a need for the product! For a nanomedicine to be developable it must be really innovative (USP) and not derivative science. There are a very few transformational nanomedicines out there… but they are radical innovations and not simply followers of fashion.

IF you do not know what precisely you are developing you are still in research and should be only be funded at the €K level. You cannot develop something that you can't define precisely. Development is quite different and distinct from Research and accepts only the top drug candidates.

The detailed technical milestones for nanomedicine developers have been discussed elsewhere – so this talk will concentrate on the top-level challenges to get useful nanomedicines to patients.

ENDOVASCULAR AND MOLECULAR TARGETING OF MESENCHYMAL TUMORS- EXAMPLES OF NOVEL THERAPIES FOR ENIGMATIC DISEASES

ELDAD ELNEKAVE

Mesenchymal tumors comprise a spectrum of soft tissue disease ranging from highly malignant sarcomas to non-metastasizing but locally aggressive desmoid fibromatoses, inflammatory myofibroblastic tumors and IgG4 related sclerosing disease. The latter group remains enigmatic etiologically and unpredictable clinically, demonstrating a combination of inflammatory and neoplastic features. A similarly heterogeneous armamentarium of drugs, from anti-inflammatory, hormonal, TKI's and cytotoxic drugs are commonly used in therapy, with only modest efficacy. Here we discuss results and prospects for novel approaches utilizing both anatomical and molecular targeting.

We describe three cases of superselective doxorubicin-eluting bead delivery into the distal vasculature of extra-abdominal unresectable pediatric desmoid tumors. Drug eluting beads permits regional delivery of high doses of the drug with virtually nil systemic distribution. We also describe our experience in using liposomal solumedrol in treating an infiltrative IgG4-related sclerosing mass within the left ventricular myometrium.

In each case, the described therapy has never been reported in the English literature. We will consider the implications for mesenchymal tumors and other diseases.

BONYPID™ – A NEW DELIVERY SYSTEM USING POLYPID TECHNOLOGY FOR THE TREATMENT OF CONTAMINATED OPEN BONE FRACTURES

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PolyPid Ltd developed a novel local drug delivery system based on self-assembly of pharmaceutically approved lipids and polymers that encapsulate drugs .The formulation is self-assembled lipid matrix via the interaction of the lipids (cholesterol and synthetic phospholipids) and biocompatible - biodegradable polymer (poly-lactic-co-glycolic). The entrapped drug is located within the anhydrous environment and therefore fully protected from both enzymatic and long-term water-exposure-related degradation.

PolyPid formulation nanostructure was characterized by different physical methods including wide angle X-ray analyses (WAXS) differential scanning calorimetric (DSC) and SEM. WAXS analyses show a strong signal in the range of 1.3-1.8 2Θº, suggesting that the polymer and lipid is a highly organized nanostructure.

The principle lipid in PolyPid technology formulations is phosphatidylcholine, which constitutes more than 85% of the overall lipid mass. It was found that the length of the acyl chains (14, 16 and 18 carbons, respectively) can significantly alter the release rate of a drug during the prolonged (4 weeks), zero-order release phase, but did not alter the release profile as demonstrated by the release of antibiotic (doxycycline).

PolyPid's first focus is BonyPid™, a local delivery platform designed to treat bone morbidities (orthopaedic and dental). BonyPid™ loaded with antibiotics was tested in humans for the treatment of contaminated or infected severe open long-bone fractures.

Gustilo type III open fractures are associated with a high infection rates in spite of instituting standard of care (SOC) consisting of intravenous antibiotics, irrigation and debridement (I&D), and delayed wound closure. Locally-delivered antibiotic has been proven to assist in reducing infection in open fractures. The aims of this study are to determine the effectiveness and safety of the new implantable and biodegradable antibacterial BonyPid™ in preventing bacterial infections and initiating bone growth in open fractures.

The osteoconductive antibacterial BonyPidTM used is a synthetic bone void filler (comprised of ≤1 mm β-tricalcium phosphate granules) coated by a thin layer (≤20 µm) of PolyPid nanotechnology formulation. Upon implantation, the coating releases doxycycline at a constant rate for a predetermined period of 30 days. One BonyPidTM vial of 10 grams contains 65 mg of formulated doxycycline. After approval, sixteen subjects with Gustilo type III open tibia fractures, were implanted with the BonyPidTM immediately on the first surgical intervention (I&D), followed by external fixation. Patients had periodic laboratory, bacteriology and radiology follow-up.

Six months results showed that no infection developed and only one BonyPidTM implantation was needed with no subsequent I&D, in the target tibia fracture. Immediate soft wound closure was done in 6/16 subjects following implantation. Out of 10 remaining subjects, 3 needed soleus muscle transfer-skin grafting and 7 required delayed primary closure; by skin grafting (5) or suturing (2). Early callus formation was seen at 8-12 weeks post-surgery, followed by bone healing seen from 16 weeks onwards. Safety of implantation was remarkable, with only one deep infection at a fibular open fracture without BonyPidTM implantation. One BonyPidTM related adverse event caused delay in skin healing due to excessive granules in the superficial soft tissues.

Conclusion: BonyPidTM demonstrate the advantages of long lasting, controlled release rate and protected reservoir a PolyPid nanotechnology in both efficacy and safety profile. BonyPid™ is effective in reducing bone infection and promoting early callus formation, resulting in early bone healing. In contrast to any other non-antibiotic bone void fillers, BonyPidTM is safe for immediate implantation into contaminated/infected severe open-bone fractures. Results support that one month release of doxycycline in a controlled manner provides an effective way for treating open fractures. This new local antibiotic delivery system is applicable in unmet medical situations associated with localized infections.
PolyPid platform technology is also used for developing of coatings for medical devices. Orthopaedic Hydroxyapatite coated metals, metal screws and pins were coated by thin layer of PolyPid formulation releasing antibiotic in a constant controlled rate for up to several months.

NANODIAMONDS FOR DRUG DELIVERY IN PRECLINICAL MODELS OF PANCREATIC CANCER

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Carbon-based nanomaterials, including carbon nanotubes, graphene-based materials, fullerenes, nanodiamonds and others are being increasingly studied as drug delivery and imaging agents (Bhattacharya et al., 2016). Nanodiamonds, in particular, display several unique properties that make them promising for biomedical applications. These include unique electrostatic properties, a chemically inert core, and a tunable surface. Moreover, recent *in vitro* and *in vivo* studies have provided evidence that nanodiamonds are non-cytotoxic and well tolerated at clinically relevant doses. Here a short overview of recent preclinical studies is provided showing that nanodiamonds are non-cytotoxic for normal immune cells and, moreover, that poly(ethylene glycol) (PEG) functionalized nanodiamonds can be employed for delivery of chemotherapeutic drugs in a 3D tumor spheroid model of pancreatic cancer. Pancreatic ductal adenocarcinoma (PDAC) remains one of the most difficult-to-treat cancers and new treatment options including appropriately tailored nanomedicines are needed (Adiseshaiah et al., 2016). The PEG-ND-doxorubicin conjugates were shown to be more effective in killing pancreatic cancer cells cultured in 3D as compared to free drug, possibly due to increased retention of the drug-nanoparticle conjugates. Further studies are required to evaluate this novel nanoscale delivery system using *in vivo* models of PDAC.

FURTHER READING

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UNDERSTANDING THE NANO-BIO INTERACTIONS: IMPROVING SYSTEMS FOR TARGETED DRUG DELIVERY AND ENABLING NEW APPLICATIONS IN MEDICINE

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The two most commonly used nanoparticles for therapeutic applications are polymeric nanoparticles and liposomes; the latter representing the majority of the clinically validated products. Optimization of the physicochemical properties of polymeric nanoparticles may confer a desired biological identity with optimal *in vivo* characteristics, which in turn can accelerate their successful clinical translation. The 1st targeted polymeric nanoparticle for cancer chemotherapy (BIND-014) entered human testing in 2011 and was evaluated in Phase 2 clinical trials. Considering the broad undifferentiated anti-tumor activity across nearly all of the clinically tested nanotherapeutics to date, recent attention has focused on a deeper understanding of the tumor microenvironment including inter- and intra-tumoral heterogeneity, in order to develop the next generation of nanotherapeutics. Emphasis has been placed on better understanding of the nano-bio interface as well as approaches for selection of patients for nanoparticle therapeutics. Furthermore, taking advantage of biophysicochemical variations between normal- and tumor-tissue, a growing arsenal of stimuli-responsive polymeric nanoparticles are under investigation. The goal of this talk is to review our efforts in the design and optimization of polymeric nanoparticles, which formed the foundation for the clinical translation of two first-in-kind targeted nanotherapeutics, and to discuss the lessons learned in this process.

NANOPARTICLE-BASED COMBINATION THERAPIES AGAINST MALARIA

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Nanomalaria Joint Unit, Institute for Bioengineering of Catalonia (IBEC), and Barcelona Institute for Global Health (ISGlobal, Hospital Clínic-Universitat de Barcelona)

The disappointing repeated failure of the therapeutic arsenal deployed against Plasmodium since the identification in 1880 by Alphonse Laveran of this protist as the causing agent of malaria, calls for a radical shift in the way we approach the treatment of this disease. To counteract the evolution of Plasmodium resistance to antimalarial drugs and their resulting loss of efficacy[1], three main protocols have been traditionally followed: development of new compounds, increase of dosage, and the introduction of drug combinations. New antimalarial drugs are being discovered at an excruciatingly slow pace, far below the rhythm at which resistant pathogen strains emerge. Apart from few remarkable exceptions[2], there is a clear lack of sufficient economic resources allocated to the research of new agents against infectious diseases which are not prevalent in the developed world. This trend does not seem likely to be reversed if we consider the poor prospects for a profit-seeking industry of obtaining high gains through investments in treatments to be mainly used in developing areas of the world, whereas public funding is also biased towards those diseases more directly affecting the citizens of the countries with highest GNPs (i.e. those usually having a larger research budget). The second approach of simply administering higher antimalarial drug amounts usually results either in side effects severe enough to discourage people from taking the medicine, or in the need to administer several doses, which is often not fulfilled; such patient noncompliance is one of the main triggers of drug resistance evolution[3]. Finally, drug combinations can delay the selection of multiple resistance[4], which however will eventually occur, ending up in loss of efficacy of even the most promising front-line drugs[5]. This scenario calls for urgent new approaches capable of breaking a century-long monotony of antimalarial therapeutics.
Rather than focusing all efforts on identifying new drugs whose efficacy is rapidly diminished by the parasite's evolution of resistance, an important and often disregarded battlefront is the implementation of targeted delivery methods capable of increasing the doses reaching the pathogen up to local levels sufficiently high to minimize this resistance emergence. Regrettably, the search for this long sought-after magic bullet against malaria has not taken off

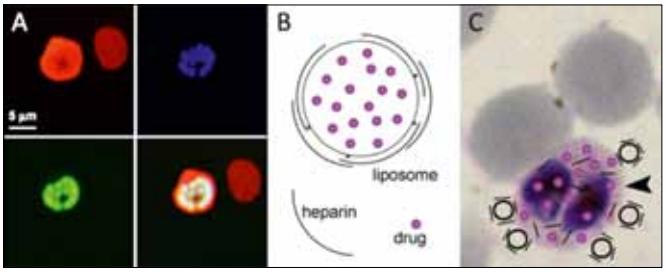
in earnest yet. However, recent data outline the feasibility of some such potential novel approaches, among which we can count new types of combination therapies where one of the activities does not act on individual Plasmodium gene products.

The known capacities of heparin as specific marker of Plasmodium-infected RBCs (pRBCs) vs. RBCs and as an inhibitor of Plasmodium growth, has been used to develop liposomal nanocarriers where heparin had a first role as a targeting molecule of antimalarial drug-loaded liposomes and a second life as an antimalarial in itself (Figure 1)[6]. This particular example of drug combination profits from the specific targeting of one of the drugs (heparin in this case) to Plasmodium-infected cells, whereby antimalarial drug-containing nanocarriers will offer a reduced drug IC50. The resulting decrease in dosage together with high drug amounts in the immediate pathogen's neighbourhood resulting from specific delivery to parasitized cells will contribute to limiting drug resistance selection. Remarkably, efforts to select for heparin-resistant parasites have proven unsuccessful[7], which places this sulfated polysaccharide as an interesting candidate in the race for finding efficient and long-lasting antimalarials.

pRBC-targeted poly(amidoamine) (PAA)-based nanocarriers have been shown to possess a role as carriers of drugs towards Plasmodium-infected cells[8]. PAAs can form nanoparticles capable of encapsulating antimalarial drugs in relatively high amounts, and which specifically interact with pRBCs (vs RBCs) without the need for incorporating targeting molecules. The mechanism of the specific interaction of PAAs with pRBCs has not been elucidated yet, although it might be similar to that of other pRBC-binding polymers such as heparin, which has been shown to bind multiple Plasmodium-encoded antigens[9]. In addition to targeting, part of the capacity of PAAs to lower the drug IC50 stems from their capability to penetrate pRBCs, likely through the tubulovesicular network built by Plasmodium to serve its needs for the intake of nutrients required for intraerythrocytic growth[10]. Finally, some PAAs have been shown to possess significant antiplasmodial activity in themselves, which is most likely based on the inhibition of RBC invasion due to the masking of necessary cell interactions by the polymer binding to parasite surfaces[8].

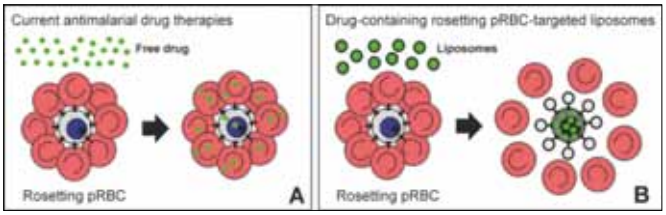
Most malaria vaccination strategies rely on antigens present in laboratory-maintained parasite strains. Because of the high clonal variability that Plasmodium exhibits in the wild[11], when these vaccines reach their clinical application after over a decade of product development and clinical trials, the original antigens might have disappeared from the parasites circulating in the human population. In addition, in each person are found several pathogen strains in a typical malaria infection in high-transmission endemic areas, which will dramatically reduce the efficacy of prophylaxis based on only a predetermined set of antigens. These could be some of the reasons why current malaria vaccines in clinical assays do not offer prospects of complete protection[12]. Experimental evidence has shown that malaria parasites are targeted by different PAAs, which in turn bind plasmodia from widely diverging malarias[8]. Because the antiparasitic mechanism of these polymers seems to operate through inhibition of Plasmodium invasion of red blood cells, the failure of egressed parasites to quickly invade a new host cell will expose the pathogen to the immune system for a longer time. Perhaps PAA-based treatments can be developed as a new therapeutic concept that requires the existence of an active malaria infection at the time of administration. This strategy is not a classical vaccine because it would not be administered before contact with the pathogen, but the resulting effect would provide protection against all Plasmodium strains infecting the patient at the moment of treatment.

Figure 1. Combination activity of heparin as targeting molecule and as antimalarial drug. (A) Heparin (fluorescein labeled, green) binds and penetrates erythrocytes infected by Plasmodium parasites (4',6-diamidino-2-phenylindole DNA stain, blue) but not uninfected cells. Cell membranes are stained with wheat germ agglutinin-rhodamine (red). (B) Schematic setup for the examination of an additive activity of heparin as antimalarial and as targeting agent towards pRBCs, where heparin is electrostatically conjugated to antimalarial drug-containing liposomes. (C) Proposed model where heparin-functionalized liposomes specifically deliver their contents (both drug and heparin) to pRBCs, lowering the drug IC50 and adding to it the antimalarial activity of heparin itself. The arrowhead indicates a pRBC besides two noninfected erythrocytes. From[6].



Recently, an immunoliposomal vehicle has been developed which exhibits a dual activity capable of specifically recognizing and disrupting rosettes while simultaneously eliminating those pRBCs forming them (Figure 2)[13]. This approach represents an innovative combination therapy for the improvement of severe malaria therapeutics having a broader spectrum of activity than either anti-rosetting antibodies or free drugs on their own.

Figure 2. Development of drug-loaded immunoliposomes for the selective targeting and elimination of rosetting Plasmodium falciparum-infected red blood cells.



The concept of antimalarial therapy has been locked for over 100 years on the administration of drugs against which Plasmodium has evolved resistance shortly after their deployment. More often than not, economy-related issues have been hampering the progress of nanotechnology-based medicines against malaria with the dubious argument that they are too expensive to be used in developing areas. Unfortunately, it is true that the application of nanoscience to infectious disease has been traditionally neglected, with most research resources overwhelmingly biased towards other pathologies more prominent in developed regions. Thus, extra ingenuity is demanded from us: malaria-oriented nanomedicines not only need to work spotless; they have to do so in a cost-efficient way because they will be deployed in low-income countries. In this regard, the use of molecular elements combining several antimalarial activities, whether drug, targeting, carrier, or booster of immune reactions, will contribute to reducing the cost of their development. The implementation of a new delivery method is usually cheaper than the process leading to the discovery of a new drug[14], and it has the additional advantage that, if well designed, these strategies can be adapted to several drugs. As an example, the direct delivery of drugs to the mosquito vector would allow a simplification of preclinical assays, thus contributing to a reduction in both the budget of product development and the bench-to-bed time of future antimalarial medicines.

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MATERIALS SCIENCE IN NANOMEDICINE: A MATERIAL'SEERSPECTIVE

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

Despite scientific enthusiasm and high hopes for nanotechnology-based progresses in new biomedical applications, several crucial challenges remain to be addressed for nanomedicines to make clinically important progress. For example, all *in vivo* applications of nanoparticles require particle chemical and colloidal stability, i.e. resistance to particle aggregation and / or dissolution in complex biological media. At the same time, particle circulation times in blood in addition to accessibility, either passively or actively to disease sites, are clearly required to improve. In the "real world" particles show size and property distributions that change during biological experiments, and are therefore not easily defined. Lack of systematic know-how makes it currently impossible to predict nanomaterials behavior under different assay condition.
Comprehensive evaluation of the
(1) composition, size, colloidal stability, surface charge, and the "real" surface after possible adsorption of e.g. plasma proteins/ lipids and the
(2) density, coverage, and stability of the surface coating under physiological conditions
are prerequisites for proper decision-making to obtain significant and consistent results from *in vitro* and *in vivo* experiments regarding nanoparticle-biology interactions, short- and long-term toxicities of nanoparticles in humans, and about their bio-distributions and clearances. In this presentation several points will be addressed in more detail, e.g. in situ particle detection, nanoparticle aggregation in complex environments, the impact of surface grafted fluorescent dyes, and the stability of nanoparticle coatings.

AN EFFICIENT SCREENING TOOL FOR LIPOSOMAL FORMULATIONS IN EARLY DEVELOPMENT OF NOVEL DRUGS

ANDREAS FISCH, Senior Fellow, Novartis Pharma AG, Basel, Switzerland

Liposomes are the most mature parenteral nanomedicine with many products already marketed or in clinical development for treatment of several diseases. By far the majority of those nanocarriers are loaded with generic drug molecules commercially available in big scale.
Novel NCEs formulated in liposomes as first presentation for clinical studies needs to start already in early discovery when only milligram amounts of the drug candidate are available.
But current methods of preparation of liposomes including the standard method of film hydration can only be performed in volumes of several milliliters and suffer from poor drug encapsulation yield in case hydrophilic drugs are integrated in the aqueous core of the liposomes. This is not compatible with the low amount of drug substance available at this development state. There is therefore a need to develop alternative screening tools for early liposomal formulation development.
Dual asymmetric centrifugation (DAC) is a new technology for production of liposomes in a scale of 1 mL or less, achieving comparable high encapsulation yields of water soluble compounds due to a high lipid content during liposome preparation using a high shear ball mill setting.
In this presentation Novartis' experience with the DAC technology in early phase development of liposomal carrier systems will be discussed.

CLINICAL UNDERSTANDING AND PRACTICAL CONSIDERATIONS FOR THE USE OF NANOMEDICINES

BEAT FLÜHMANN, Vifor Pharma Ltd, Switzerland, Member of the Non-Biological Complex Drugs Working Group steering committee hosted at the non for profit public private partnership Lygature, Netherlands

Nanomedicines are important representatives of non-biological complex drugs (NBCDs). They are becoming increasingly available. Up to 23 nanomedicines are approved, and approximately 50 are in clinical development.
Iron sucrose similar have entered as first nanosimilars the European market by application of the generic approval pathway. Post launch significant clinical differences have been observed between the copies and the reference product. Many hospital pharmacists are unaware of the specific characteristics, the *in vivo* profile of nanomedicines and the potential clinical consequences. This lack of awareness leads to a not appropriate substitution practice with

products that are not therapeutically equivalent endangering safe and effective therapy.
Evaluation criteria for rational decision making for the inclusion of nanomedicines into the hospital formulary were discussed in a consensus round table with an international panel of experts and hospital pharmacists. Special emphasize was put on criteria for evaluation of substitutability and interchangeability. On top of previously published criteria for biosimilars, a set of seven criteria, that specifically apply to nanosimilars, were identified and incorporated into an evaluation tool. These include particle size and size distribution (1), particle surface characteristics (2), fraction of uncaptured bioactive moiety (3), stability on storage (4), bioactive moiety uptake (5) and distribution (6), and stability for ready-to-use preparations (7).
Such an evaluation tool can assist the responsible hospital pharmacist to have the necessary data and criteria applied for the nanomedicine evaluation
in early phase development of liposomal carrier systems will be discussed.

THE BRIDGE BETWEEN SYSTEMS AND LOCAL COMPLEXITY – SWITZERLAND AS PACE SETTER TOWARDS NANOMEDICINE AND PERSONALIZED MEDICINE?

GERD FOLKERS

Innovation in the field of chemical and biological therapeutics is one of the most rewarding, both financial and social, may be even intellectual endeavors mankind has started some thousand years ago. Today, hypotheses and premises have fundamentally changed and are mainly based on a bio-physical understanding of a living organism. This is inevitable accompanied by a reductionist perspective on a human being, a patient respectively on his and her biochemistry and physiology. Rigorous rational design suffers from the complexity of local intervention and complexity of systems effects (Matthias Adam 2009).
At the phenomenological level this complexities culminate in the fundamental debate about a healthy individual. English provides a slightly different but more sophisticated vocabulary in discerning illness from disease, or the mental from the somatic part. Modern research realizes the strong entanglement of both, a feeling that Virginia Woolf already was musing about in her essay “On being ill.”
At the molecular level and the nano-level scientists have gained huge knowledge and understanding about disease. This knowledge created a high-level chemotherapy with tremendous success rates in infectious diseases, since the last two decades even in cancer. But, especially in the latter, war is not over. Even when the Time Magazine announced in May 1998 that the problem of cancer is solved at that it will be eradicated soon, the complexity of a “starving” cancer by angiogenesis inhibitors (escape to metastases, resilience and resistance) made the campaign rather a hype than really a hope. Some three monoclonal antibodies and some five small molecule tyrosine kinase and m-TOR inhibitors and the re-positioned Thalidomide try to conquer the market. Some 150 molecules are in the pipelines.
Hence new concepts are needed and being sought, cancer immune therapy being among the most sophisticated and promising. Cutting edge research emerges around Immune Checkpoint Inhibitors. Much of a deeper insight is however needed before eliciting a hype again. To quote a recent review: “The way forward for this class of novel agents lies in our ability to understand human immune responses in the tumor microenvironment.“ (Sharma & Allison 2015)
This is exactly again the problem of understanding local interaction complexity, one of the barriers to neat rational design of new drugs. Maybe that biologicals provide a solution, since their creation implicates a (hidden) optimization of the stereoelectronic control of target interaction. This might pave the way to a real personalized medicine even. At the moment hopes focus on precision medicine. There still remain some open questions. To what extent, an individual is going to be “normalized” by its measurable parameters into a certain cohort? What set of parameters is adequate? What about individual responsibilities and social integration?
If not in Switzerland, where else such research and its consequences may be tackled? The Swiss are world leaders in innovation (per capita), very probably because of stable funding paralleled by entrepreneurship. This situation is still ideal along the lines of the old humboldtonian idea of spill-overs, or framed in a more modern wording by the visionary facilitater of modern science: “As long as scientists are free to pursue the truth wherever it may lead, there will be a flow of new scientific knowledge to those who can apply it to practical problems.“ (Vannever Bush 1945).
Who is going to pay? It is not only the tense financial situation of private and public funding, which demands to spend the money in a more focused manner. Also politics asks for the societal benefits of science, exhibiting growing utilitaristic perspectives. This led to innovation centers and parks, which are believed to work as accelerators for closing the gap between “basic science” and “applied science” and innovation and market entry. This has also led to a kind of generalized peer review system applied both in public and in private funding. Sponsors have nearly completely replaced benefactors or philanthropists. Economic behavior in science is more likely the rule than the exception.

IN VITRO AND IN VIVO EVALUATION OF LIPOSOMES FOR MYOCARDIUM TARGETED DELIVERY

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ABSTRACT

A variety of carrier systems have been investigated for the myocardial delivery of biopharmaceuticals. Here, we aimed to evaluate the biocompatibility of liposomes in the context of cardiac delivery. Liposomes were successfully produced by lipid film hydration. Cytocompatibility was assessed using an Alamar Blue assay in L929 fibroblasts and H2c9 cardiomyocytes. No toxicity was determined at liposomal concentrations ranging from 120 – 9.3 µM. For *in vivo* evaluation, intramyocardial delivery of fluorescent liposomes was performed by ultrasound-guided transthoracic injection in isoproterenol-infarcted mice. Three days later, liposomes were visualized in heart tissue with a slight inflammatory reaction and absence of fibrosis. Importantly, heart retention of liposomes was confirmed by fluorescent microscopy, indicating their ability to remain in the myocardium for 72 h. No fluorescent signal of liposomes was observed in the kidney, lungs, liver and spleen, indicating absence of their biodistribution. These findings demonstrate the potential of liposomes as versatile carriers of biopharmaceuticals for myocardium-targeted delivery.

1. INTRODUCTION

Myocardial infarction (MI) remains a leading cause of morbidity and mortality worldwide[1]. Despite of benefits with drugs, surgical reperfusion and revascularization procedures, the overall efficacy of these approaches is limited.
In the past decade, there was growing investigation on new strategies for regeneration of the injured myocardium, including gene therapy[2], cell therapy[3] and the use of growth factors[4]. These in-

novative approaches have been challenged by hindrance to achieve the heart tissue and promote therapeutic benefits. For example, growth factors clinically failed due to the short circulating half-life and high instability of proteins when systemically administered[5].
Different carrier systems such micro and nanoparticles have been investigated for delivery of biopharmaceuticals into the myocardium[4]. In particular, liposomes have been widely used as nanosized drug delivery carriers, but a few number of studies demonstrating the feasibility of cardiac delivery of liposomes in animal models of MI have been reported[6,7].
Herein, we aimed to evaluate the *in vitro* compatibility of a liposomal formulation in cardiomyocytes and fibroblasts. In addition, the *in vivo* retention and biocompatibility of these liposomes was assessed in an experimental model of myocardial infarction induced by isoproterenol in mice.

2.MATERIAL AND METHODS

2.1 Preparation and characterization of liposomes
Liposomes were prepared using the method of hydrating the lipid film[8] using the lipids soybean phosphatidylcholine and cholesterol (117.6 mM) at 8:2 ratio. Liposomes were characterized by particle size, zeta potential and transmission electron microscopy (TEM).

2.2 In vitro assays
Murine L929 fibroblast cells and H2c9 cardiomyocytes were used for cytocompatibility tests, as previously described[9, 10]. Cell viability was evaluated with liposomes at the following concentrations for 72 h by AlamarBlue® reagent: 120, 72, 43.2, 25.9, 15 and 9.3 µM.

2.3 In vivo experiments
2.3.1. Myocardial infarction model
Animal experiments were performed using a model of isoproterenol induced-myocardial infarction in male C57BL/6J mice (150 mg/kg/day, SC)[11]. Cardiovascular monitoring was performed by electrocardiogram (ECG) and mice with evident alterations were included in the study.

2.3.2 Intramyocardial delivery of liposomes
Twenty-four hours after inducing of MI, myocardium delivery of liposomes was performed by ultrasound-guided transthoracic injection (Vevo 770, VisualSonics, Toronto, Canada). Briefly, sedated mice (1-3% isoflurane mixed with 0.5-0.8 L/min 100% oxygen) were placed onto animal platform to receive a transthoracic injection by a 29G needle/transducer.

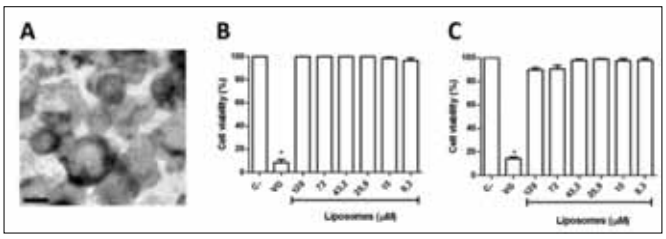
2.3.3 Histological assessment of myocardial tissue after liposome administration
Mice were euthanized 72 h after liposome injection and their hearts were collected for histology, as described elsewhere[12]. Hematoxylin–eosin (HE) and Picrosirius red (PSR) stainings was performed to visualize tissue structure. Samples from other organs were also analyzed for eventual biodistribution.

2.3.4 Tissue retention of liposomes on infarcted myocardium
Rhodamine B isothiocyanate (0.5 mg/mL) was used as a fluorescent marker to localize the injected liposomes by fluorescent microscopy in the heart tissue.

3. RESULTS AND DISCUSSION

3.1 Liposome characterization
Liposomes with different sizes were successfully produced by the method of hydrating the lipid film: 165 nm, 470 nm, 1550 nm and 2000 nm. Zeta potential values varied from – 1,09 mV to – 8,47 mV, for small and large liposomes, respectively. Morphological observation by TEM displayed that liposomes presented a regular and uniform spherical shape (Figure 1A).

Figure 1. TEM image of liposomes. Bar = 500 nm (A). Effects of liposomes on percentage viability of L929 fibroblast (B) and H2c9 cardiomyocyte (C) cell lines by AlamarBlue® assay. Pararosaniline chloride (VG) was used as positive control for cytotoxicity, while untreated cells were used as a negative control (cultivated with DMEM only). The data are representative of two repeats of n = 4. Error bars indicate SEM.



3.2 Cytocompatibility of liposomes
No toxic concentrations were determined in the range 120 – 9.3 µM, where liposomes did not alter significantly the cell viability of L929 fibroblasts and H2c9 cardiomyocytes (Figures 1B-C).

3.3 Ultrasound-guided transthoracic injection of liposomes
Using isoproterenol-infarcted mice, we set up an ultrasound-guided injection of liposomes into myocardium (Figures 2A-B), which allowed the real-time visualized injection of liposomes on heart tissue (Figures 2C). This approach provided a minimally invasive method to achieve successfully the myocardium, as previously validated by transthoracic injection of Evan's blue dye (Figure 2D).

3.4 Histopathological evaluation
HE staining revealed low-grade inflammation at different zones as function of particle size. Large liposomes induced inflammation in both epicardium and myocardium. In contrast, inflammation limited to myocardium was noticed in mice injected with small liposomes. For all liposome sizes, a slight inflammatory reaction was observed (data not shown). On the other hand, no fibrosis was visualized in tissue sections by PSR staining.

3.5 Liposome deposition on myocardium
Heart retention of rhodamine-labeled liposomes was confirmed by fluorescent microscopy, indicating their ability to remain in the myocardium for 72 h. A more intense fluorescent signal on heart tissue was located in mice injected with 470 nm-sized liposomes (Figure 2E). No fluorescent signal of rhodamine-marked liposomes was observed in other tissues such as kidney, lungs, liver and spleen, indicating no migration of the liposomes toward solid organs.

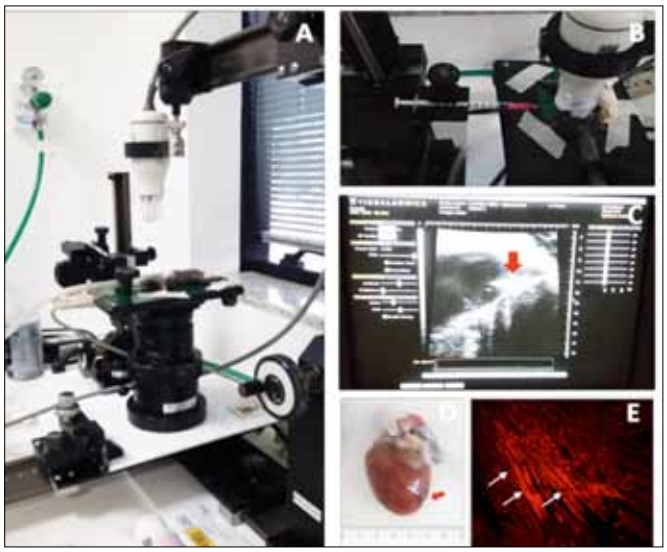


Figure 2. Experimental setup for assessment of myocardium delivery of liposomes. Overview of the animal platform with a transducer for ultrasound-guided transthoracic injection (A). Positioning of a 29G needle syringe filled with fluorescent liposomes under the transducer to enter the thoracic cavity (B). 2D imaging of echocardiography displaying the intramyocardial injection in the left ventricular wall (red arrow) (C). Validation of transthoracic injection using Evan's blue dye, with the injection site visualized on the epicardium (red arrow) (D). Fluorescence microscopy image showing the tissue reten-

tion of fluorescent liposomes (arrows indicating rhodamine fluorescent signals on heart tissue).

4. CONCLUSIONS

Injectable liposomes were successfully produced and our results indicate their cytocompatibility and heart retention. This study shows the feasibility of cardiac delivery of liposomes in a convenient and non-surgical manner. Taken together, these findings demonstrate the potential of liposomes as versatile carriers of biopharmaceuticals for myocardium-targeted delivery, supporting the development of further research on delivery systems for heart disease.

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CONTINUATION OF PROJECTS AFTER THE END OF EU FUNDING

SIEGHARD FRISCHMANN

For research project about 18 billion € are provided in average each year. In the last few years small and middle size enterprises were in the focus for awarding grant fund money. It is estimated, however, that about 80 % of all EU funded projects never lead to a product on market or services in a community. This would mean that of the 75 billion € invested in Horizon 2020 program, 60 billion € will not lead to a product or concept on the market. This situation is worth for a more closer view on the reasons for such a low output efficiency. Not all funded projects of course are directly related to a product idea or service, there is an significant amount of work and money invested in basic research programs.

In general, however, project durations are underestimated. At the end of the project period about 90% of intended work has been finished. The remaining work may require more than 10 % of financial and personnel resources. Certain national programs help to close the financial gap finishing the project task successfully.

Even after a successfully finished project, companies may fail to bring the developed technology or product to market. Companies with a new developed e.g. diagnostic product at hand must start a validation period to get all legal requirements for CE marking or other regulatory issues completed. Depending on the product group this process may take 1–2 years. If products are to be marketed in countries outside the EU, then further national registration demands have to be met.

Another often unconsidered point are patents owned by organizations which were not part of the consortium. Such patents involve certain components which are crucial parts in a project. So enzymes, certain components / procedures for stabilization, DNA / RNA targets, etc. are covered quite frequently by patents. During the project work the use of such patented raw materials is free, but when it comes to commercialization they become a major threat for a successful market launch. The royalties together with a common upfront payment are major issues which often torpedo calculations for a projected market price level.

The total project time from idea, over application to approval, development work and registration easily exceeds a period of 5 years. During such time periods reimbursement, market trends and regulatory issues can change delaying if not hindering the market launch at all.

In order to get some benefits out of a funded project – under the assumption that parts of the concept fail due to unexpected incidents in the marketing schedule, some key findings of the project's development should be fit into existing products, which have found their customers already.

EXTRACELLULAR VESICLES AS SMART CARRIERS FOR SMALL MOLECULE DRUGS

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Extracellular vesicles (EVs) are cell-derived lipid membrane particles decorated with surface and membrane proteins [1]. EVs are nature's way to deliver information as they transfer protein and nucleic acid based cargoes selectively to their target cell. Moreover, EVs feature a naturally derived composition; can potentially bypass complement activation and coagulation factors leading to reduced immunogenicity and increased stability in biological fluids. In addition, they often transit to their specific target cell rendering them promising candidates for drug delivery applications in cancer [2], inflammation [1] or infection research [3]. In order to harness these properties, new ways of encapsulation of drugs into EVs need to be developed. Although EVs have been investigated to deliver RNA-based therapeutics [2], their use as carriers for small molecule drugs has not been studied in detail.

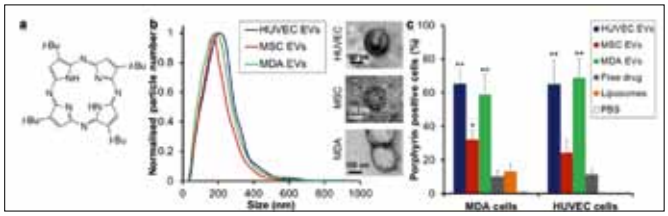

*Figure 1. (a) Chemical structure of hydrophobic porphyrin. (b) Size and morphology of EVs analysed by nanoparticle tracking analysis and electron microscopy. (c) Cell uptake of EV and liposome loaded porphyrin, or free drug (1 µM) in MDA or HUVEC cells (*p<0.05, **p<0.01 vs. free drug, ANOVA, Tukey post-hoc test).*

In this work, we discuss the potential of EVs as smart drug delivery systems [4]. EVs from various cell types (endothelial HUVEC, stem MSC and cancer MDA cells) were isolated and compared regarding size distribution and morphology. Subsequently, they were loaded with model compounds (porphyrins) of different hydrophobicities (Fig. 1a). Porphyrins are currently investigated as potent drugs for photodynamic therapy (i.e., light activatable cytotoxic drug) but their cellular uptake under physiological conditions is often poor. Here, we show successful loading of porphyrins into EVs using various active and passive encapsulation techniques and assessed their therapeutic efficiency. First, EVs from various cell types showed an average diameter of 171-197 nm (single population) as confirmed by TEM (Fig. 1b). Subsequently, EVs were loaded with hydrophobic porphyrin at high ratios and more efficiently than into standard liposomes. EV-mediated delivery of encapsulated porphyrin significantly increased its cellular uptake in cancer and endothelial cells. EVs from MDA and HUVEC cells were more efficient to bring the hydrophobic porphyrin into the cells compared to MSC EVs but all EVs induced a significantly better drug uptake in cancer cells compared with free or liposome encapsulated porphyrin which indicat-

ed their potential for drug delivery applications (Fig. 1c). Upon light illumination, cells that had previously taken up EV-encapsulated porphyrin had a significantly increased cumulative risk to undergo cell death compared to free drug and control cells. Our results indicate that EVs are promising carriers that are able to overcome physiological barriers and deliver drugs with high efficiency and at the cellular level.

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SAFETY REQUIREMENTS IN NANOMEDICINE AND THE SAFE INNOVATION APPROACH

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Innovative nanotechnology applications in both medicinal products and medical technologies are expected to have a continuously growing impact on healthcare. New applications promise advantages such as more effective and less toxic therapeutics, improved and earlier diagnostics and more personalized interventions. On the other hand, the safety evaluation of nanomedical products could pose specific challenges associated with the particulate character of their formulations. We have investigated developments in both types of medical products. We have carried out a horizon scan to identify nanomedical devices that are currently available on the market or under evaluation in clinical studies, and we addressed the various aspects of safety evaluation and risk assessment of medical devices containing nanomaterials. In a different study, we identified nanomedicinal products on the market or in the pipeline. Such products may have distinct physicochemical properties and pharmacokinetics when compared to non-nanomedicinal products. Therefore, we started a follow-up investigation to identify which information would be needed on these aspects for a benefit-risk assessment of nanomedicinal products based on current scientific insights. In addition, we analysed available non-clinical toxicity data as well as reported clinical side effects for a limited set of products. We also performed a review of the accumulating knowledge on potential immunotoxic effects of nanomedicinal products, and in relation to this we did an additional review on assays that are available today for endotoxin determination in nanomaterials and nanomedicinal products and possible pitfalls as a result of the interaction of the testing samples with the assays. Finally, we considered our findings in relation to existing regulatory guidance and testing requirements for medicinal products.
It is important to have a thorough understanding of any specific properties that nanomedical devices and nanomedicinal products may have. Safe innovation, as for example currently being propagated in the NanoReg2 project, requires improved interactions between innovative product developers and regulators throughout the entire innovation process. In this approach, concepts of safe-by-design and regulatory preparedness are combined. At the same time, it is imperative that input from other stakeholders like clinicians and patients is taken into account.

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PIPERINE LOADED PEG-PLGA NANOPARTICLES: PREPARATION, CHARACTERIZATION AND TARGETED DELIVERY FOR ADJUVANT BREAST CANCER CHEMOTHERAPY

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Current anti-cancer drugs are either very toxic or ineffective because of drug resistance development; thus, there is an urgent need to find safe and effective solution for drug resistant cancers. Herbs have shown enormous therapeutic potential without any side effects. Herbal molecules have evolved evolutionarily; their potent activities against a number of diseases make them excellent drug lead candidates for cancer therapy. Curcumin (active component of turmeric) and Piperine (an isolate from black pepper), both has significant anti-cancer activity against drug resistant cancer cells. Piperine has ability of enhancing the bioavailability of co-administered drug, therefore, combination studies of free curcumin and free piperine were done using MCF-7 breast cancer cell line, which demonstrate the enhanced anti-cancer activity as compared to monotherapy. However, hydrophobicity and short half-life of curcumin; central nervous system depression and reproductive toxicity of piperine hinder the therapeutic applications of these two herbal molecules. To overcome above mentioned limitations, targeted delivery of curcumin and piperine was done by using aptamer conjugated-PEG-PLGA-nanoparticles (A-PEG-PNP). Nanoparticles were prepared using single emulsification and solvent evaporation method. Electron microscopy and Zetasizer demonstrated round shape and 130nm size for the prepared PEG-PLGA-nanoparticles. Biophysical characterizations by FT-IR and X-Ray diffraction confirm the amorphous nature of prepared nanoformulations. Confocal microscopy using Lysotraker exhibited the lysosomal escape nature of PEG-PLGA-nanoparticles. FACS analysis using FITC-annexinV indicated the apoptotic effect of both nanoformulations, and western blotting experiments specified the involvement of Bcl2 family proteins in apoptosis. In vitro studies demonstrated that curcumin or piperine encapsulated in Aptamer-PEG-PLGA-nanoparticles have enhanced cellular uptake and higher anti-cancer activity as compared to free form molecule. Bioavailability studies of curcumin-PEG-PLGA-nanoparticles in mice showed enhanced bioavailability of 160ng/ml as compared to 60ng/ml for free curcumin, which further increased to 185ng/ml with combination of Piperine-PEG-PNP. Overall, combination

of Curcumin-PEG-PLGA-nanoparticles and Piperine-Aptamer-PEG-PLGA-nanoparticles may find potential application as an effective as well as safe nanoformulation combination for the treatment of drug resistant breast cancers.

CURRENT STATUS AND FUTURE DIRECTIONS IN NANOTECHNOLOGIES FOR CANCER – VIEW FROM THE US NCI ALLIANCE FOR NANOTECHNOLOGY IN CANCER

PIOTR GRODZINSKI, Director, NCI Alliance for Nanotechnology in Cancer, National Cancer Institute

Nanotechnology has been providing novel, paradigm shifting solutions to medical problems and to cancer, in particular. In order to further these research goals, NCI formed a program called Alliance for Nanotechnology in Cancer which was initiated in 2004. The program funds Centers of Cancer Nanotechnology Excellence – translational arm of the effort, smaller grants focused on fundamental mechanisms of delivery and device characterization, and cancer nanotechnology training programs. An intramural arm of the Alliance - Nanotechnology Characterization Laboratory provides a characterization support to evaluate clinically promising nanomaterials and establish their physical, pharmacological and toxicological characteristics.
In this presentation I will discuss a current status of cancer nanotechnology efforts funded by the program and also describe future opportunities and strategies in this field. Further progress is likely to follow two parallel tracks. First one will be associated with ongoing translation to the clinical environment; while the second with the development of new tools and techniques in research arena. It is expected that small molecule drugs in nanoparticle-based formulations currently undergoing clinical trials will be joint by other modes of therapy including siRNAs, kinase inhibitors, and others. Active targeting, when appropriate will be used more frequently. In order to make translational efforts more wide spread, access to reliable GLP characterization and GMP manufacturing facilities will need to become more available.
Imaging techniques based on nanoparticles will be designed to operate in multi-functional manner; whether it is ability to probe and monitor tumor microenvironment in addition to imaging tumor mass itself, capability of multi-modality imaging, or use of theranostic functions of diagnosis and subsequent treatment. The use of nanotechnologies in intra-operative imaging to guide real time surgery is also expected to expand. In vitro diagnostic devices have matured to a stage in which the development of additional device modalities with new transduction methods does not seem necessary. These devices will be however, increasingly used to collect data for sophisticated multi-parameter analysis allowing to correlate levels of different biomarkers, optimize reliable panels which are required to determine presence of the disease, and determine response of individual patients to different modes of therapies.

CURRENT STATUS AND FUTURE DIRECTIONS IN NANOTECHNOLOGIES FOR CANCER – VIEW FROM THE US NCI ALLIANCE FOR NANOTECHNOLOGY IN CANCER

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Albumin is the most major protein in plasma, playing a key role in regulation of plasma colloidal osmotic pressure and transportation of numerous endogenous compounds. Kinds of cargo moieties including water, cations (e.g., Ca^{2+}, Na^{+} and K^{+}), hydrophobic molecules (e.g., lipophilic vitamins and hormones), and drug molecules are bound to albumin during their blood circulation. Over the past decades, albumin thereby has been extensively explored as a versatile nanocarrier for drug delivery, due to its perfect biocompatibility, lack of toxicity and immunogenicity, as well as its great storage and *in vivo* stabilities. A wide spectrum of drugs has been successfully incorporated with albumin via hydrophobic interaction, electrostatic interaction, or covalent conjugation, resulting in a variety of albumin based nanomedines for diseases diagnosis and therapy.
Inspired by the advantageous properties and appealing development of albumin, we have fabricated several albumin based theranostic nanomedicines with integrated diagnostic and therapeutic functions for multimodal imaging guided combination therapy of cancer. For example, we prepared Gd:CuS@BSA hybrid nanomedicines with bovine serum albumin (BSA) as biotemplates via an environment friendly biomimetic strategy. The as-prepared Gd:CuS@BSA hybrid nanomedicines demonstrated prominent cancer contrasted MRI/photoacoustic imaging performance, as well as efficient hyperthermia ablation and enhanced positive immune response against cancer. Photo induced transformable mBiS/PTX@HSA hybrid nanomedicines were also successfully prepared by encapsulating multi-BiS nanorods and paclitaxel (PTX) into human serum albumin (HSA). The mBiS/PTX@HSA hybrid nanomedines will undergo disintegration upon near infrared laser irradiation, resulting efficiently activated PTX release and single BiS nanorod embedded HSA (sBiS@HSA). In this way, the as-prepared mBiS/PTX@HSA nanomedicines showed enhanced retention and penetration ability as well as controllable drug release, achieving accurate CT/photoacoustic dual-modal imaging diagnosis and efficient chemo/photothermal combination therapy against cancer. Additionally, we also fabricated activated macrophage targeting ICG/MTX@BSA-FA theranostic nanomedicines, pursuing fluorescence/photoacoustic dual-modal imaging guided chemo/photothermal synergistic therapy against rheumatoid arthritis.

RNA NANOMEDICINES FOR INTRAVENOUS INJECTION: CONTROL OF TARGETING SELECTIVITY TO THE ORGANS

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

Targeted delivery of pharmaceutically active compounds for therapeutic purposes has been in the focus of basic research and pharmaceutical development for several decades. For successful translation of such concepts into clinical practice, it is helpful to gain accurate insight and control of all steps of delivery, from injection to the final pharmaceutical effect at the target site. The necessity for such control is even more obvious for nucleotide-based strategies of therapeutic intervention, such as in case of DNA-based *in vivo* gene transfer, and for products based on siRNA, micro RNA or messenger RNA (mRNA).
Here we give insight into the targeting coherencies of intravenously injectable mRNA lipoplex nanoparticle formulations which can be applied for tumor vaccination and further therapeutic applications. We correlate the organ selectivity with molecular characteristics of the nanoparticle products and reveal information on the microscopic distribution pattern in the target organs, the targeting-to-expression ratio, as well as the cell type specificity inside the organs to detail. Such quantitative information on the targeting

specificity *in vivo* can provide a valuable contribution for evaluation and decision making in the course of clinical translation of mRNA delivery vehicles.

RNA LIPOPLEX NANOPARTICLE PRODUCTS FOR TUMOR IMMUNOTHERAPY: CLINICAL UPDATE PHASE 1

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

TBioNTech has developed a technology platform of injectable RNA nano-medicines for tumor immunotherapy, where tumor antigen-encoding messenger RNA (mRNA) is delivered into antigen presenting cells (APCs) in order to induce T-cell mediated antitumoral responses. Pharmaceutical products for local (intranodal) and systemic (intravenous) administration of mRNA vaccines have been successfully brought into clinical testing.
The intravenously injectable RNA vaccines consist of RNA lipoplex nanoparticle formulations made up from lipid excipients, denoted RNA(LIP), which account for high biopharmaceutical availability as well as efficient and organ-selective expression of the RNA. The first product on this basis that has made its way from benchtop to bedside is based on a fixed set of four liposome formulated RNA drug products (DPs) each encoding one shared tumor antigen for (Lipoplex Melanoma RNA Immunotherapy, "Lipo-MERIT"). In the meantime, several further studies have been initiated or are in preparation. This is being approached also for the therapeutic concept of an active personalized cancer vaccine, following identification of every patient´s tumor-specific mutations.
Here we give an update on the advancement of the clinical trials which meanwhile have been initiated on this basis.

PATIENTS AS ADVISORS AND DRIVERS IN MEDICINE DEVELOPMENT PLANNING

DAVID HAERRY, Treatment Writer and Conference Reporter

Patient engagement in early drug development - how much is it wishful thinking, how much is it reality? Two examples from the HIV field show both the benefits and the challenges. The first case is about the first phase III study combining two investigational compounds in a single pivotal study. The intention was to provide a sustainable new therapy to multidrug resistant patients with HIV. By providing access to two NCA from different classes, it was expected that patients could find a way out of the vicious cycle of continuous mono-exposure. Very good collaboration was required to have the trial approved and enrolling both in the US and in Europe.
The second case involves the same actors on both sides - Tibotec (later bought by Janssen) and the European AIDS Treatment Group on the other side. The company had found a new delivery mechanism via depot formulation, which would address adherence challenges and gastro-intestinal side effects. There was also the question of testing the formulation in prevention of HIV acquisition. Very early consultation paved the way for having both interventions developed.

FROM LAB TO BEDSIDE: CLINICAL TRANSLATION OF TARGETED LIPOSOMA DOXORUBICIN TALIDOX/TLD-1

STEFAN HALBHERR, Manager Research and Development, InnoMedica

BACKGROUND:

InnoMedica is a young company with focus on clinical translation of nanomedicine. The company is developing its own liposome platform technology and bridges the gap from bench to bedside. In a first approach, the lead-formulation Talidox/TLD-1 (targeted liposomal doxorubicin) was developed in order to ameliorate chemotherapy for patients, taking into account the patterns of drug-biodistribution in the entire organism as well as nanoparticle-cell interactions and subcellular drug localisation.

METHODS:

Besides physico-chemical characterizations, the pharmacologic properties of TLD-1 were investigated *in vitro* and *in vivo*, while special emphasis was placed on the parallel comparison of the TLD-1 formulation with commercially available free-drug doxorubicin as well as Caelyx/Doxil. In all animal models tested, total amount of applied doxorubicin and treatment regimen for each comparator group was the same, allowing for bridged efficacy conclusions without dosing variations. Animal studies of cancer included immune-competent hosts carrying murine metastatic breast cancer 4T1, and immuno-suppressed hosts carrying human triple negative breast cancer MDA-MB231, A2780 human ovarian cancer, and patient derived xenografts. Drug toxicity was assessed preclinically by body-weight measurements of treated animals and macroscopic examination of tissues prone to display hand-foot-syndrome such as conjunctiva, ears, and tails. Finally, GLP-compliant toxicity testing in rats was initiated and is currently ongoing. The phase 1 open-label non-randomized clinical trial for patients with advanced solid tumors is set to begin in July 2017.

RESULTS:

In 2D and 3D cell culture assays, TLD-1 showed a marked increase in cytotoxicity compared to Caelyx and was similar to the free drug formulation. The tumor growth inhibiting activity of TLD-1 was superior to that of free doxorubicin and subtly outperformed Caelyx/Doxil. Importantly, Hand-Foot-Syndrome symptoms were absent in TLD-1 treatments but observed after Caelyx/Doxil administrations.

CONCLUSIONS:

The sum of all nanoparticulate features of TLD-1 liposomes are believed to jointly attribute to the antitumor activity. Cell culture data indicates that TLD-1 liposomes are readily taken up by cancer cells and release their drug load into the cytoplasm, while commercial liposomes as in Caelyx/Doxil seem to remain outside of cells. TLD-1 conferred markedly reduced side effects compared to both free drug or Caelyx/Doxil whilst greater anticancer efficacy was achieved. This represents a step forward towards the refinement of current state of the art chemotherapy, allowing for reduction of adverse drug reactions and opening ways to better therapeutic outcomes.

PERSONALISED HEALTHCARE – IMPLEMENTATION CHALLENGES AND SOLUTIONS

ANSGAR HEBBORN
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Advances in personalized healthcare (PHC) have led to important discoveries and new treatments that are tailored to specific characteristics of individuals, such as a person's genetic makeup. PHC promises to revolutionise healthcare and has the potential to sub-

stantially improve patient care and patient outcomes, while supporting efforts aiming at more efficient healthcare systems. PHC uses diagnostic tools to identify specific biological markers that can help assess which medicinal treatments and procedures will work best for each patient. By combining this information with an individual's medical history and circumstances, PHC allows doctors and patients to develop targeted prevention and treatment plans.
However, patient access to innovative PHC technology varies dramatically between health care systems in different countries. If the potential of PHC is to be realised, changes will be necessary in the way medicines are developed, regulated, assessed (HTA) and paid for. It is necessary to make policymakers and payers realise that investing now in these advanced therapies and technologies, as well as in adequate regulatory and payer decision making frameworks, will be a key pre-requisite to seeing the full potential of PHC in terms of improved patient outcomes and more efficient health care systems.
The necessary steps to improve the situation range from better coordination and collaboration models between stakeholders and decision makers at various stages within the bench-to-bedside timeframe and across countries to more sophisticated pricing, reimbursement and funding mechanisms as well as effective forms of utilisation management to address the inherent complexity of PHC technologies and services.

NEW LIFE SCIENCES RESEARCH MODELS: US AND CHINA

MICHAEL HEHENBERGER, HM NanoMed

The impact of the Human Genome Project (HGP) from 1988 to 2003 has been studied extensively. It was concluded 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.
However, the US Government has since reduced investments and is increasingly counting on the private sector to fund new Life Sciences breakthroughs. Another emerging trend is the role played by Charitable Foundations, either focused on patient advocacy or on donations by extremely wealthy individuals.
China has made enormous progress since 1999, the first year of China's participation in the Human Genome Project. In 2003, when the completion of HGP was announced by President Bill Clinton and PM Tony Blair, China's contribution was only 1% of the total investment. However, only ten years later, the Chinese BGI organization had taken the global lead in DNA Sequencing. In 2016, China launched the National GeneBank, a $1B project, and started the first Gene Editing (CRISPR) based lung cancer trial.
While current US Government policies de-emphasize science, the Chinese government considers Science a national priority. Will China overtake the West in generating future Life Sciences breakthroughs?

FACE CHARGE AND FLUORESCENCE: BIOCHEMICAL ANALYSIS OF LIPOSOMES AND EXTRACELLULAR VESICLES BY NANOPARTICLE TRACKING ANALYSIS

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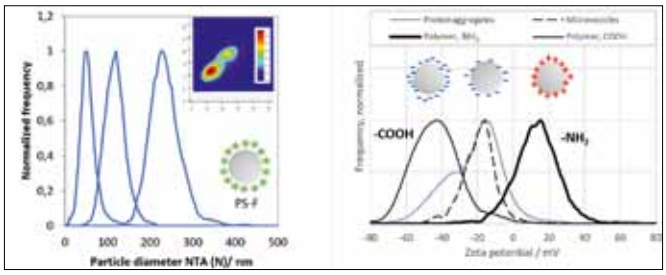
Nanoparticle Tracking Analysis (NTA) measures size and concentration of particles in the size range from 10 nm to 1 µm[1]. While getting a particle size distribution, the user typically cannot discriminate e.g. whether the particle is a lipid or an inorganic precipitate. NTA was combined with surface charge measurement and fluorescence detection capability. This enables the user to gain bio-chemical information about the particle surface and increases resolution and discrimination power.
The spectrum of biological particles ranges from rather small proteins, viruses, virus-like particles (VLPs), vesicles, liposomes protein aggregates to cells. Classical particle analyzers performing sizing and concentration are typically techniques based on the principles of light scattering. Physical techniques detect particles, but a discrimination between biological or inorganic particles such as e.g. dust, nanobubble, metal-oxide particles or precipitates from buffer is typically not achieved. To overcome this limitation, NTA was combined with fluorescence detection[2] and surface charge discrimination techniques[3]. Performance of NTA fluorescence detection was verified for both concentration and size by means of fluorescent nanoparticles (Fluo-Sphere® beads, Fisher Scientific GmbH, Schwerte, Germany). In figure 1 even small particles of 40 nm are detected. When using quantum nanodots (QD), the lowest detected size is even smaller. Several membrane specific dyes have been evaluated in this study: PKH67, DiO, CMO and Alexa Fluor® 488 with either isolated and purified EV samples or liposomes. Stability studies, protocols and calibration procedures will be discussed on selected examples.

Figure 1 (Left): Fluorescence labelled polystyrene particles of nominal 40, 100 and 200 nm.
(Right): Zeta potential distribution is dominated by the surface chemistry of the nanoparticle.



The surface charge is determined by the zeta potential, revealing type and amount of charge. Figure 2 shows zeta potential distributions of nanoparticles bearing positive or negative surface charge depending on the specific groups.

Conclusion: Protocols for quantitative NTA fluorescence detection of isolated EVs and liposomes are presented. By Zeta potential measurements stability and characterization of charge add specific information.

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PERSONALIZED MEDICINE & CANCER: THE ENABLING ROLE OF NANOTECHNOLOGY

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Analysis of somatic mutations has led to the identification of a compendium of aberrations that confer cancer development and has in turn enabled development of drugs that specifically target hallmarks of neoplasia. In the recent years, selection of patients (so-called responders) out of bigger patient cohorts has proven to

be beneficial with regard to drug efficacy and patient outcome and has ushered in the era of precision medicine. Although perceived with much excitement by the scientific community, per-sonalized therapy strongly relies on initial genomic profiling and the constitutive monitoring of bi-omarkers, which require cost-efficient analysis of massive amounts of data; hence few personalized medicines have entered clinical routine. To date, physicians get disproportionally higher rates for pro-cedure-oriented services rather than for evaluation and monitoring of patient outcome [1]. This leads to a financial disincentive to run diagnostic tests to prescreen patients only to find that further treat-ment is ineffective or unnecessary. At the same time, this has led to the resumption of the ethically highly controversial debate whether patients should pay for their participation in clinical research. Be-tween all these conflicting priorities, there are a number of scientific opportunities that may contribute to the easing of the situation by opening new markets, leading to a redistribution of costs and revenues between the various stakeholders. Nanotechnology-enabled carrier and reporter systems may assist in the collection of patient-specific data noninvasively by giving access to unprecedented spatiotemporal resolution [2]. Additionally, nanotechnology-enabled systems may assist in close-meshed data collec-tion as well as compliance monitoring at reduced cost, hence reducing the overall burden to the healthcare system.
In this presentation, I will discuss the role of nanotechnology as an enabling technology facilitating transition from traditional "one fits all" to personalized therapy.

Figure 1:

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IRON OXIDE NANOPARTICLES: ASSESSMENT OF THE STATE OF THE ART AND OUTLOOK

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Superparamagnetic iron oxide nanoparticles (SPIONs) are recognised as promising advanced materials for various biomedical applications, such as targeted drug delivery, contrast agent for imaging, cell tracking, and transfections [1-6]. γ-Fe_2O_3, is of special interest because of the approved biocompatibility of these nanoparticles (NPs), including the well-understood metabolism of the NPs in the liver [7]. Their outstanding magnetic properties makes them versatile candidates for molecular resonance imaging (MRI) or hyperthermia[8].
One of the promising minimally-invasive cancer treatments is magnetically-mediated hyperthermia, which is based on the sensitivity of cancer cells at temperatures above 41°C. Heat is generated by MNP placed in an external alternating magnetic field. In other words, part of the body or the whole patient, having MNP in the tumour, is placed in the coil providing the alternating magnetic field with frequency and amplitude. Due to both the maximal energy to which humans can be exposed and temperature fluctuations at high and/or non-uniform fields, there are limits for the applied frequency and magnetic field strengths. Therefore the nanoparticle properties has to be adapted so that the enough heat is generated by MNPs and dissipated in the surrounding cancerous tissue inducing cell death or tissue sensitisation to increase the efficacy of other anti-cancer therapies. Therefore, the development of this type of therapy is difficult and needs continuous exchange between material science, bioengineering, pharmacy, engineering and clinical researchers. The heating ability of MNPs is quantified by the rate of heat dissipation per unit mass of MNPs, called the specific absorption rate (SAR). It is still a need to develop MNPs having higher SAR values for improved treatment with reduced injected dose, but in allowed magnetic field conditions safe for the patients. SAR is also a complex non-linear function of the magnetic anisotropy constant, the magnetic volume and the saturation magnetization, where optimisation of the first two variables has the largest impact on the SAR increase. The behaviour of the particles in the body (after systemic administration, is of high importance but not very well understood. In this presentation the potential of this type of particles for clinical applications, especially for hyperthermia is highlighted and achievements, future needs, open questions and developments will be discussed.

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WITNESSING THE SOLUTIONS AND TACKLING THE HURDLES OF NANOMEDICINE

PATRICK HUNZIKER

Nanomedicine, the application of the nanotechnologies to the benefit of human health, was mostly a topic of science fiction in the late 20th century, a topic of intense basic research in the first decade of the new millennium, and has now developed into a field of considerable promise in translational and clinical research.
Nanomedicine designates a new paradigm to understanding the processes of life, for diagnosing diseases and treating them in a targeted fashion, unlike many traditional, often organ-related medical specialties.
This key concept sheds new light on the processes at a dimension larger than individual molecules, but smaller than cells. Key tools include innovative imaging tools that allow discerning and analyz-

ing nanoscale objects and processes, new synthetic approaches that assemble materials at the supramolecular scale and lead to multifunctional materials that have manifold advantages compared to the single molecules that are still a mainstay of pharmaceutical medicine.
Innovation and complexity does not come for free, however. Understanding nanomaterials and nanoscale processes and their interaction with life requires a new, broadly interdisciplinary approach in basic science as well as in academic-industrial translation. The interdisciplinary collaboration mandated by the development of nanomedicine is an entry price to be paid, but is followed by a huge reward: The strong focus on interdisciplinarity in basic science and in applied science is closing the gap that has often developed in scientific fields that have increasingly diverged in focus, in scientific language, and in longterm goals.
In its 10th anniversary ,this conference therefore witnesses the solutions to the benefit of human health that can be achieved by overcoming the challenging hurdles in this exciting fields.

ERADICATION OF ATHEROSCLEROSIS

PATRICK HUNZIKER

Atherosclerosis is one of the most prevalent, most deadly and most costly diseases of the developed countries. It's eradication could contribute I a major fashion to improving public health and reducing health care costs.
This talk explores the state of the art 2017 of atherosclerosis eradication and examines the role of Nanomedicine to achieve this worthy goal. To this end, it considers new developments in diagnostics as well as progress in nanomedicine and targeted delivery. It also raises the question of costs in view of current healthcare expenditures, using the example of Switzerland.
It turns out that eradication of atherosclerosis appears to be an attractive and major long-term goal of medicine, and nanomedicine in particular.

NANO-ONCO: BIOPRAXIS ROADMAP TO NANOMEDICINE TRANSLATION FOR CANCER TREATMENT

OIHANE IBARROLA AND ANGEL DEL POZO

BIOPRAXIS faces drug development for rare cancers, including glioma and pancreatic cancer, using nanotechnology and biological molecules like proteins and peptides. Moreover, is also working in controlled release systems through nanoparticles (Solid Lipid Nanoparticles, Magnetic Nanoparticles, Lipid Nanovesicles), which improve therapeutic action of APIs and allow protection of biological molecules and targeting, imaging and future theranostic of different diseases including cancer. BIOPRAXIS and some European partners have existing working expertise in this area, which, more recently, it culminate into HEATDELIVER project: Heat and Drug Delivery nanosystem with active tumor targeting features (Eurotransbio project).
Through the development of the "nanocancer projects", Biopraxis has identified the main challenges about GMP scaling up and nanomedicine translation to patients, and has developed a technological road map to overcome those challenges and get products to the market.
Biopraxis developments are mainly based on "open innovation approaches". We will show how open innovation is crucial to succeed in this context, and will present two examples extracted from 2 European funded projects where Biopraxis, as industry, is enabling scale-up of nanomedicine at GMP level: Theraglio (FP7) and NoCanTher (H2020).
Assessment and implementation of the proposed production processes at GMP conditions will be the crucial role for Biopraxis, in order to get a formulation ready to be tested in a Phase I clinical trial.

EXTENSIVE GLOMERULAR FILTRATION OF GRAPHENE OXIDE THIN SHEETS: IMPACT ON KIDNEY PHYSIOLOGY

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Graphene-based materials have attracted a great deal of interest recently due to their unique properties that have led to a great potential for use in many fields including biomedicine [1]. In this study graphene oxide (GO) derivatives were administered intravenously in C57BL/6 mice. Extensive urinary excretion was one of the main biological end-fates, suggesting an interaction with the kidney glomerular filtration barrier (GFB) [2]. Analysis of the kidney function, histopathology and ultrastructure was carried out. Serum and urine analyses revealed no impairment of kidney function up to one month after injection of GO at doses up to 10 mg/kg. Histological examinations suggested no damage to glomerular and tubular regions of the kidneys. Ultrastructural analysis by transition electron microscopy showed the absence of any damage, with no change in podocyte slit, endothelial fenestra sizes or glomerular basement membrane width (figure 1). This suggests that these rather large GO sheets with sizes that exceed several times the GFB cut-off (< 40 nm) were excreted, by structural re-modelling of the thin and flexible GO sheets. Furthermore, an *in vitro* investigation of how the two highly specialized kidney cells (glomerular endothelial cells and podocytes) interact and uptake this unique 2D material at doses 100 times higher than that expected to be received by the kidney was investigated in this study. The study provides a better understanding of how the unique 2D materials interacts with biological barriers and shows that GO has a potential for various biomedical applications, such as diagnostic imaging and drug-delivery systems.

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COMPLEX GENERICS: THE FDA PERSPECTIVE

WENLEI JIANG, Ph.D., Senior Science Advisor, Office of Research and Standards, Office of Generic Drugs, Center for Drug Evaluation and Research, US FDA

FDA strives to make generic versions available for all product categories by applying a science-based regulatory approach. Complex drug products including those with complex active ingredients (e.g., peptides, mixture of natural products), complex formulations (e.g., liposomes), and drug-device combinations (e.g., inhalation products) posed regulatory challenges for equivalence demonstrations and generic drug development. 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.

THE INTERNATIONAL PHARMACEUTICAL REGULATORS FORUM (IPRF) AT CLINAM 10/2017 IN BASEL, SWITZERLAND

MICHAEL JOHNSTON

As a growing product category, nanotechnology-based therapies have become of more interest for many regulatory agencies. In this regard, the International Pharmaceutical Regulators Forum (IPRF) working group for nanomedicines was established to discuss nanotechnology-related issues relevant to regulated products that may contain nanoscale materials. This session will encompass the working group's annual face-to-face meeting focusing on the open exchange of information on issues of mutual concern for regulatory agencies who are members of the IPRF working group as well as interested parties. A work plan for 2017/2018 will also be discussed.

TARGETING SMALL RNAS FOR CARDIAC REGENERATION

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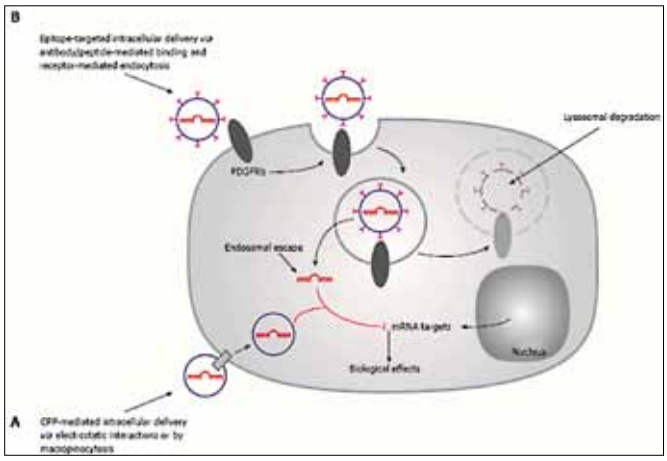
Acute myocardial infarction (aMI) results in the massive loss of cardiomyocytes and subsequent heart failure. The capacity for endogenous regeneration of heart tissue is limited. Several therapeutic approaches to increase endogenous cardiomyocyte regeneration including the application of cell cycle activator and/or growth factors have been pursued. Also, stem cells and progenitor cells are applied to increase cardiomyocyte numbers and although all these therapies improve cardiac function to some extent the overall effects are relatively limited. Therefore, there is a necessity for the development of new therapeutic strategies that aim for increased numbers of cardiomyocytes and decreased scar formation at the site of MI.

Here we propose a targeted drug delivery approach applying cellular plasticity to reprogram cardiac fibroblasts into cardiomyocytes, while we inhibit the cardiac endothelial to mesenchymal transition. The knowledge of these processes has grown enormously the last decade and allows us to specifically pharmacologically interfere in these. Recently, several microRNAs have been identified as key regulators for cardiac fibroblast reprogramming to functional cardiomyocytes. However, to apply microRNAs for reprogramming fibroblasts *in vivo* we require advanced delivery strategies. Current developments in targeted drug delivery open up opportunities for the therapeutic delivery of small RNAs to cardiac fibroblasts and also to activated endothelial cells in the diseased heart. In figure 1, cellular uptake and processing of a liposomal drug delivery system for small RNAs (microRNA or siRNA) is schematically depicted[1].

Figure 1 Passive and active targeted drug delivery systems for small RNA delivery. A: Passive targeting by cell-penetrating peptide-coated nanoparticles are internalized by receptor-mediated endocytosis; B: Active targeting by PDGFRb-targeted liposomes. Liposomes interact with cell surface receptors (PDGFRb) and internalized via receptor-mediated endocytosis. The endocytotic vesicles fuse to form early endosomes which ultimately become part of the lysosomes, where proteins and nucleic acids are degraded by acid hydrolases. To achieve target gene silencing, microRNAs need to be released from the liposome and escape from the endosomes into the cytoplasm, where the microRNA directs the cleavage of target mRNAs.



Small RNA delivery to activated endothelial cells is gaining more and more interest in chronic inflammatory diseases and in tumor angiogenesis. Application of such delivery systems in aMI to inhibit or decrease scar formation would be a logical approach. The NFκB pathway has been shown to play a pivotal role in endothelial-mesenchymal transition[2] and therefore inhibition of NFκB activity in cardiac endothelial cells is expected to be beneficial. Previous studies in mice have shown that local inhibition of NFκB activity in diseased kidney endothelium significantly decreases disease progression[3].

Advances of our basic understanding in the processes involved in aMI, have provide new therapeutic approaches for the amelioration of damage from aMI through delivery of small RNAs for which advanced delivery systems are required. Although there are technical challenges ahead we feel that this targeted and multidisciplinary approach for therapeutic intervention in aMI will become beneficial for patients in the future.

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ALBUMIN BASED RADIONUCLIDE THERAGNOSIS TARGETING FOR SPARC EXPRESSING GLIOBLASTOMA

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Albumin is the most abundant plasma protein and its plasma half-life is 19 days. It is a carrier protein for steroids, thyroid hormone, retinoids, other lipophilic hormones, and lipophilic drugs. When Evans blue-albumin complex was injected into tumor bearing mice via a tail vein, it gradually accumulated in the tumor and retained in the tumor for a long period. This phenomenon is called the enhanced permeability and retention (EPR) effect on the tumor.

^{99}mTc-human serum albumin (HSA) was injected in a tumor bearing mouse, and radioactivity accumulated slowly in the tumor. It has not yet been elucidated why albumin accumulates in the tumor. Some mechanism other than the EPR effect is suggested. A secreted protein, acidic and rich in cysteine (SPARC) is highly expressed in malignant cells and secreted into interstitial space. SPARC may play a role in albumin accumulation in the tumor. We investigated correlation between SPARC expression and HSA uptake by using radiolabeled and fluorescence labeled HSA in U87MG glioblastoma tumor xenograft mouse models.

To confirm SPARC dependent HSA uptake, we prepared a human glioblastoma cell line, U87MG cells which is known to express SPARC and SPARC knock down (KD) U87MG cells. We labeled fluorescence dye FNR648 on HSA by click chemistry reaction. After i.v. injection of FNR648-HSA in U87MG and SPARC KD U87MG xenograft models, the fluorescent signal was detected by IVIS Lumina II *in vivo* and by a confocal microscopy ex vivo. In xenograft models, serial fluorescence imaging revealed that the FNR648-HSA was more accumulated in U87MG tumors than SPARC KD U87MG tumor tissues. While micro-distribution of FNR648-HSA was broad in U87MG tissue, it was confined to endothelial cells in SPARC KD U87MG one.

A radionuclide ^{177}Lu emits both β and γ-ray at the same time. β-ray kills surrounding cells in the tissues and γ-ray penetrates tissues which can be detected by gamma camera. We attempted a radionuclide theragnosis which is combining therapy and *in vivo* imaging by injecting ^{177}Lu labeled HSA in U87MG xenograft mouse models. Serial follow up imaging in a SPECT/CT (single photon emission computed tomography/computed tomography) showed accumulation of ^{177}Lu-HSA in the tumor and tumor growths were slower than control. We visualized tumor targeting of HSA in a glioblastoma model and HSA uptake was dependent on SPARC in the tissue.

STRATEGIES TO DEVELOP NANOPARTICLES FOR CLINICAL IMAGING AND THERAPY

FABIAN KIESSLING, University Hospital Aachen, Director of the Institute for Experimental Molecular Imaging (ExMI) RWTH Aachen University, Member of the directorate of the Helmholtz Institute for Biomedical Engineering, Aachen (D)

If you ask me about the right strategy to develop nanoparticles for clinical imaging and therapy my advice is first to learn thinking like a clinician, then as a health insurance company and then you can add your knowledge about nanoparticle design and synthesis.

In this context, the major question asked by clinicians will be whether the intended nanomedicine strategy will have a chance to improve the therapeutic outcome of patients. This sounds trivial but often this important question is not sufficiently addressed. By the way, this question also holds true for diagnostic probes because if these do not improve the therapeutic outcome clinicians will not use them and insurance agencies will not reimburse the costs.

There were shortcomings in the past, which explain many failed research efforts: For example, there are numerous targeted nanoparticles that have been suggested for molecular imaging purposes but almost none of the targets has been validated in clinical samples with respect to sensitivity and specificity. In addition, it is often not clear which advantage a nanoparticle will have over a small molecule if it comes to diagnostic applications where fast inter-compartmental exchange is mandatory. Furthermore, imaging modalities need to be chosen appropriately. For example, PET-MRI has been developed because PET lacks sufficient anatomical detail but MRI is not sensitive enough for most molecular probes. Taking these circumstances under consideration, does it make sense to develop bi-functional MRI-PET nano-probes? Rather it would make sense to combine PET and optical imaging if a fluorescence-guided surgical intervention follows the whole body PET examination. Another critical question is whether a probe that strongly accumulates via EPR or that is taken up by the RES can be suitable for targeted imaging purposes, where the clinician wants to get a fast and precise information about the expression of a certain target in the tissue? Certainly not, because the unspecific background signal will be too high and elimination of the unbound fraction will be too slow.

The potential of nanoparticles as therapeutics is higher than as diagnostics. It has been clearly shown that many tumors with high EPR better respond to nanomedicines than small drug molecules. However, EPR is heterogeneous and strategies need to be defined to select the ideal cohort of patients. In this context, companion diagnostics and theranostics may be required. Unfortunately, also in the therapeutic fields many nanomedicines convince more by their structural complexity than by their practicability. Pharmacokinetic properties, biodistribution and tissue penetration are key determinants of successful probes. Thus, their optimization should be the primary aim. Secondly, one should strictly distinguish systems that may release their payload in the interstitial space from those that need to enter cells. The first kind of systems should maximally benefit from the EPR effects but can only be used for drugs, which after their release penetrate the tissue and are capable to enter the cells. In case of drugs with low diffusion capacity, high toxicity, and low cellular uptake (and there are many such drugs that were not successful in clinical trials) active targeting may be necessary. However, actively targeted probes need to be small enough to deeply penetrate the tissues and much smaller drug delivery systems than liposomes or micelles may be required. In this context, we hypothesize that the size of antibodies may be ideal.

With these considerations, you have already addressed some important aspects that determine the selection of an appropriate strategy. On top of these questions, if it comes to commercialization and translation, further aspects need to be discussed like protection of intellectual property, market potential and the producibility of the nanomedicine including upscaling.

KILL 'PATIENT CENTRICITY' – THE ETHICAL REQUIREMENT TO INVOLVE PATIENTS AS PARTNERS

INGRID KLINGMANN

Research and development of new treatments are expensive due to a number of factors and even after having achieved marketing authorisation the commercial success is not sure. More focus on patients' needs all through the development process is considered important to maximise the chance for success. "Patient centricity" is interpreted and implemented in different ways by different pharma companies. But is this enough? Which role should patients play in the product development process? At what stage should they be involved? Are they prepared for their role?

Can better selection of drugs or products based on patient-driven, upfront defined, optimal outcome definitions be a step in the right direction? Involving patients into this strategic decision and the development planning and performance is widely debated – but in reality it only works to a very limited degree. There is uncertainty on industry and patient organisation side on how to make this collaboration work. What are the problems and how can these be overcome? This session will provide suggestions and examples for solutions.

HYALURONAN NANOPARTICLES SELECTIVELY TARGET PLAQUE-ASSOCIATED MACROPHAGES AND IMPROVE PLAQUE STABILITY IN ATHEROSCLEROSIS

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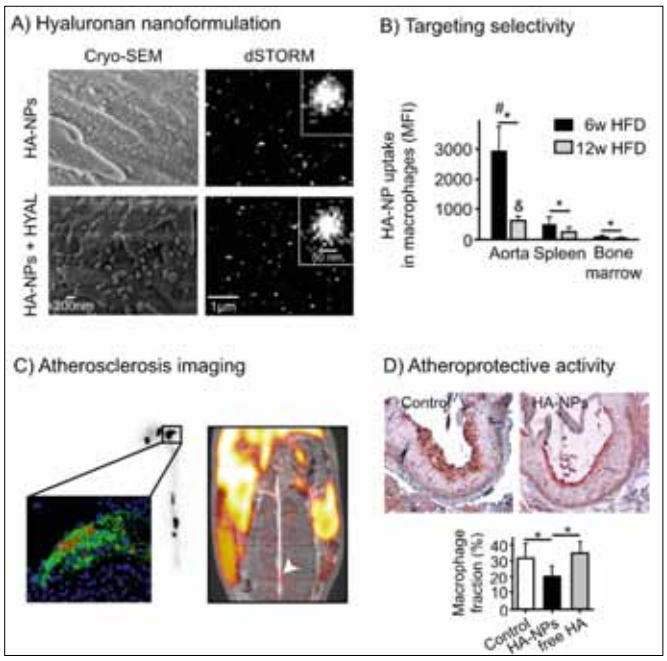
Hyaluronan is a biologically active polymer, which can be formulated into nanoparticles. In our study, we aimed to probe atherosclerosis-associated inflammation by using hyaluronan nanoparticles and to determine whether they can ameliorate atherosclerosis.
Hyaluronan nanoparticles (HA-NPs) were prepared by reacting amine-functionalized oligomeric hyaluronan (HA) with cholanic ester, and labeled with a fluorescent or radioactive label. HA-NPs were characterized *in vitro* by several advanced microscopy methods. The targeting properties and biodistribution of HA-NPs were studied in apoe-/- mice, which received either fluorescent or radiolabeled HA-NPs and examined ex vivo by optical or nuclear techniques. Furthermore, three atherosclerotic rabbits received ^{89}Zr-HA-NPs and were imaged by PET/MRI. The therapeutic effects of HA-NPs were studied in apoe-/- mice, which received weekly doses of 50 mg/kg HA-NPs during a 12 week high-fat diet feeding period. Hydrated HA-NPs were circa 90 nm in diameter and displayed very stable morphology under hydrolysis conditions. Flow cytometry revealed 6 to 40-fold higher uptake of Cy7-HA-NPs by aortic macrophages compared to normal tissue macrophages. Interestingly, both local and systemic HA-NP-immune cell interactions significantly decreased over the disease progression. ^{89}Zr-HA-NPs-induced radioactivity in atherosclerotic aortas was 30% higher than in wild-type controls. PET imaging of rabbits revealed 6-fold higher standardized uptake values compared to the muscle. The plaques of HA-NP-treated mice contained 30% less macrophages compared to control and free HA-treated group. In conclusion, we show favorable targeting properties of HA-NPs, which can be exploited for PET imaging of atherosclerosis-associated inflammation. Furthermore, we demonstrate the anti-inflammatory effects of HA-NPs in atherosclerosis.

Figure 1: A) Hyaluronan nanoparticles (HA-NPs) (upper panel) and HA-NPs after hyaluronidase (HYAL) treatment (lower panel) visualized by cryo-scanning electron microscopy (Cryo-SEM) (left panel) and direct stochastic optical reconstruction microscopy (dSTORM) (right panel). B) The uptake efficacy of Cy5.5-HA-NPs in aortic, splenic and bone marrow macrophages measured by flow cytometry. Black and grey bars represent the data obtained for mice fed with a high fat diet for 6 (6w HFD) and 12 weeks (12w HFD), respectively. Symbol "" indicates inter-group differences, whereas "#" and "&" indicate significantly higher median fluorescence intensity (MFI) compared to all other macrophage populations within 6w HFD and 12w HFD group, respectively, and at $p < 0.05$. C) The accumulation of fluorescent (shown in red) or radiolabeled HA-NPs in atherosclerotic plaques assessed by confocal microscopy (left panel) or autoradiography (middle panel), respectively. Left panel displays a representative PET/MRI fusion image of an atherosclerotic rabbit 12 hours after the administration of ^{89}Zr-HA-NPs. PET signal hot spot can be observed in the abdominal aorta (white arrowhead). D) Representative images of aortic roots from mice that received either PBS (Control, left image panel) or HA-NPs (right image panel) during a 12-week high-fat feeding period. The sections were stained with a macrophage-specific antibody (MAC-3). Bar charts display the percentage of plaque area containing macrophages in the investigated treatment groups.*



IMAGING INTEGRITY OF LIPID NANOCARRIERS IN BLOODSTREAM AND TUMOR OF LIVING MICE

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Lipid nanocarriers are promising candidates for drug delivery and cancer targeting because of their low toxicity, biodegradability and capacity to encapsulate drugs and/or contrasting agents. However, their biomedical applications are currently limited because of a poor understanding of their integrity *in vivo*. It remains unclear whether these nanocarriers are able to bring their cargo (drug) to the destination (tumor), or the cargo is lost and distributed non-specifically in healthy tissues, producing adverse effects. To address this problem, we developed nano-emulsion droplets of 100 nm size encapsulating lipophilic near-infrared cyanine 5.5 and 7.5 dyes. Excellent brightness and efficient Förster Resonance Energy Transfer (FRET) inside lipid nanocarriers enabled for the first time

quantitative fluorescence ratiometric imaging of nanocarrier integrity directly in the blood circulation, liver and tumor xenografts of living mice using a whole-animal imaging set-up. This methodology revealed that in blood circulation of healthy mice, the integrity of our FRET nanocarriers is preserved at 93% after 6h of post-administration. Due to enhanced permeability and retention (EPR) effect, they accumulate rapidly in tumors, while preserving their integrity (77% after 2h), and then further disintegrate with a half-life 4.4h. Thus, we propose a robust methodology for quantification of nanocarrier integrity *in vivo*. Moreover, we show that the nano-emulation droplets are remarkably stable nano-objects that can preserve their integrity in the blood circulation and release their content mainly after entering target tumors.

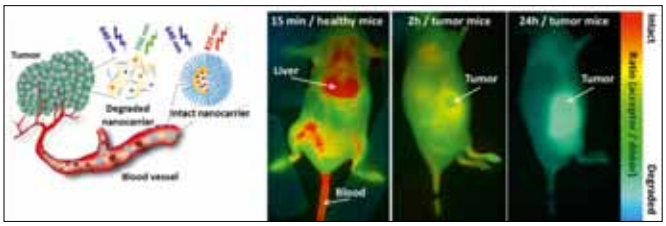

Figure 1. Concept of FRET nanocarriers that can report on their integrity in vivo by change in their emission color. FRET imaging of nanocarrier integrity in healthy and tumor-bearing nude mice.

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IMMUNOREACTIVITY OF FUNCTIONALIZED GOLD AND ALBUMIN NANOPARTICLES

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In the past 30 years, several nanoparticulated drugs and contrast agents were approved for clinical practice to improve the biodistribution versus tumor accumulation, reduce severe toxicity to certain organs such as cardiotoxicity of doxorubicin by liposomal formulation (DOXIL), and allow an enhanced contrast in magnetic resonance or theranostics by iron oxide nanoparticles. The benefit for the patients was significant but unfortunately, with the new nanoformulation of drugs also a novel toxicity, the C activation-related pseudoallergy (CARPA) or hypersensitivity reaction (HSR)[1] was observed in patients. Basing on this first observation for DOXIL, a great number of nanoparticulate drug formulations were tested systematically and were found above a threshold dose to induce more or less severe CARPA in a validated pig model[2,3,4].

Among the nanoparticles designed and synthesized for medical applications, gold nanoparticles (AuNPs) have some attractive features such low toxicity due to the inert metal core, good biocompatibility, photothermal properties and easy functionalization with ligands or other especially thiolated molecules. We tested surface-structured AuNPs coated by self-assembly with hydrophilic mercaptoundecansulfonate (MUS) and hydrophobic octanthiol (OT) in a 2:1 ratio[5,6] for their immunoreactivity along with albumin NPs in 20–30kg pigs in increasing doses.

For albumin we found at low concentrations no or low immune reaction while the aggregates of albumin observed at high concentration induced a significant response. In contrast, the MUS:OT AuNPs showed no immune reactivity at all even at a high concentration. The MUS:OT AuNPs represent therefore a new class of drug carriers that shows no immune reactivity in the pig model, suggesting potentially CARPA–free use in humans.

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NANOBODIES AS THERANOSTIC TOOLS

TONY LAHOUTTE

Our research is focused on the development of radiolabeled cancer targeting probes to image and treat cancer. We apply probes that are based on single domain antibody fragments derived from heavy chain only antibodies also called nanobodies or single domain antibody fragments (sdAbs). We developed nanobodies against the human epidermal growth factor receptor subtype 2 (HER2) as a theranostic tool for breast cancer. Recently these probes were evaluated in a phase I clinical trials. The presentation will give an overview of the development of the anti-HER2 nanobodies and their translation into a clinical application.

CHARACTERISING LENTIVIRAL VECTOR PARTICLES BY SIZE AND COMPOSITION

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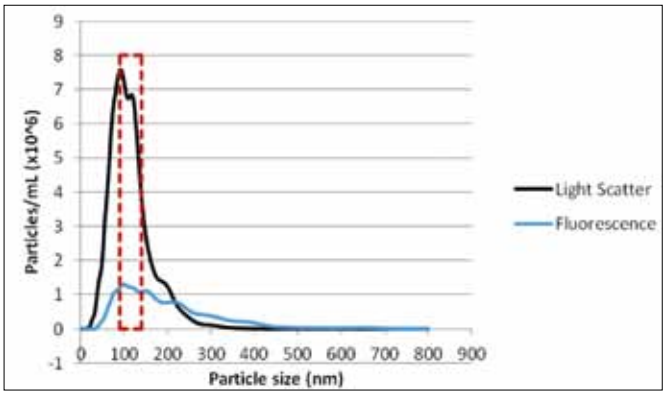
Oxford BioMedica is currently developing several gene therapies that use its Equine Infectious Anaemia Virus (EIAV)-based vector platform technology. Detailed characterisation of lentiviral vector particles is critical to ensure the quality and consistency of batches, and also for manufacturing development purposes. In addition to evaluation of titre, there are several 'indirect' methods available for the characterization of these vectors including vector RNA quantification and evaluation of reverse transcriptase (RT) activity. However, additional information regarding the physical characteristics of vector particles is highly desirable not only for the characterization of clinical batches, but for manufacturing process evaluation and development.

Nanoparticle tracking analysis (NTA) technology was used to analyze lentiviral vectors with comparison to data obtained from flow cytometry and RNA copy number analysis. NTA is a unique tech-

nique for visualising and analysing particles in liquids from 10 nm to1000 nm depending on the material optical properties. The sizing method is based on the Brownian motion of particles. NTA tracks all random particle movements and calculates Diffusion Coefficient. Then using the Stokes-Einstein equation, the particle size and precisely the hydrodynamic diameter is calculated.
HEK293T cells are transfected with three plasmids containing product specific vector genome, codon-optimised Gagpol cassette required for virion production, and VSV-G envelope to allow pseudotyping of vector particles. Preparations however, may contain a mixture of particles as a result of this process and a method that can characterize and distinguish between different particle types would be advantageous.
Fluorescence allows differentiation of VSV-G positive vector particles from other small particles present in the conditioned media shown by the agreement in particle numbers once the non-VSV-G particles are subtracted from the count. Alongside the NTA, samples were analyzed by flow cytometry to quantify particle number.

Figure 1. IH61 crude harvest analyzed with 1/1000 diluted antibody with light scatter(black) and Fluorescence (blue) gated between 90-140 nm (red)



Sample	Detection method	Particles/ml
293T Conditioned media	Light Scatter (gated)	5.09 x 10^8 (105-140nm)
IH61 Clarified Harvest	Light Scatter (gated)	6.46 x 10^8 (105-140nm)
IH61 Clarified Harvest	Fluorescence (gated)	1.26 x 10^8 (105-140nm)
IH61 Clarified Harvest	Fluorescence (ungated)	7.48 x 10^8

Table 1: Illustration of the specificity of VSV-G positive particles in crude harvest using the fluorescence capabilities of NTA

NTA has been used to characterize total particle concentrations and size distribution profiles of lentiviral vector samples. In addition, the use of fluorescence to detect particles that have been immune labelled for VSV-G has been used to increase specificity of detection. Total and VSV-G-positive particle concentrations measured using NTA agree well with estimates based on measurements of RNA copy number.
There were marked differences in VSV-G positive particle concentrations measured using flow cytometry and NTA. It is hypothesized that the lower concentrations returned using flow cytometry are attributable to a combination of high levels of fluorescent background and the fact that measurements are being taken close to the limit of detection. As a consequence, thresholds are set such that some VSV-G positive particles are excluded by the analysis.
Both methods present desirable attributes for the characterization of lentiviral vector samples. NTA can be used to determine particle size with a greater degree of resolution than flow cytometry. However, flow cytometry does have the capability for the development of multiplex analysis due to the large number of lasers and detectors available for simultaneous use. The use of multiplex assays could further increase specificity for the vector particles of interest.

GRAPHENE FOR RNA DETECTION

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Detection for intracellular RNA ranges from mRNA for coding genes and non-coding RNAs for non-coding genes. Quantification of mRNA and detection of usually small amount of non-coding RNAs (ncRNA) have been challenging (Hwang, 2017). Graphene oxide has a high affinity for single stranded nucleic acid and fluorophore-labelled microRNAs (miRs) could be detected in multiplex in cultured cells (Min, 2014). A long non-coding RNA (lncRNA), brain cytoplasmic 1 (BC1) abundant in the brain could be detected using relevant siRNA labeled with fluorophore (Kim, 2016). For this detection, sponge effect of lncRNA was exploited.
In microfluidic chip, cell-non-autonomous differentiation of neural stem cells via exosome from earlier-differentiated cells was also proven using graphene-miR-193-fluorophore sensor. Increased miR-193 of donor differentiated neurons was delivered to recipient neural stem cells via exosomes on microfluidic chip (Oh, 2017). This utility of graphene oxide for RNA sensing was made possible by the advantage of graphene oxide capable of nearly complete quenching by minimizing non-specific background.
For RNA detection in cultured cells in the wells or on the microfluidic chip, to reveal the microRNA and lncRNA expression in the cytoplasm, scheme of graphene oxide-single stranded RNA-fluorophore biosensor is proposed to be an excellent nanoplatform.

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FROM SUPRAMOLECULAR CHEMISTRY TOWARDS ADAPTIVE CHEMISTRY BIOORGANIC AND BIOMEDICAL ASPECTS

JEAN-MARIE LEHN, ISIS, Université de Strasbourg

Supramolecular chemistry aims at constructing and implementing highly complex chemical systems from molecular components held together by non-covalent intermolecular forces. It has relied on the development of preorganized molecular receptors for effecting molecular recognition, catalysis and transport processes. A step beyond consisted in the design of systems undergoing self-organization, i.e. systems capable of spontaneously generating well-defined functional supramolecular architectures by self-assembly from their components Supramolecular chemistry is intrinsically a dynamic chemistry in view of the lability of the non-covalent interactions connecting the molecular components of a supramolecular entity and the resulting ability of supramolecular species to exchange their components. The same holds for molecular chemistry when the molecular entity contains covalent bonds that may form and break reversibility, so as to allow a continuous modification in constitution by reorganization and exchange of building blocks. These features define a Constitutional Dynamic Chemistry (CDC) on both the molecular and supramolecular levels. CDC operates on dynamic constitutional diversity and performs component selection to achieve adaptation, in response to either internal or external factors, thus opening towards an Adaptive Chemistry. De-

velopments in bioorganic chemistry, drug discovery and biomaterials will be presented.

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NANOMEDICINES FOR OVERCOMING MICROBIAL BARRIERS: HOST CELL MEMBRANES, BIOFILMS AND THE GRAM NEGATIVE BACTERIAL ENVELOPE

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INTRODUCTION

Infectious diseases represent an increasing thread to human health. This is at the one hand caused by increasing occurence of drug resistant bacterial strains, and on the other hand by the fact that research for novel infectives had been abandoned by many pharmaceutical companies during the past decennia. According to recent epidemiological forecasts, worldwide fatalities due to antimicrobial resistance disease might in some years even outnumber those caused by cancer. In order to change this trend, increased research efforts for novel antiinfectives are urgently needed. In order to be successful on the long run, some paradigm shifts are also necessary. Rather than aiming for radically killing microorganisms, which is the principle of classic antibiotics, novel so-called pathoblockers merely aim to make bacteria changing their metabolism and behavior, like e.g. to stop the formation of biofilms or the production of disease-causing virulence factors. The hypothesis behind such approaches is that the bacteria will experience less of evolutionary pressure under such therapy and a selection of resistant strains will not occur.

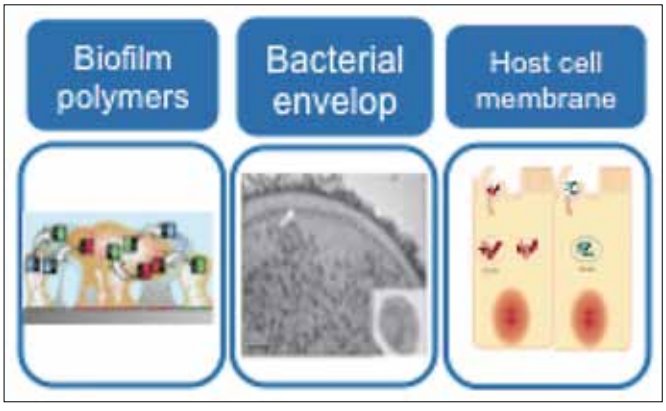

Figure 1: Biological barriers encountered by anti-infective drugs

In contrast to other drugs, for which most receptors are located on the outer cell membrane and thus readily accessible provided sufficiently high drug concentrations are reached in the blood plasma, the site of action for anti-infective drugs is often shielded by some additional biological barriers, which must be taken into account and be adequately addressed by innovative delivery strategies: First, in order to hide and shield themselves, the preferential habitat of some bacteria is the cytoplasm of non-phagocytotic host cells, such as e.g. epithelial cells, sometimes even surrounding themselves by an additional membrane forming a so–called vacuole. Other bacteria produce hydrogel-forming polymers, leading to the formation of so-called biofilms, which nicely protect them both from attacks by the cellular or humoral immune system. At the same time, biofilms are likely to represent a significant diffusion barrier also to anti-infective drugs and drug carriers. Finally, as opposed to the mammalian host cells, bacteria are surround by a more robust cellular envelope ("cell wall"), which besides featuring some protective efflux systems, especially in case of gram-negative bacteria consists of two membrane bilayers and an interspersed proteoglycan shell.

PSEUDOBACTERIAL NANOCARRIERS FOR INTRACELLULAR DELIVERY OF ANTI-INFECTIVES

The poor membrane permeability of many anti-infective drugs poses a significant hurdle to the effective treatment of intracellular infections. Incorporation of such anti-infectives into nanosized particulate carriers and functionalizing their surface with invasive moieties derived from entreo-invasive bacteria may represent a potential way to overcome this difficulty. With this in mind, we have decorated the surface of liposomes with invasin, an outer membrane protein of Yersinia species, which promotes bacterial uptake into intestinal epithelial and M cells [1]. Invasin-functionalized liposomes loaded with the poorly permeable antibiotic gentamicin, which is notorious for being effective only against extracellular bacteria, caused significant killing of both vacuolar and cytoplasmic intracellular bacteria [2,3]. In forthcoming studies, rod-shaped polymeric nanocarriers are being designed to also to mimic the aspherical nature of common enteropathogenic bacteria.

MODELING THE GRAM-NEGATIVE BACTERIAL CELL ENVELOPE IN VITRO FOR PERMEABILITY ASSESSMENT OF NOVEL ANTI-INFECTIVES

Gram-negative bacteria possess a unique and complex cell envelope, composed of an inner and outer membrane separated by an intermediate cell wall-containing periplasm. This tripartite structure acts intrinsically as a significant biological barrier, often limiting the permeation of anti-infectives, and so preventing such drugs from reaching their target. We therefore decided to develop *in vitro* permeation based on Transwell® filter inserts. In a first step, the filter was coated with bacteria derived lipids to model the inner membrane. Permeability investigations of model compounds as well as anti-infectives allowed to clearly detecting differences in comparison to an analogous comparator model based on mammalian lipids [4]. In more recent studies, the model was extended to mimic all three essential structural elements of the Gram negative bacterial envelop: the inner membrane, the periplasmic space as well as the asymmetric outer membrane. Quantitative and time-resolved permeation data as measured for some novel anti-virulence compounds with this in-vitro model were found to be predictive for bacterial uptake and could explain different in bacterio activities in spite of similar receptor affinities.

MODELING AND COMBATTING RESPIRATORY INFECTIONS

Quorum sensing inhibitors represent a novel class of anti-infectives. They are aimed to reduce the pathogenicity of biofilm forming P. aeruginosa by interfering with this peculiar a cell-to-cell communication system which also involves the virulence factor pyocyanin. To improve the delivery of these small, but poorly water-soluble molecules across non-cellular barriers, such as mucus and bacterial biofilms, ultra-small solid lipid nanoparticles (us-SLNs), were prepared by hot melt homogenization and found suitable for aerosolization. Testing the anti-virulence efficacy by measuring by pyocyanin formation in P. aeruginosa cultures showed up to seven-

fold superior anti-virulence activity to the free compound [5]. Interestingly, the plain SLNs exhibited anti-virulence properties themselves, probably due to some anti-virulence effects of the emulsifiers used. These startling findings represent a new perspective of ultimate significance in the area of nano-based delivery of novel anti-infectives.
In another study we have developed clarithromycin microparticles as aerosolizable dry powder using leucine and chitosan. After aerosol deposition on Calu3 monolayers, the integrity of the epithelial barrier was maintained while antimicrobial activity against Gram positive and Gram negative bacteria could be demonstrated [6]. The same drug was also incorporated into poly(lactic-co-glycolic acid) nanocapsules, partially also coated with the mucoadhesive polymer chitosan. Antimicrobial activity of nano-encapsulated clarithromycin was evaluated in Staphylococcus aureus infected macrophages. The macrophages internalized the nanocapsules without evident cell toxicity, while survival of intracellular S. aureus was significantly reduced, the effect being even more pronounced in presence of a chitosan coating.

In contrast to animal experiments, cell and tissue based *in vitro* models allow detailed investigation of cellular processes occurring at the air blood barrier, which is normally not accessible to direct observation *in vivo*. When based on human cells and tissues, such models moreover avoid problems related to species differences. Our team has recently succeeded in generating a first human alveolar epithelial cell line by lentivirus-mediated immortalization (hAELVi) [7]. This cell line displays AT-1 characteristics and tight intercellular junctions (<2000 Ohm cm^2) over several passage (<30) and is commercially available. Autologous coculture of human alveolar epithelial cells and macrophages could be established by using both cell types originating from the same donor [8]. The establishment of a co-culture model of human pulmonary epithelial cells and various bacteria is currently in progress, with the aim to monitor both the antimicrobial efficacy as well as respiratory safety of novel aerosolizable antiinfectives and nanocarriers.

CONCLUSIONS:

Besides the exploration of novel modes of actions and new molecular structures, the efficient delivery of anti-infective drugs to the site of action remains essential for their bioavailability. In this context, both nanomedicines as well advanced human cell- and tissue-based *in vitro* models are valuable tools.

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PRECISION MEDICINE: THE VIRTUAL SELF AS THE BASIS OF TRULY PERSONALISED THERAPY AND PREVENTION

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Every patient is different. In particular, every tumor is different. Even subgroups of tumor cells can react differently to specific therapies, due to the heterogeneity of many tumors. Drug therapies therefore typically only help a fraction of patients; many patients do not respond, with some suffering sometimes severe side effects of ineffective treatments.
The ability to identify effects and possible side effects of different drugs on individual patients will, in our view, require highly detailed molecular, clinical, imaging and sensor analyses ideally covering a wide range of different techniques of every individual patient and his/her individual disease; data that is integral to generating individualized computer models, which can then be used to test the effects of drugs (or other therapies) on the individual. In oncology, for example, we minimally need data on low coverage genome, deep exome and transcriptome of the tumor, low coverage genome and deep exome of the patient, but many types of additional information could help a lot (e.g. epigenome, proteome, metabolome, spatially resolved transcriptome of the tumor, deep immune status of the patient, sequences and methylation status of free DNA etc.).
This will, on one hand, provide a basis for a truly personalized selection of therapies optimal for the individual patient, first in cancer patients, but increasingly also in other areas of medicine and prevention. It will, however, also open the way to an increasing virtualization of the drug development process, by e.g. virtual clinical trials of drug candidates carried out throughout the development process.

NANOMEDICINE FOR TARGET-SPECIFIC IMAGING AND TREATMENT OF ATHEROTHROMBOSIS – OUTCOMES OF THE EU-FUNDED PROJECT "NANOATHERO"

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Atherothrombotic diseases remain the main cause of morbidity and mortality with clinical manifestations of angina, heart attack and stroke. There is a need for new approaches for early diagnosis and improved therapies. This is the focus of NanoAthero, an European large scale project, started in February 2013 (Letourneur & Trohopoulos, 2014). The aim is to demonstrate that nanotechnologies can be developed and clinically proven to be effective in tackling cardiovascular diseases (Chauvierre & Letourneur, 2015; Juenet et al., 2015; Zheng & Stroes, 2016).
NanoAthero combines in-depth knowledge of nano-carrier 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

(16 partners from 10 countries - see http://www.nanoathero.eu/). NanoAthero project integrates several key elements: GMP production, the initiation of clinical investigations in patients at high cardiovascular risk, 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 (Matuszak et al., 2016) were studied and evaluated *in vitro* and *in vivo* (Almer et al, 2014; Suzuki et al., 2015; van der Valk FM et al., 2016). Using GMP liposomal nanoparticle (Lobatto et al., 2015), the clinical studies of liposomes encapsulating prednisolone in atherosclerosis were already performed (van 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 molecular 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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DEVELOPING FUNCTIONAL NANOMATERIALS FOR SENSITIVE BIOIMAGING APPLICATIONS

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The plasmonic nanomaterials have been extensively investigated as a promising tool for various bio applications such as in-vitro biosensing, bioimaging and therapeutic applications. Rationally designed nanomaterials with the unique optical properties of plasmonic nanomaterials could be a promising tool that can overcome the key limitations of current technology. In this talk, I am going to introduce three different plasmonic nanomaterials designed to solve the sensitivity issues of Raman-based cell imaging, photoacoustic imaging and radionuclide-based imaging applications. Raman-based live cell imaging can be a powerful spectroscopic technology for high throughput and high content drug screening, but currently it requires the dramatic increase of signal sensitivity and reproducibility that can be attained with the sophisticated instrumental setup and/or signal amplification strategies (i.e., SERS) to obtain enhanced Raman spectrum. In this regard, the recently reported the intra-nanogap gold nanostructure (Au-NNPs) has a strong potential for high resolution and high speed Raman imaging [1].

Photoacoustic imaging is another promising tool to overcome the low resolution of ultrasound-based imaging technology by use of light as an excitation source, but it essentially requires the use imaging agent to obtain high resolution imaging. The rationally designed hybrid structure composed of plasmonic nanoparticle and graphene could be a good PA imaging agents because of the enhanced photothermal effect and subsequently enhanced photoacoustic amplitude of the hybrid nanomaterial [2].

For nuclear imaging, the imaging agent with high signal sensitivity and stability in-vivo are also required to sensitively monitor the disease state of target organ. Here we used DNA-modified AuNPs as a multivalent substrate for simple and straightforward radio-labelling chemistry that can generate strong and stable radio-activity for nuclear imaging.

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THE CLINAM NETWORK FOR ADVANCING NANOMEDICINE

BEAT LÖFFLER, MA; CEO of the European Foundation for Clinical Nanomedicine

The aim of the CLINAM foundation is the research and development of nanomedicine with regard to its use as an innovative technology, better medical care in the future and the establishing an international network in nanomedicine and related fields. This speech will show the background of the idea and the possible pathways how networks influence the possibilities to get funding for research projects. Some cooperation projects that occurred out of the CLINAM-network will be shortly presented.

DELIVERY OF RNA WITH EXTRACELLULAR VESICLES

IMRE MÄGER

Extracellular vesicles (EVs), nano-sized vesicles secreted by the cell, are important mediators of cell-to-cell communication. By trafficking their cargo such as miRNA and other regulatory RNA, EVs can affect the biological state of their recipient cells. However, sequencing of EV cargo reveals that despite mediating a wide range of biological effects upon transfer, miRNA represents only a very small proportion of EV-loaded RNA. EV proteome is consistent with this observation. Whereas RNA binding proteins are abundant in EV samples, miRNA binding proteins are relatively rare. These findings are consistent with the reported high heterogeneity of EVs and, importantly, reveal new insights on different aspects of the overall RNA delivery with EVs.

GETTING FROM CELLS TO MICE TO MEN WITH NANOMEDICINES IN CANCER THERAPY: HOW TO CHOOSE WHAT NANOMEDICINES CAN DO FOR CANCER THERAPY

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Nanomedicine has promised to make a huge impact in the way we diagnose and treat human diseases. Among these cancer treatment has been a prime target for many groups. Not only because it is a pressing field of research with so many people getting older in the developed countries but also because the first nanocarriers developed for applications in medicine (DOXIL®) has been in this field. Nonetheless the translation of experimental observations and with all the highly promising nanocarriers brought forward in the scientific literature the translation into a product is not only hampered by the hurdles in the industrial-pharmaceutical process of getting these products through the phases of clinical trials. The translation from cell culture into animal models and finally into a clinical trial can only be successful when meaningful questions are chosen as well as appropriate test systems *in vitro* and *in vivo* are implemented. Only then the results can be translated into a clinical trial.
The hurdles of translating ideas in the field of nanocarriers from bench to bedside will be discussed from the materials of nanocarriers used, the testing *in vitro* and finally *in vivo*. This will bring forward the questions of cell types and media as well as media supplement used. As a prominent example the choice of media supplement has been largely disregarded for a long time and has been shown to be a critical aspect. This demonstrates that the role of such factors which are invariantly used by many groups and are therefore considered "good scientific practice" may lead to non-transferable results. Also the choice of animal models is of critical importance. Pitfalls in choosing and applying animal models also are becoming critical components. With all this together we should come up with more relevant answers and procedures which are transferable into clinic.

NANO/MICRO SIZED CONTRAST AGENTS FOR IMAGING APPLICATIONS: THE CRITICAL FACTORS FOR THEIR CLINICAL TRANSLATION

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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, nano- & micro-technology in medical imaging reefers to the use of nano & micro-particles 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).
Recently other new imaging modalities are emerging for a potential larger clinical applications like Optical Imaging (OI) and Photoacustic Imaging (PAI).
The increased attention in the development of multifunctional nano & micro sized imaging probes is principally due to their versatilities offered over the conventional contrast agents. In fact, the availabilities of several surface chemistries, unique magnetic properties and tunable energy absorption and emission properties make the nanoparticles an exciting technology opportunity for all the imaging techniques. However, among others issues, the clinical interpretation of an imaging signal coming from nanoparticles may be extremely cumbersome. Indeed the whole signal could be the result of several mixed mechanisms where both active and passive interactions with heterogeneous tissue localizations can affect the total signal in an unpredictable way. The translation of the registered static or dynamic signal to an information with a clear diagnostic value is not straightforward. In contrast with the considerable amount of papers published on the use of nanoparticles for functional and molecular imaging, currently there are only two remarkable examples of nano-sized contrast agents available on the clinical market. The first example is a sulfur based colloidal formulation of 99tmTc used for the staging of breast cancer and, more recently, melanoma. The second one is represented by the off-label use of Ferumoxitol as an MRI angiography agent in patients with renal failure who cannot be given gadolinium and in clinical trials for the characterization and mapping of metastatic lymph nodes and hepatic masses.
Differently from the complexities encountered in the development of nano-sized systems for imaging purpouses, micro-sized systems like micro-bubbles are largely used as contrast agents for Ultrasound imaging and their use is continuously growing in the clinical practice. Their clinical value has been established and offer several translation advantages due to their simplified biodistribution properties triggered by their blood pool behavior and low half-time. Moreover, with micro-bubbles, active targeting strategies may be finely tuned and can be efficiently used to probe virtually any abnormality in the membrane composition of endothelial cells. On this respect the information regarding organ perfusion and endothelium aberrations are derived independently and can be used to stratify pathological tissues. The aim of this talk is to provide an update on the latest advances designed to overcome the main challenges encountered in the translation of nano- & micro-sized particles for medical imaging applications.

PERSONALIZED MEDICINE FOR ATHEROSCLEROSIS – RELEVANCE, PROSPECTS

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Myocardial infarction and stroke are caused by unstable, vulnerable atherosclerotic (AS) plaque lesions of the vascular wall. An early diagnosis and therapeutic stabilization of these lesions may effectively prevent fatal clinical endpoints. As the scenario of vulnerable AS-plaques encompasses interlocked pathological conditions like dyslipidemia, immune-inflammation, disturbed balance between clotting and bleeding, genetic aspects, oxidative stress, metabolism-microbiom interactions, the individual clinical outcome differs significantly. An improved sharpened recognition of the individual pathological profile will give a better basis for a personalized medicine of atherosclerosis.
Vulnerable AS plaques are frequently non-stenotic, they remain

preclinical undetectable by conventional imaging modalities. Blood lipids (triglycerides, small, dense LDLs, fatty acids), C-reactive protein, and interleukin-6 may be increased, but are insufficient for a personalized diagnostic assessment. Some biomarkers (e.g., troponin, copeptin, heart-type fatty acid binding protein, natriuretic peptides, soluble ST2, etc.) indicate acute coronary syndrome or cardiac insufficiency, but not the preclinical phase of a critical destabilization of AS lesions in coronary or carotid arteries. Thus, valuable time that could be used to treat the patient is wasted.
Inflammation including macrophage and T-cell polarization, innate- and adaptive immune responses are critically involved in the process of destabilization. New biomarkers of interest comprise Pentraxin 3, Angiopoietin-like proteins, Trimethylamine N-oxide (TMAO) and microbiome interactions. Nevertheless, the main challenge of a feasible personalized management remains: which person should be screened? At which time? Furthermore, it is essential to act therapeutically specific, effective, without side effects because the "patient" feels healthy at the time of prediagnosis.

GOING BEYOND THE DISCOVERY OF ASSOCIATIONS

A 3C- Categorization, Classification & Clustering- strategy, was developed in our lab, as part of the Medical Informatics efforts in the Human Brain Flagship Project. It was applied to the above described Parkinson's disease cohort and to the Alzheimer's disease Neuroimaging Initiative (ADNI) cohort. The 3C approach aims to provide a comprehensive insight through a stepwise process, based on supervised and unsupervised algorithms. The approach incorporates medical expert knowledge in a structured way into the analysis process of the disease manifestations and potential biomarkers. The preliminary study applying the "3C strategy" to the ADNI cohort suggests, for example, new sub-classes, with clinical and biomarker characteristics different from those assigned in the ADNI database. We therefore believe it has the potential to move us, toward personalized reliable prediction and treatment. These case studies have led to insights on effective exploitation of large databases in the study of disease. We are convinced that the resulting methods will help us move forward toward our goals of personalized medicine and healthy aging.

GETTING FROM CELLS TO MICE TO MEN WITH NANOMEDICINES IN CANCER THERAPY: HOW TO CHOOSE WHAT NANOMEDICINES CAN DO FOR CANCER THERAPY

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The premise of personalized and precision medicine depends on the ability to define the broad, comprehensive and reliable signature of a disease. The term "Disease Signature" is widely used these days, but a systematic literature review reveals no proper definition and no clear concept for this term. Many articles define disease signature as a single (often genetic) biomarker, mainly in neurology and oncology.
Thus, our target is a comprehensive definition of "Disease Signature" that relates to all relevant personal micro and macro environmental features, physical, mental, cultural and environmental of the human body functioning in his surroundings. It might include parameters such as: age, gender, clinical tests, biological markers, medical history, genetics, imaging, lifestyle, physical and sociological environment, etc.
New advanced technologies have greatly enhanced our ability to capture, analyse and translate these parameters through profound insights on the human body and how it functions. Still, integration knowledge and data from different domains encounters many barriers, for example, dealing with various sets of data originating from different sources, missing values, privacy, security and the special concern of dealing with the lack of consistency in the final diagnosis. Involving and combining various hospital data creates additional barriers of concepts, language, modes of treatments, missing values, etc. A Parkinson's disease hospital cohort is one case study we have analysed, including diagnosed patients and their family members, and containing genetic, cognitive and environmental measures.
The pre- processing stage in the Parkinson case was crucial and included identification of invalid values, correlated variables and those with the same medical interpretation or redundant information. An imputation scheme was developed for addressing missing values while segregating 'missing at random' from 'missing not at random' cases and relating to the characteristics of the observations. Statistical analysis could commence only after these important stages of data cleaning. As a second stage the large database is screened while controlling the average false proportion rate over the selected families using the Benjamini & Bogomolov (2013) proposal for multiple testing of families. This yielded new discoveries regarding the association between genotypes and Parkinson's disease clinical data as well as guarantees for replicable results, in spite of the fact that they were discovered after intensive search.

SWARMS OF MAGNETIC BACTERIA COULD BE USED TO DELIVER DRUGS TO TUMORS

SYLVAIN MARTEL

To achieve optimal deliveries to regions leading to the maximum therapeutic effects for a given molecular construct while minimizing systemic toxicity for the patient, a new and disruptive approach with the capability to enhance significantly existing therapies is needed to target active cancer cells in primary tumors or in relocated metastatic tumors. As such, a new delivery agent with the right specifications and functionalities to target and deliver therapeutics directly to regions of active cancer cells using the shortest physiological routes is required.
Presently, three main obstacles prevent most therapeutic agents to reach regions of active tumor cells. The first one is systemic circulation which yields a low therapeutic index with increased systemic toxicity to healthy organs and tissues. The second obstacle is the diffusion limit of passively-drifting molecular constructs caused by the tumor interstitial fluid pressure [1] preventing such agents to reach regions of active cancer cells located deep in tumoral tissue. Finally, the third obstacle is the lack of a sensory-based displacement capability to reach and target regions of active cancer cells.
As smart bombs relying on engineering principles that replaced carpet or saturation bombings yielding more precise targeting with less collateral damages, engineering could potentially play an increasing role in improving the delivery of existing therapeutic payloads in terms of accuracy and effectiveness, hence providing a means to increase the therapeutic efficacy of existing molecular constructs while decreasing systemic toxicity. Indeed, looking more carefully at existing obstacles to achieve more efficient therapeutic deliveries, one could see that they correspond exactly to the types of problems that are often investigated and solved in robotics but at a larger scale. For instance, navigation along a trajectory is commonly used in robotics and could be adapted to allow non-systemic deliveries using the shortest physiological routes separating the injection site to the regions to be treated. Actuation in the form of a propelling force is also widely used in robotics and if implemented at a sufficiently small scale, could not only enable the aforementioned non-systemic deliveries but also propel the navigable therapeutic agents well beyond the diffusion limit of present molecular constructs and deep enough to reach regions of active tumor cells. Lastly, sensory-based displacement as often encountered in robotics and supported by an appropriate homing capability embedded in each delivery agent would allow effective and autonomous targeting of regions of active tumor cells.
The main problem is that such sophisticated agents supporting these advanced robotic functions cannot be implemented with current technologies at the scale required to transit through the physiological microenvironments and inside tumors. Instead, the mag-

neto-aerotactic bacteria Magnetococcus marinus strain MC-1 cells [2] have been harnessed to implement such delivery agents (Figure 1). When operating in an artificially generated magnetic environment in a special interventional platform dubbed the magnetotaxis platform which has been developed by our group, these bacteria behave as "smart" delivery agents with the robotic functionalities required to deliver non-systematically therapeutic payloads to active tumor cells. Each therapeutic loaded MC-1 cell is sufficiently small to transit through the interstitial spaces and the intercellular openings of less than 2 µm between endothelial cells within solid tumors [3]. Each cell is self-propelled by two bundles of flagella connected to rotary molecular motors. Navigation is achieved by applying a directional torque on a chain of intracellular iron-oxide nanoparticles synthesized in the cell during cultivation that acts like a microscopic compass needle [4]. Such direction is set towards the target region from a weak magnetic field generated by the magnetotaxis platform. Once in the tumor, the magnetic field strength is adjusted to a sufficient low level to allow the bacteria to seek tumor oxygen depleted hypoxic regions [5]. Such tumor hypoxic regions occur when a sufficiently large number of tumor cells duplicate at a rapid pace, consuming oxygen faster than the supply of oxygen. By following a decreasing oxygen gradient, the bacteria autonomously search for a low level of 0.5% oxygen as they do in nature [6] and which corresponds to the level of oxygen expected in regions of active cancer cells.

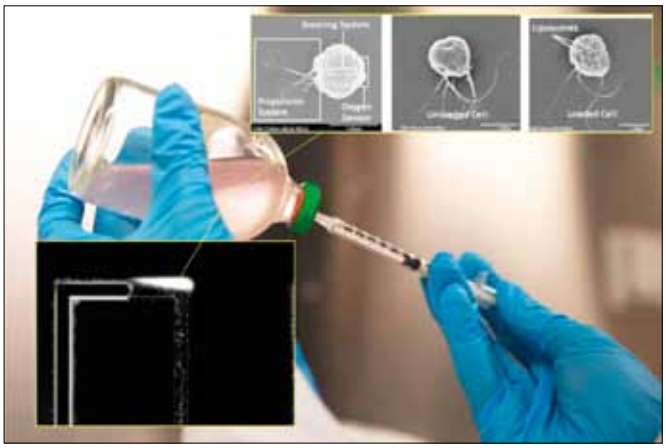

Figure 1 – The MC-1bacteria can be harnessed to become sophisticated therapeutic delivery agents with the robotic functionalities required to directly target active cancer cells.

We showed with initial *in vivo* experiments conducted in mice that 55% of the 100 million drug-loaded MC-1 cells [7] injected peritumorally successfully targeted and delivered therapeutics to tumor hypoxic zones [8]. With promising safety tests conducted in mice and rats, a human-scale magnetotaxis interventional already built, and the fact that many therapeutic modalities could take advantages of such delivery agents, translational efforts are underway to bring such new technology to the clinics.

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NEW MODELS OF DRUG DEVELOPMENT FOR NEGLECTED TROPICAL DISEASES

PASCAL MÄSER, Swiss Tropical and Public Health Institute, Basel

Tropical diseases such as African sleeping sickness, leishmaniasis or Chagas' disease are neglected in the sense that they mainly affect patients who cannot afford to pay for treatment. These diseases therefore do not represent attractive markets for the pharma sector, in spite of the fact that there is a high need for new drugs. Vaccination against trypanosomatid parasites is not possible and the currently available drugs are impractical and toxic. New collaborative models are required to translate the breakthroughs in infection biology basic research into better and safer chemotherapeutics. Here I shall present successful developments that have boosted drug R&D for neglected tropical diseases, ranging from new chemotherapeutic approaches over different kinds of public-private partnerships to new financial incentives such as FDA priority vouchers.

FROM THE ENHANCED PERMEABILITY AND RETENTION (EPR) EFFECT TO CANCER STROMAL TARGETING THERAPY (CAST), WITH RESPECT TO THE CHARACTERISTICS OF CLINICAL CANCER TISSUES

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BACKGROUND

Drug delivery system (DDS) formulations, including monoclonal antibody (mAb)-based drugs, are too large to pass through the normal vessel wall but can easily be extravasated from leaky tumor vessels and retained in solid tumor tissues for long periods of time because of the enhanced permeability and retention (EPR) effect. Moreover, mAbs can target tumor cells actively. Therefore, to date, numerous mAbs recognizing molecules on tumor cell surfaces have been developed and conjugated with anticancer agents (ACAs), radioisotopes, or toxins. However, the use of high molecular weight agents presents a dilemma for cancer therapy, since the very properties that favor their high accumulation in a lesion also results in the low diffusion of these macromolecules within a tumor. Indeed, most human solid tumors possess abundant stroma that forms a barrier preventing macromolecular drugs from directly attacking cancer cells.

In the 19th century, the French physician Armand Trousseau described thrombophlebitis in patients with stomach cancer for the first time. Today, a large body of clinical evidence supports the conclusion that abnormal coagulation occurs in a variety of cancer patients. It is now known that tissue factor (TF), a trigger protein of extrinsic blood coagulation, is highly expressed on the surface of almost all human tumor cells. Therefore, TF may be involved in tumor-related abnormal blood coagulation. Above all, any malig-

nant tumor can erode the surrounding normal tissue, and the more erosive types of cancer have more destructive actions. If these cancer clusters erode adjacent normal or tumor vessels, microscopic hemorrhage can occur at any place and at any time within or adjacent to the cancer tissues, and fibrin clots immediately form in situ to stop the bleeding. These fibrin clots are subsequently replaced by collagenous stroma in a process similar to that which occurs during normal wound healing and in other non-malignant diseases. Fibrin clot formation in non-malignant disorders, such as cardiac infarction, brain infarction, injuries, and active rheumatoid arthritis, should form only during the onset or active stage of disease and should subsequently disappear within a few weeks because of plasmin digestion or replacement with collagen, a process that is accompanied by some symptoms. On the other hand, fibrin clot formation in cancer lasts for as long as the cancer cells survive in the body and occurs silently.

MONOCLONAL ANTIBODIES TARGETING CANCER STROMA

We successfully developed a mAb that reacts only with human insoluble fibrin, and not with human fibrinogen or fibrin degradation product (FDP). Another advantage of this mAb is that it cross-reacts with mouse insoluble fibrin, but not with mouse fibrinogen or FDP. The production of a mAb that can distinguish a fibrin clot from fibrinogen, soluble fibrin, and FDP is a major breakthrough, since these proteins have common amino acid sequences. As a result of the unique properties of the mAb, it is not neutralized by soluble fibrinogen or soluble fibrin products in the body.

Determining the epitope was difficult because the insolubility of fibrin clots prevented an X-ray or NMR analysis. However, we finally succeeded in determining the epitope. Our anti-fibrin clot mAb recognizes an unexplored hole that is only uncovered when a fibrin clot forms. The epitope in the hole is a hydrophobic region on the Bβ-chain that interacts closely with a counterpart region on the γ-chain in a soluble state.

ANTIBODY DRUG CONJUGATE (ADC) FOR CANCER STROMAL TARGETING (CAST) THERAPY

Antibody and ADCs: Anti-insoluble fibrin IgG (Fbn-ADC) and a control (Control-ADC) were prepared in our laboratory.

In vivo experiments: Bulb/c nude mice were subcutaneously inoculated with 5×105 5-11 cells into their backs and used in the experiments when the tumor volume reached approximately 200 mm3. Mice received PBS, 0.3 mg/kg MMAE, and 20 mg/kg ADCs (0.3 mg/kg, MMAE equivalent) twice a week, for 12 times in total.

Eight-week-old spontaneous pancreatic tumor-bearing KPC mice were also treated with PBS and ADCs according to the same regimen as that used for the subcutaneous model.

RESULTS AND DISCUSSION

In the subcutaneous tumor model, Fbn-ADC significantly inhibited tumor growth compared with PBS, MMAE and Control-ADC ($P < 0.01$). In the KPC mice, Fbn-ADC significantly improved survival, compared with PBS and Control-ADC ($P < 0.05$).

The ADC selectively extravasates from leaky tumor vessels and forms a scaffold as it is captured by the tumor stromal network, resulting in a high antitumor effect by damaging both tumor cells and tumor vessels.

The present discovery, which arose from a combination of knowledge gained in the fields of tumor stromal biology and physiology with knowledge of organic chemistry, may open a new field of drug design with the potential to produce many useful treatment modalities, especially for stromal rich and refractory cancers such as pancreatic cancer, stomach cancer, and glioma.

NEAR INFRARED (NIR)-NANOPROBES FOR TRACKING OF HUMAN MESENCHYMAL STROMAL CELLS AFTER IMPLANTATION IN BRAIN AND SKIN

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Human mesenchymal stromal cells (hMSC) have therapeutic potential for cell-based therapies. The ability to monitor the fate of transplanted cells by non-invasive imaging enables the development of improved cell-based therapies, as it allows cell tracking in host tissue microenvironments and informs on engraftment efficiency and biodistribution1. In this study we aimed to develop liposome-based NIR nanoprobes to label hMSC without influencing the normal cell function and eliminating the need for injection of contrast agents or cell genetic modification. By formulating ICG into liposomes, as previously described2, we aimed to achieve long lasting labelling of hMSC for *in vivo* tracking with a non-invasive imaging optical technique. Furthermore, the stability of ICG in cell culture media was considerably improved when formulated into liposomes, in comparison to the dye itself. Using liposome-labelling, we showed that hMSC spheroids engrafted into subcutaneous or brain tissues were retained for at least 2 weeks, even better in some of the transplanted animals for up to 3 weeks. Labelling of hMSC before transplantation could enable tracking of cells after administration and would thus be a powerful tool for the assessment of cell-based therapies, design of clinical trials and monitoring in clinical practice.

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DEVELOPING NANOPARTICLES-IN-MICROSPHERE POLYMER (NIMP) PARTICLES USING SCALABLE HIGH SHEAR FLUID PROCESSING TECHNOLOGY

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INTRODUCTION

Nano- and micro-sized particles are promising drug delivery platforms. With their excellent abilities to protect degradations from the bodily systems, polymer particles made with biocompatible and biodegradable polymers are ideal for targeting and delivering therapeutic agents to the gastrointestinal tract. Among those particles, nanoparticles-in-microsphere polymer (NiMP) particles have great potential for co- encapsulating and delivering hydrophilic and hydrophobic therapeutic agents. This study aims to develop an efficient and scalable process for producing NiMP particles with a unique high pressure, high shear fluid processing technology.

METHODS

The NiMP particles were produced using a double emulsion-evaporation method. The primary emulsion was formulated by dissolving poly (lactic-co-glycolic acid) (PLGA) in dichloromethane (DCM) (20 mg/ml) and mixed with the first aqueous phase to form a 2% W1/O emulsion. This emulsion was subsequently added into a second

aqueous phase to form W1/O/W2 double emulsion with a total of 5% oil phase. In most tests, both emulsions were prepared by passing through a Microfluidizer® high pressure processor. The process pressure and number of passes were varied to achieve optimum results. To study the effect of preparation method, a rotor-stator mixer was also used to prepare the second emulsion. The residual DCM in the final solutions was then evaporated at room temperature to allow hardening of the NiMP particles. The particle characterizations were performed with laser diffraction instrument and scanning electron microscope (SEM).

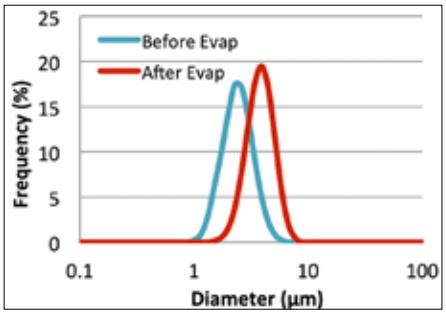


Figure 1. NiMP particle size distributions.

Figure 2. SEM image of NiMP particles created using Microfluidizer for both emulsions.

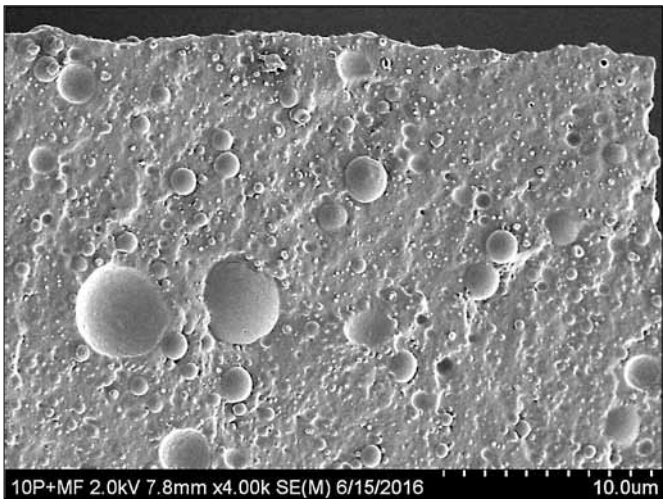
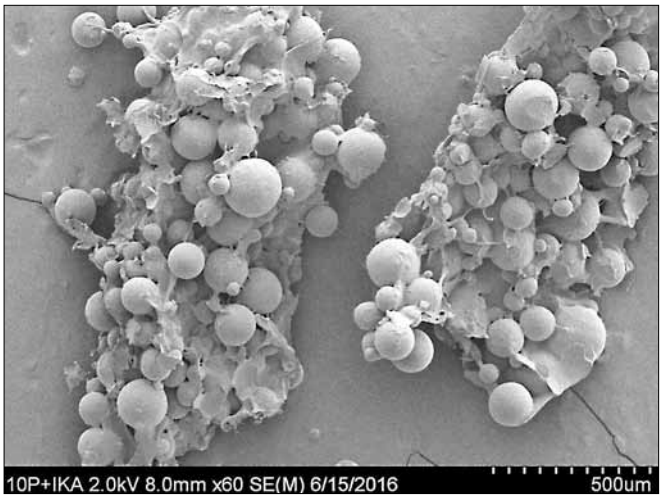


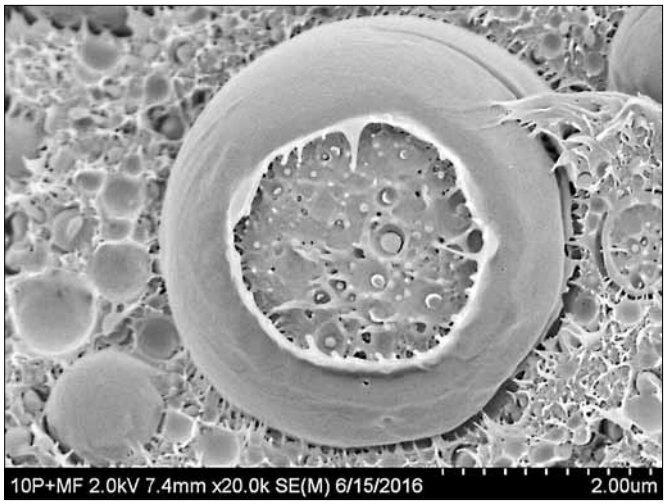
Figure 3. SEM image of NiMP particles created using rotor-stator mixer for the 2nd emulsion.



RESULTS

NiMP particles with size up to a few microns were successfully assembled through above method. The size distributions of Microfluidized NiMP particles before and after solvent evaporation are shown in Figure 1. Figure 1 indicates that the particle became slight larger after evaporating the solvent, which may be caused by fusion of small particles with the large ones. The SEM images shown in Figures 2 and 3 represent NiMP particles prepared using Microfluidizer for both emulsions and using rotor-stator mixer for the second emulsion, respectively. Both methods were able to produce spherical particles with smooth surfaces. However, Microfluidization was able to create much smaller and more uniform particles due to its higher mixing rate. On the other hand, rotor-stator generated a wide range of particles of up to several hundred microns. Figure 4 shows the internal structure of NiMP particles presented in Figure 2 and confirms the encapsulation of the first aqueous phase.

Figure 4. SEM image shows internal structure of NiMP particles from Figure 2.



CONCLUSIONS

The NiMP PLGA particles were successfully fabricated through the double-emulsion evaporation method using a high pressure Microfluidizer® processor. Compare to traditional rotor-stator mixer, Microfluidization was able to produce much smaller and more uniform NiMP particles. SEM images also confirmed the encapsulation of the first aqueous phase inside the micro spheres. The type and concentration of surfactants used to form both emulsions, polymer concentration, and process conditions were found to affect the size and size distribution of the final NiMP particles, which require further formulation and process optimizations. Encapsulation efficiency and release profiles are currently under investigatio

NANOMEDICINE DEVELOPMENT: THE JOURNEY FROM PUBLICATION TO PRECLINICAL

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Nanotechnology has been at the forefront of cancer research for more than a decade now. Yet, few nanomedicine products have made it to the commercial market in this time. Nanomedicines are no exception to the idea that drugs, in general, take years to develop, push through clinical trials and bring to market. A look at the number of nanomedicine products in clinical trials shows that there are in fact hundreds of products in all stages of clinical evaluation. Several nanomedicine formulations have been approved or have successfully completed clinical trials and are nearing approval. Follow-on versions of nanomedicines are also beginning to reach the market, but need improved methods to evaluate similarity with innovator products. This talk will highlight the successes of nanomedicine and discuss the different avenues by which nanomedicines are evaluated for success, including engineered properties to decrease toxicity or improve pharmacokinetics, promoting synergy of drug combinations, and improved patient benefits and quality of life.

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THE US-NCL VIEW

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Nanomedicines are complex formulations, posing unique challenges in evaluating and quantitating drug release. Unlike traditional small molecules drugs, nanomedicine drugs exist in a complex equilibria consisting of the (1) nanomedicine-encapsulated drug, (2) unencapsulated protein-bound drug, and (3) unencapsulated, free and unbound drug. Although only the unencapsulated, free unbound drug is generally considered biologically active, all three fractions represent an important part of the pharmacokinetic understanding of the formulation. This talk will highlight new bioanalytical techniques aimed at separating and quantitating these various drug fractions. Novel approaches to measure the drug fractions in complex nanomedicine formulations are at the forefront of nanomedicine research, striving to address unmet regulatory needs as follow-on generic nanomedicine products are nearing the market.

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Funded by NCI Contract No. HHSN261200800001E.

PRELIMINARY RESULTS ON THE USEFULNESS OF INSECTICIDE-DOTADED NANOPARTICLES TO CONTROL MOSQUITOES AND FLIES

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Mosquitoes (Figs. 1, 2) belonging to the genera Aedes, Anopheles and Culex are able to transport and inject into humans the agents of severe, often deadly diseases such as Yellow fever, Dengue fever, Japanese encephalitis, West Nile fever, malaria and filariasis etc.. Flies (Fig. 3) are known (but still underestimated) as vectors in the mechanical transmission of viruses, bacteria, protozoan cysts as well as worm eggs (Figs. 4, 5). Thus control of such ectoparasites is essential in the houses of humans and in animal stables. In the case that insecticides are sprayed directly onto the fur of the animals or on the walls of stables respectively dwellings the health of humans and animals is endangered, since they come in constant contact to the insecticide by touching or inhaling.

Thus the use of insecticide-dotated nanoparticles promises protecting effects, since the insecticide starts only working, if the insects squeeze these particles with their legs and thus their nerves get in contact with the insecticide.

The present paper reports preliminary effects in tests with 50 nm sized nanoparticles containing the insecticide cypermethrin @ ZrO(mdp)@ZrO(HPO4). The tests were done with aquaeous probes containing a particle concentration of 0.6 mg/ml, 1.6 mg/ml or 2.8 mg/ml containing either 30 µmg, 70 µg or 115 µg cypermethrin or 52.7 µg, 123 µg or 201.1 µg. All concentrations were shaken before spraying onto the walls of plastic vessels. After one hour of drying large amounts of mosquitoes of the species Aedes aegypti (Fig. 1), Anopheles stephensi (Fig. 2) or Culex quinquefasciatus as well as Calliphora erythrocephala (Fig. 3) were transferred into the vessels. Then it was observed starting after one hour, whether the insects were still alive. The mosquitoes, which had been placed into vessels one hour after the vessels had been sprayed, were found dead. When the mosquitoes of all three species were placed in vessels that had been sprayed 4 weeks before, it took 2-3 hours until all specimens of all Aedes and Anopheles mosquito species were dead, while it took 6-8 hours in the case of the Culex specimens.

The adult Calliphora stages, however, survived 10 days inside the sprayed vessels, when having access just to water. Thus further experiments are needed with respect to the length of the killing effects on mosquitoes at low dosages of the insects and of the stability of the nanoparticles as well as to evaluate increased insecticide dosages in order to get also killing effects on Calliphora flies respectively other fly species. Furthermore tests with further insecticides should be done, too.

Fig. 1 Macrophoto of Aedes mosquitoes.

Fig. 2 Scanning electron micrograph of a female of Anopheles stephensi.

Fig. 3 Macrophoto of an adult Calliphora fly and a pupa.

Fig. 4 Culture vessel with one fly starting foot contacts.

Fig. 5 Culture vessel showing the growth of bacteria after one Calliphora fly had been placed thereon for 1 minute (Results obtained from Gestmann et al. 2012 and Förster et al. 2012).

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ANALYTICAL ULTRACENTRIFUGATION IN THE CHARACTERIZATION OF NANOMEDICINE PRODUCTS

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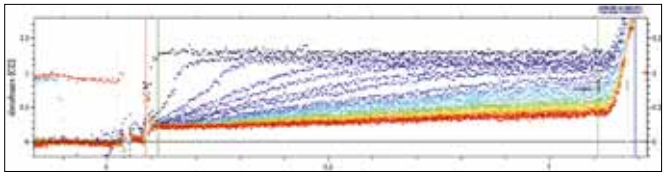
Since its discovery, analytical ultracentrifugation (AUC) has grown to be a well-established and widely used technique in protein research. Recently, with the latest software developments [1, 2] the method has become easier to use and applications in nanoparticle characterisation have also appeared [3-5].

In our work we demonstrate that besides of the typical use of determining mass or diameter of nanoparticles, AUC might be able to provide additional, precious information on nanomedicine products by simple and fast real time optical monitoring of particle sedimentation. Examples of characterising sample purity, size distribution, density, free drug content and drug release are presented, compared to results of golden standard methods and discussed also in the context of quality control and regulatory needs.

Figure 1 illustrates the typical AUC sedimentation profile curves of daunorubicin loaded vesicles. Absorption values at various time points are marked with different colours changing from dark blue (first time point) to red (last time point). The absorption signal remaining after the sedimentation of the liposomes corresponds to a species not sedimenting at this speed but absorbing at the same wavelength: free drug outside the liposomes.

Figure 1: typical AUC sedimentation profile curves of daunorubicin loaded vesicles in the presence of free daunorubicin in the particle suspension in PBS, at 1000 rpm, 490 nm, 20 °C.



The AUC analysis of the liposome suspension at 490 nm wavelength suggests the presence of about 17.1 ± 1.7 % free daunorubicin in solution.

The same measurement provides also size distribution results, revealing the bi-modal nature of this sample. Figure 2 shows the size distribution results calculated from the measurement at 514 nm.

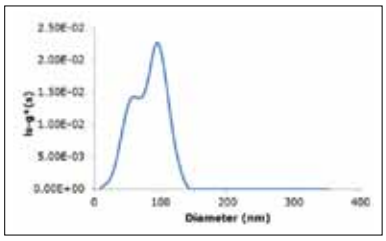


Figure 2: Size distribution of the daunorubicin loaded liposome suspension

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BIO-INVASIVE NANOCARRIERS FOR ORAL DELIVERY OF COLISTIN

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ABSTRACT SUMMARY

Nanocarriers with bio-invasive ability consisting of liposomes containing colistin and further surface functionalized with the bacterial invasion protein “Eap” from Staphylococcus aureus were developed and characterized in order to facilitate the oral delivery of colistin. Colistin-loaded liposomes (CL) were tested in different simulated media to mimic the harsh conditions of the gastrointestinal (GI) tract. The results showed that the liposomes were able to withstand the GI tract environment. In vitro cellular uptake studies showed that, functionalization of CL with Eap improves the internalization of liposomes into HEp-2 cells.

INTRODUCTION

The treatment of intracellular bacterial infections remains a considerable challenge, due to the commonly poor permeability or retention of anti-infectives in mammalian cells. This problem is compounded by the ability of many invasive infectious organisms

to take refuge in intracellular 'shelters' such as membrane-bound vacuoles, which prove difficult for anti-infective agents to access[1]. Nanocarriers such as liposomes encapsulating therapeutic agents and surface functionalized with specific moieties were an innovative approach which has been developed over the past years to overcome drug delivery difficulties. Recent work performed in our laboratory has demonstrated the potential of the surface functionalization of liposomes with invasive moiety "invasin"; an outer membrane protein and invasion factor of Yersinia spp. to facilitate a more effective intracellular anti-infective delivery than non-functionalized liposomes[2, 3]. Nevertheless, the oral administration of such nanocarriers remains an unsolved obstacle due to the aggressive environment in the GI tract including low pH in the stomach and the enzymatic cocktail in the intestine. Therefore, the aim of the current work is to develop and characterize bio-invasive nanocarriers able to facilitate the oral delivery of anti-infective drugs for intracellular infection treatment. For this purpose, liposomal nanocarriers encapsulating the poorly permeable drug colistin were formulated and surface functionalized with Extracellular Adherence Protein "Eap", a bacterial invasion protein of Staphylococcus aureus which mediates the adherence and the internalization of the bacteria into mammalian cells.

EXPERIMENTAL METHODS

Liposomes consisting of 1,2-dipalmitoyl phosphatidylcholine and 1,2-distearoyl-sn-glycero-3-phosphocholine with 1,2-dipalmitoyl-sn-glycero-2-phospho-ethanolamine and Cholesterol (1:1:0.2:1 mol%) and incorporating colistin were prepared by lipid film hydration method[2]. The stability of the liposomes in the GI tract was investigated by incubating the liposomal suspensions in simulated gastric and intestinal fluids in fasted and fed states. Liposomes were functionalized with the bacterial protein Eap either via an adsorption of the protein on the liposomal surface or via a covalent-coupling. The formulated liposomes were characterized in terms of size distribution and surface charge, as well as their colistin entrapment and Eap functionalization efficiencies.

In vitro uptake studies were conducted using epithelial cells of HEp-2 cell line to investigate the effect of Eap on the intracellular delivery. Cells were incubated with rhodamine-labeled Eap-functionalized or non-functionalized liposomes for 2 h at 37 °C and 5% CO_2. Afterwards; HEp-2 cells were washed and stained using fluorescein for the cell membrane and DAPI for the nucleus. Flow cytometry was used to determine the uptake efficiency and confocal laser scanning microscopy (CLSM) was used to visualize the internalization of liposomes into HEp-2 cells. Cell viability assays were performed to assess the cytotoxicity of liposomes on HEp-2 cells.

RESULTS AND DISCUSSION

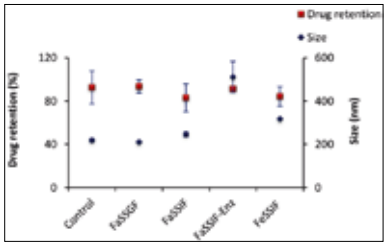
The developed formulations exhibited a mean diameter of approximately 200 nm, a narrow polydispersity index and they were negatively charged (Table 1). The colistin entrapment and Eap functionalization efficiencies were approximately 35% and 60% respectively.

Table 1. Physicochemical characteristics of Eap-functionalized and non-functionalized colistin-containing liposomes, PDI = polydispersity index, ZP = zeta potential, EE = entrapment efficiency, FE = functionalization efficiency.

Sample	Size (nm)	PDI	ZP (mV)
Unloaded liposomes	198.3±4.5	0.07±0.01	-28.2±1.2
CL	214.1±4.4	0.06±0.03	-23.7±0.7
Eap-CL	215.4±3.6	0.09±0.01	-20.0±0.9

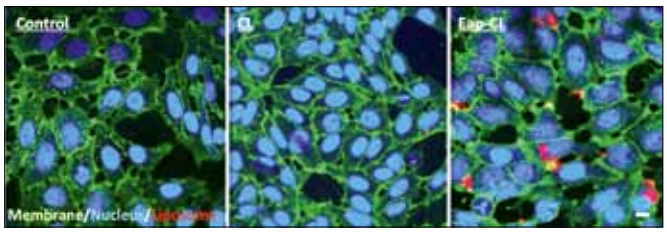
Colistin-loaded liposomes showed a good stability in simulated gastric and intestinal fluids in fasted and fed states, in terms of colloidal parameters as well as drug retention. Whereas in fasted states simulated intestinal fluid containing pancreatin, the size of liposomes increased and the drug retention was around 80% (Figure 1).

Figure 1. Stability of colistin-loaded liposomes in simulated gastric and intestinal media in fasted and fed states after 5 h incubation at 37°C.



The cellular uptake studies showed a significant increase of the uptake efficiency when cells were treated with Eap-functionalized liposomes containing colistin compared to non- functionalized formulation after 2 h incubation. CLSM imaging results strongly support the findings of flow cytometry studies; a red fluorescence was detected on CLSM images after treating HEp-2 cells with Eap-functionalized liposomes, containing colistin for 2 h (Figure 2).

Figure 2. Representative confocal microscopy images of HEp-2 cells treated with colistin-loaded liposomes (CL) and Eap-functionalized liposomes containing colistin (Eap-CL). Scale bar: 10 µm.



CONCLUSION

A bio-invasive formulation consisting of colistin-loaded liposomes and functionalized with Eap was developed and characterized. Stability studies in simulated GI fluids showed suitability for an oral application. Functionalization of liposomes with Eap increased the uptake of the nanocarriers into epithelial cells; indicating the promise of such formulation as a strategy for intracellular delivery of anti-infectives.

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MICROFLUIDIC ASSEMBLY OF NUCLEIC ACID LOADED NANOPARTICLES

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Despite several new discoveries in gene editing and post-transcriptional gene silencing in the 2000s, such as CRISPR and RNAi, therapeutic nucleic acids have only sparsely made it into clinical trials. The major hurdle is their efficient delivery to target tissues and cells. And nanoparticles are amongst the most promising delivery strategies for therapeutic nucleic acids. However, reproducibility, batch-to-batch variability, polydispersity, scalability and shelf-life are just a few of the limitations encountered with nanomedicines in general. One approach to addressing these limitations is the use of microfluidics for the assembly of nanoparticles. Here, we describe the preparation of triblock copolymer based micelles capable of complexing with nucleic acids for gene delivery. Micelleplex preparation, scalability, and their physico-chemical characteristics as well as *in vitro* and *in vivo* fate were studied following microfluidic preparation of siRNA nanoparticles compared to the routinely used batch reactor mixing technique. Microfluidic nanoparticles were prepared in large batches and showed a reduction of overall particle size as well as a more uniform size distribution when compared to batch reactor pipette mixing. Stability studies at room temperature, +4°C and -20°C revealed excellent stability and shelf-

lives of > 6 months at +4°C. Confocal microscopy, flow cytometry and qRT-PCR displayed the subcellular delivery of the microfluidic formulation and confirmed the ability to achieve mRNA knockdown. Intratracheal instillation of microfluidic formulation resulted in a significantly more efficient ($p<0.05$) knockdown of GAPDH compared to treatment with the batch reactor formulation which was attributed to the smaller size of the microfluidic formulation.

EVALUATION OF IRON SPECIES IN HEALTHY SUBJECTS TREATED WITH GENERIC AND REFERENCE SODIUM FERRIC GLUCONATE

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Patients with chronic kidney disease induced anemia are treated with intravenous iron (IV) products in the clinic. In the US, one of these IV iron products – ferric iron gluconate – is available as both an innovator (Ferrlecit) and a generic product. Ferric iron gluconate is a complex, colloidal nanoparticle composed of an iron-hydroxide core and a carbohydrate shell. There is concern, based upon reports by the EMA of toxicity from generic iron colloid products, that the innovator and generic formulations release iron differently. The biological target for the released iron is the protein transferrin, which delivers iron to cytoplasmic proteins for use or storage. Iron overload leads to saturated transferrin, and the remaining iron, termed labile iron, is transported into the cell where it can participate in chemistry with oxygen species leading to DNA, RNA and protein damage (Fig 1). We are planning a two-way crossover pharmacokinetic study administering each drug in healthy volunteers to determine if there are differences in iron release between the innovator and generic drug products. To quantify the concentration of various iron species in the blood plasma of our clinical samples, we have developed a robust and highly sensitive bioanalytical approach. The approach involves coupling size exclusion chromatography to inductively coupled plasma mass spectrometry (ICP-MS). This strategy allows us to measure all of the iron species in the plasma - total iron (TI), transferrin bond iron (TBI), drug bound iron (DBI) and labile iron (LI) simultaneously (Fig. 2). In addition, methods to identify potential biomarkers (secondary assays) including oxidative stress, inflammation and lipid peroxidation via quantitative mass spectroscopic approaches have also been developed. The innovative bioanalytical methods of LI and potential biomarkers will be used to compare the oxidative stress and toxicity caused by innovator and generic iron colloid products. The novel bioanalytical method of measuring DBI of iron colloid products in the human plasma is developed and reported for the first time. The pharmacokinetics of these iron species will provide a clear picture comparing the safety and efficacy profiles between innovator and generic iron colloid drug products. Our current bioanalytical approach for quantifying various iron species in the plasma as well as physiochemical characterization for the two drug products will be presented in support of our ongoing clinical trial.

Fig. 1. Left: Normal iron uptake pathway. Fe binds to the protein transferrin and is then imported into the cell to be utilized by proteins as a co-factor. Right. Iron overload conditions leads to the presence of labile iron, which is adventitiously, imported into the cell where is causes DNA, RNA and protein damage.

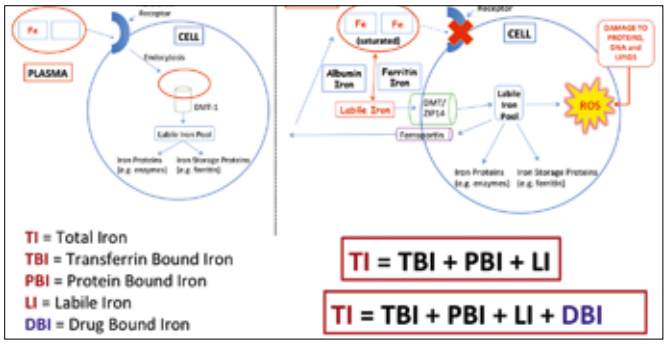
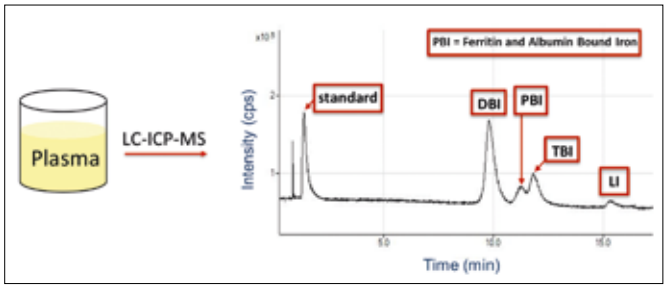


Fig.2 Illustration of the LC-ICP-MS method developed to measure the iron distribution in blood plasma. DBI = drug bound iron, PBI = ferritin and albumin bound iron, TBI = transferrin bound iron and LI = labile iron.



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OVERCOMING ADVERSE INJECTION REACTIONS TO NANOMEDICINES

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Intravenous injection/infusion of nanoparticles/nanomedicines (including stealth nanoparticles) into some human subjects induces acute cardiopulmonary adverse reactions (Nanomedicine, 2013, 9:458–460). Complement activation has been suggested to play a role, however, the majority of these nanoparticles/nanomedicine are poor/moderate activators of the complement system compared with zymosan (a gold standard) and their administration is unlikely to deplete complement and generate sufficient quantities of anaphylatoxins. Furthermore, these nanoparticles still incite complement in many of non-responders. Adverse reactions to nanomedicines, however, disappear by slowing down the infusion rate. Acute injection/infusion reactions in humans are reproducible in pigs. Unlike humans, pulmonary intravascular macrophages (PIM) are abundant in pig lungs, which rapidly intercept intravenously injected particulate matters (Nucleic Acid Ther., 2016, 26:67–72). It has long been known that robust phagocytosis of particles by PIM results in immediate release of large quantities of mediators that correlate with periods of peak cardiopulmonary disturbances. Not

so surprisingly, on PIM destruction, nanoparticle (including stealth entities)-induced cardiopulmonary disturbances disappear. Over 20 years ago, I demonstrated robust clearance of the so-called "stealth nanospheres" by murine macrophages *in vivo* under different pathophysiological conditions and independent of opsonization processes (J. Leukocyte Biol., 1993, 54:513–517; J. Natl. Cancer Inst., 1996, 88:766–768; Clin. Sci., 1997, 93:371–379); an era when nanospheres where mostly referred to as "microspheres". It is therefore likely that PIMs might express receptors capable of recognizing polymer coatings (or blood proteins intercalated in polymers) of stealth nanoparticles. These collective findings not only raises questions on relevance of the pig model to human cases, but also sheds doubt on validity of the complement activation-related pseudoallergy (CARPA) hypothesis as a possible regulator of the cardiopulmonary distress. However, it is highly plausible that there could be a transitional link from robust clearance of nanoparticles by strategically placed macrophages (as in PIMs) in systemic circulation to adverse haemodynamic reactions. These macrophages (whose phenotype yet to be mapped) may be abundant in sensitive individuals either in their lungs or elsewhere (e.g., liver, spleen). In this presentation, I will provide evidence supporting the link between robust phagocytosis and adverse cardiopulmonary distress to various nanoparticles and introduce simple nanoengineering initiatives, and without immunological and pharmacological manipulations, that overcome adverse injection reactions in the porcine model, including from administration of complement-activating nanoparticles (Nature Nanotechnol., 2017, in press). Finally, I suggest that while some sub-populations of macrophages act as major players in modulating nanoparticle-mediated injection reactions, the exact role of complement needs to be explored further (e.g., a synergistic effect of C5a on some macrophage receptor signalling processes). Nevertheless, we should be cautious in extrapolating pig responses to humans; simply establishing nanomedicine safety in pigs may not necessarily translate to human safety, as the responsive human macrophages may still respond and perform differently in their own microenvrionment.

RECENT DEVELOPMENTS WITH NON-BIOLOGICAL COMPLEX DRUG (NBCD) PRODUCTS: COMPLEX DRUGS IN A COMPLEX ENVIRONMENT

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NBCD products represent a growing number of synthetic innovator-driven drug products introduced successfully over the last decades and their copy versions. NBCD products typically also use nano-technology to exert their clinical effect. Examples include drug carrying liposomes and emulsions, iron-carbohydrate complexes and glatiramoids. NBCD products are indicated for a broad spectrum of acute and chronic diseases. Their complexity is high and their characteristics are defined by a complicated manufacturing process which has to be well-controlled and robust to reproduce the drug product. These NBCD (nano-)structures cannot be fully characterized by physicochemical analyses [1].
In contrast to a well-defined small, low molecular weight drug, the comparability exercise of a NBCD follow-on version with the reference innovator drug product is difficult and the therapeutic equivalence assessment of the two drug products is challenging. A totality of evidence approach and a documented absence of clinically meaningful differences (critical attributes) will define the place in therapy as a therapeutic alternative or even interchangeable/substitutable drug product in patients. In contrast to the regulatory equivalence evaluation which is well established for classical generics and biosimilars, the approval and post-approval standards for NBCD products are still debated and globally not aligned (fig.1) (http://www.nyas.org/Events/Detail.aspx?cid=07082904-d593-4f0e-ada7-446ec8e7444b)

Fig. 1 The landscape of drug follow-ons (adapted from e-briefing NYAS):

Intravenous iron sucrose (IS) formulations present a good example of a NBCD product family where different regulatory strategies for follow on versions were and still are being followed in different parts of the world. In the early 2000 iron sucrose similars (ISS) have been authorized based on the generic paradigm in a number of EU countries (decentralized procedure). This still occurs in Asia. IS is considered as a simple small molecule and the complex, nano-colloidal character of the IS dispersion is not recognized. In the post-approval period non-clinical and clinical comparison studies with ISS and the ISinnovator were published showing significant differences between the drug products [2]. In 2011 EMA published a reflection paper (RP) on iron-based nano-colloidal products developed with reference to an innovator drug which was updated last time in 2015. This RP indicating that quality characterization on its own would not provide sufficient assurance of the similarity between the two products. Quality, non-clinical (biodistribution) and human PK studies are needed for the evaluation. In case of minor differences in these tests a therapeutic equivalence study might be necessary to address the impact on efficacy and safety (http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2015/03/WC500184922.pdf). The RP on parenteral iron nano-colloidals has to be read in connection with other EMA documents e.g. on liposomes and with ICH guidelines for biotechnological drug products.
FDA also has issued industry guidance documents on intravenous iron colloidal dispersions to address the complexity of these products and the challenges when using the equivalence paradigm of generic complex drug products. Differences in physicochemical properties may result in differences in stability or distribution pattern *in vivo* like in iron-carbohydrate complex drugs [3].
In general, when speaking about NBCD nanomedicines, scientific gaps have to be filled to understand the observed and reported differences between the performance of innovator and follow-versions. This science base should help all stakeholders: regulatory scientists, industry scientists, academics, medical professionals to develop globally aligned science base standards for approval of NBCD follow-on products to ensure NBCD product efficacy and safety for the patient.

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APPLICATION OF SWNT FOR BIOMARKER DISCOVERY AND THERAPEUTIC EVALUATION

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Key Words: SWCNT, Angiogenesis, Biosensors, ROS, RNS.

Reactive species, specifically nitric oxide (NO) and hydrogen peroxide (H_2O_2), activate signal transduction pathways during angiogenesis and other biological systems and therefore play important roles in physiological development as well as various pathophysiologies. Herein, we utilize a near-infrared fluorescent single-walled carbon nanotube (SWNT) sensor array to measure the singlemolecule efflux of NO and H_2O_2 from human umbilical vein endothelial cells (HUVEC) in response to angiogenic stimulation. Two angiogenic agents were investigated: the pro-angiogenic cytokine, vascular endothelial growth factor A (VEGF-A) and the recently identified inorganic pro-angiogenic factor, europium (III) hydroxide in nanorod form. The nanosensor array consists of a SWNT embedded within a collagen matrix that exhibits high selectivity and sensitivity to single molecules of specific reactive species. We find that the production of H_2O_2 following VEGF stimulation is elevated outside of HUVEC, but not for stimulation via nanorods, while increased generation is observed in the cytoplasm for both cases, suggesting two distinct signaling pathways. In addition, we are able to detect the spatial resolution of NO in HUVEC cells in response to VEGF. Moreover, by employing transmission electron microscopy, confocal fluorescent microscopy, and UV-vis spectroscopic analysis, we have confirmed the internalization of DNA-SWCNT in HUVECs. Additionally, by using pharmacological inhibitors as well as genetic approaches, we have found that SWCNT is endocytosed through Rac1- GTPase mediated macropinocytosis in normal endothelial cells. Our work reveals a unique mode of entry of SWCNT in cells and might help to properly formulate SWCNT as nanovectors in biological systems.

NANOSCIENCE AND NANOTECHNOLOGY FOR HUMAN HEALTH: MECHANO-SENSITIVE LIPOSOMES FOR TARGETED DRUG DELIVERY

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In 2017, the publisher WILEY has issued a book that covers the materials aspects of nanomedicine [ISBN 978-3-527-33860-3]. The related, medically driven, interdisciplinary research activities embrace the most relevant diseases such as cancer, caries, and neuro-degeneration. The leading cause of death in Europe, North America, and Asia relates to cardiovascular diseases [Circulation 119, e21 (2009)]. Therefore, not only cardiologists, internists, immunologists, and other medical experts but also natural scientists and engineers including materials scientists targets current research toward non-systemic treatments of stenosed vessels. Currently, vasodilators such as nitroglycerin are administered to widen the constricted atherosclerotic arteries in a systemic fashion. The vasodilator drug widens all vessels with serious side effects including a drastic blood pressure drop. Therefore, the dose has to be kept limited. Specific biomarkers, which may help to target the stenosed parts of the vessels, do not exist for this prevalent inflammation. Therefore, we have proposed to exploit the wall shear stress, which is significantly increased at constricted arteries with respect to the healthy parts, as purely physical trigger to release drugs from mechano-sensitive containers of nanometer size [Nature Nanotechnology 7, 536 (2012)]. This nanotechnology-based innovation is sweeping the established treatments, especially the ones applied before the patient reaches the operating room and endovascular devices implantation for intra-arterial clot lysis, stent placement or arterial balloon dilatation can be performed [Cardiovascular Research 99, 328 (2013)]. Before this innovation could become supportive in patient treatment, detailed studies including *in vitro* and *in vivo* experiments on the immuno-response of the artificial liposomes have to prove the suitability. First experiments have demonstrated a surprising lack of complement activation [Nanomedicine: NBM 12, 845 (2016)]. Consequently, the nanometer-sized mechano-sensitive liposomes, to be used as containers for targeted drug delivery, are promising for the efficient treatment of cardiovascular diseases.

DRUG DELIVERY IN THE 21ST CENTURY

SESHA NEERVANNAN, Senior Vice President, Pharmaceutical Development, R&D, Allergan Plc

Drug Bioavailability at therapeutic concentrations within the target tissues is a 'must-have' for efficacy. However, the journey of drug to target is filled with challenges and often side-effects from unwanted exposure to other tissues/organs overwhelm the intended consequence of therapy. Many pharmacologically relevant molecules don't become "drugs" for this very reason.
Drug Delivery technologies have played a pivotal role in enabling drug to reach the target and has gone through various evolution over the decades, from "macro" to "micro" to "nano" systems. Key advances in material science is pivotal in targeted delivery of drugs to specific tissues. In addition, the therapeutic advances, especially with Biologics therapeutics bringing in enormous specificity and selectivity to treat critical diseases, has provided both opportunities and challenges. Delivering Biologic drugs opens a whole new need for technologies and innovation that should energize the entire scientific community. Success depends on close collaboration between scientists in the fields of materials, pharmaceutical sciences, engineering, biology and clinical medicine to form a deeper appreciation for the highly complicated interfaces between drug, human body, and the disease.

APPROACHES TO DEVELOP SCIENTISTS AND PROFESSIONALS FOR A COMPLEX PHARMACEUTICAL R&D ENVIRONMENT

SESHA NEERVANNAN, Senior Vice President, Pharmaceutical Development, R&D, Allergan Plc

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TITLE: 21ST CENTURY APPROACHES TO NANO EHS AND SAFER NANOMATERIALS

ANDRÉ NEL, M.B. Ch.B., Ph.D., Distinguished Professor of Medicine, Associate Director California NanoSystems Institute, Chief of Nanomedicine and Director of the Center for Environmental Implications of Nanotechnology, UCLA, Los Angeles, CA (USA)

In 2007 the National Academy of Sciences issued "Toxicity Testing in the 21st Century: A Vision and a Strategy". That report called for a paradigm shift from conventional descriptive approaches for hazard assessment in animals to one based on an understanding of cellular response pathways that, when triggered by chemical substances, could initiate key biological events that lead to adverse outcomes at the individual or population level. This new paradigm, relying on non-vertebrate, alternative testing strategies (ATS), utilizes mechanism-based *in vitro* assays and in silico predictive tools for expedited screening of the hazard potential of chemical substances and engineered nanomaterials (ENMs) at significantly less cost. With funding from NSF, EPA and the NIH, we have developed tools for the use of ATS by the Nano EHS and nanotherapeutics communities for the assessment of nanomaterials along the same line as being discussed for chemical regulations by OECD. The talk will demonstrate, by way of a few key examples (such as carbon nanotubes, graphene and mesoporous silica nanoparticles), the utility of ATS for mechanism-based high throughput and high content screening, which relies on adverse outcome pathways (AOPs) for ranking and profiling of pristine and commercial ENMs. I will discuss how the data can be used for dose-and exposure-relevant tiered testing development, as well as data acquisition and submission for regulatory consideration. The discussion will address how the connection between a molecular initiating event, tied to ENMs physicochemical properties, and key intermediary responses can be linked to apical health outcomes. This allows predictive hazard profiling of a large categories and numbers of nanomaterials to provide a reference grid against which new materials can be introduced, ranked, categorized, and safer designed. Finally, the talk will also address pitfalls that need to be overcome for the acceptance of ATS as an integral risk assessment tool in the new Toxic Substances Control Act in the U.S.

TITLE: WHAT IS THE AMOUNT OF DRUG BIODISTRIBUTION AND RELEASE BY NANOCARRIERS AT THE TUMOR SITE – OPTIMISM VS DESPAIR?

ANDRÉ NEL, M.B. Ch.B., Ph.D., Distinguished Professor of Medicine, Associate Director California NanoSystems Institute, Chief of Nanomedicine and Director of the Center for Environmental Implications of Nanotechnology, UCLA, Los Angeles, CA (USA)

It has recently been proposed by Chan and co-workers that only 0.7% (mean) of the administered nanoparticle dose in DDS is delivered to solid tumors. Since a delivery problem could signify negative consequences on the translation of nanotechnology for human use from the perspective of therapeutic efficacy, cost, toxicity and use of nanocarriers for the treatment of cancer, a debate has ensued regarding the interpretation of this position. My brief introduction will provide a quick overview of the divergent opinions regarding this controversial matter, including the role of dosimetry in assessment of therapeutic outcome.new Toxic Substances Control Act in the U.S.

NANOPARTICLE BEHAVIOR IN STEM CELLS – IMPLICATIONS FOR REGENERATIVE MEDICINE

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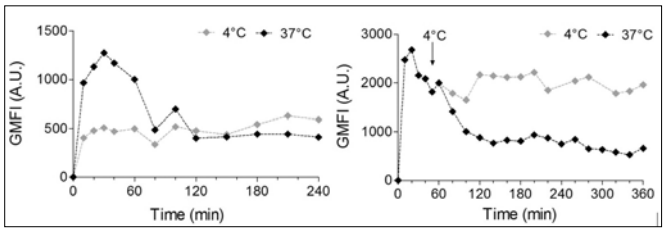
The use of nanotechnology in biomedical applications is a field of high potential, and the interaction of nanostructures with the human body is a crucial aspect in it. Therefore, knowledge of the modulation of basic cellular and molecular functions induced by nanoparticles (NPs) is essential. The mechanism by which NPs enter and are transported within the cell predominantly determines their intracellular fate and, consequently, their biological impact. In the present study we focused on the developing immune system by investigating uptake of engineered NPs by ex vivo derived hematopoietic progenitor cells (HPC). CD34-positive HPC were derived from human cord blood and were exposed to well-defined, 40-nm size fluorescent carboxylated polystyrene NPs. Nanoparticle uptake kinetics was assessed by measuring fluorescence intensity at short time intervals using flow cytometry. The HPC were found to promptly accumulate NPs within the first hour after exposure after which the uptake declined. We demonstrated that this transient interaction requires an energy-dependent cellular process (Figure), suggesting active loading and release of NPs by HPCs.

Figure: Fluorescent polystyrene nanoparticles (NPs, 40 nm) loading and release are energy-dependent. Human CD34+ hematopoietic progenitor cells were exposed in vitro to 50 µg/ml NPs at 4°C or 37°C (left), or for 1 hour at 37°C followed by further incubation at 4°C to inhibit all active processes (right). Representative data of a single donor-derived cell culture are shown.



These findings are in contrast to reported observations in other cell types, such as phagocytic cells or cell lines, and suggests that CD34+ progenitor cells handle NPs by using distinct mechanisms. This novel observation offers a unique approach to transient NP delivery to HPCs, with potential applications for *in vivo* monitoring of therapeutic stem cell transplants. Further research will be conducted to investigate the processes underlying the observed kinetics, such as induction of ATP binding cassette transporters or vesicle trafficking. Moreover, further experiments with NPs of different size, composition or surface functionalization will provide further insights on the application domains.

PRECISION DESIGN OF NANOMEDICINES TO TARGET TUMOR MICROENVIRONMENT AND OVERCOME LOW CHEMOSENSITIVITY FOR PANCREATIC CANCER TREATMENT

GUANGJUN NIE[&], Yinlong Zhang[#,&], Xiao Zhao[&], Tianjiao Ji[&], Ying Zhao[&], Yanping Ding[&]

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In spite of the promising therapeutic potential exhibited by the numerous antitumor nanomaterials, it remains a major challenge for particulate-based therapeutics to effectively transport into solid tumors, especially in stromal enriched tumors. The recent progress on understanding the critical roles and the underlying mechanisms of tumor microenvironment on tumor rapid growth and metastasis has made targeting the tumor microenvironment becomes a feasible strategy to improve the effectiveness of nanomaterials-based cancer diagnosis and therapy. Expression of stromal fibroblasts-specific enzymes and pathological changes of tumor vasculature are the most remarkable hallmarks of tumor microenvironment in almost all types of tumors and can be considered as good specific triggers or targets for design of broad-spectrum and local-environment responsive functional nanomaterial-based platform. Here we report novel biomimetic nanostructures based on oligopeptide self-assemblies that could quickly response and regulate the key components of tumor microenvironment. With such a strategy, the optimal formulations were developed, showing the high activation efficacy and antitumor efficacy. The tailor-made self-assembled biomolecule nanomaterials have the potential to be used in early and late stages of solid tumors, especially for stromal enriched solid tumors, which is expected to be of crucial importance for clinical tumor therapeutics. Although additional research is urgent needed to develop robust methods for targeting and regulating nanomaterials to tumor sites and the supporting environment, the applications of tumor microenvironment-based nanotechnology for safer and more effective antitumor nanomedicine have so far been proven to be successful and will eventually revolutionize the current landscape of cancer therapy [1-5].

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FOLATE-TARGETED NANOPARTICLES FOR RHEUMATOID ARTHRITIS THERAPY

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Rheumatoid arthritis (RA) is the most common inflammatory rheumatic disease, affecting almost 1% of the world population. Although the cause of RA remains unknown, the complex interaction between immune mediators (cytokines and effector cells) is responsible for the joint damage that begins at the synovial membrane (Figure 1).

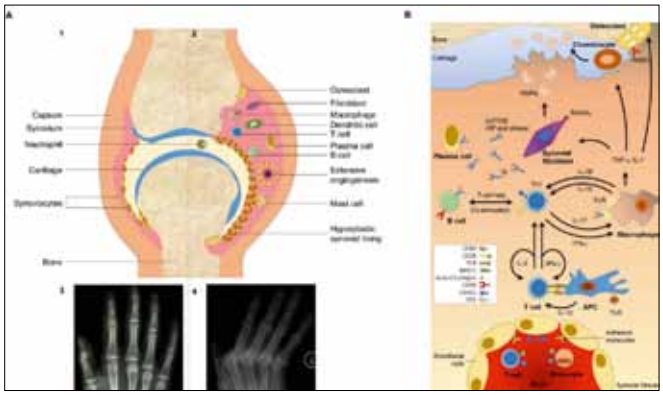
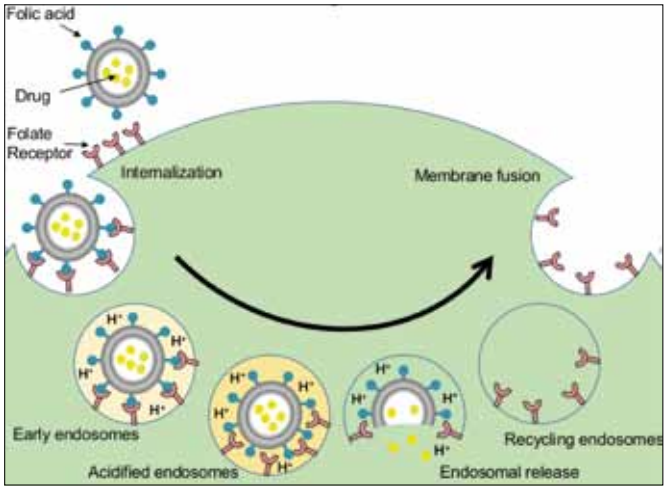


Figure 1. (A) Schematic view of (1) a normal joint and (2) its changes in RA. The "radiographic joint space" of metacarpophalangeal joints in (3) a normal hand and (4) from a patient with established RA. (B) Schematic representation of events occurring in RA.

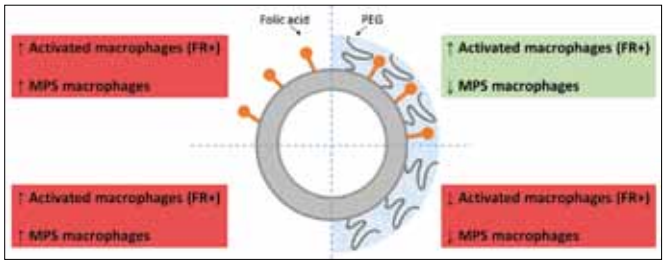
Activated macrophages are critical in the pathogenesis of RA and showed specifically express a receptor for the vitamin folic acid (FA), folate receptor β (FRβ). This particular receptor allows internalization of FA-coupled cargo (Figure 2).

Figure 2. Schematic representation of folate mediated endocytosis.



In this work we will address the potential of nanoparticles as an effective drug delivery system for therapies that will directly target activated macrophages. Special attention will be given to stealth degree of the nanoparticles as a strategy to avoid clearance by macrophages of the mononuclear phagocytic system (MPS) (Figure 3).

Figure 3. Influence of stealth degree in specificity of folate target nanoparticles to FRβ activated macrophages.



This work summarizes the application of FA-target nanoparticles as drug delivery systems for RA and proposes prospective future directions.

ORAL DOSE REDUCTION FOR ANTIRETROVIRAL DRUGS BY FORMATION OF SOLID DRUG NANOPARTICLES

ANDREW OWEN, Professor of Pharmacology, University of Liverpool

Advances in antiretroviral therapy over the past two decades have transformed HIV from a terminal to a chronic disease. However, low and middle income countries (L&MIC) still carry the major burden of disease and strategies to reduce cost of therapy while maintaining efficacy will greatly facilitate the WHO 90:90:90 ambition. Namely, to diagnose 90% of HIV-positive people, supply 90% of these with sustained antiretroviral therapy, and maintain viral suppression in 90% of these patients. Using emulsion-templated freeze drying to form solid drug nanoparticle (SDN) formulations of efavirenz (EFV) and lopinavir (LPV), we screened approximately 4500 formulations to determine candidates with the *in vitro* potential for augmented bioavailability. The ability of a lopinavir and efavirenz SDN formulation to improve pharmacokinetic exposure in preclinical species was subsequently confirmed, and the formulations entered GMP manufacture using an optimised spray drying approach. First in human pharmacokinetic data conducted at the St Stephens AIDS Trust in a small number of healthy volunteers showed encouraging evidence that a 50% reduction in dose may be able to maintain therapeutic exposure to both drugs. The approach has the potential for huge cost savings, while also freeing up manufacturing capacity that could be used to meet drug demand in L&MIC. Working with partners at the Medicine Patent Pool and the Clinton Health Access Initiative, and funded by the USAID OPTIMIZE project, we are now applying this approach to develop and translate multiple antiretroviral SDN formulations.

PHARMACOGENETICS: TOWARDS STRATIFIED NANOMEDICINE DEPLOYMENT

ANDREW OWEN, Professor of Pharmacology, University of Liverpool

The genetic basis for variability in pharmacokinetics and pharmacodynamics of small molecule therapeutics has been extensively studied for decades. There are numerous examples of how single nucleotide polymorphisms (SNPs) can influence drug exposure, which in turn influences efficacy and concentration-dependent toxicity. Such SNPs occur within drug metabolising enzymes (e.g. cytochrome P450 enzymes; CYPs), or drug transporter proteins (e.g. the organic anion transporting polypeptides; OATPs) that influence either drug absorption, distribution or clearance. Moreover, SNPs that occur within the coding sequences of other proteins may also influence response to medication in terms of efficacy or safety (I.e. when they occur within genes that mediate the primary pharmacology of the molecule or a protein involved in an off-target toxicity). The ultimate goal of pharmacogenetics is to provide clinical tools for predicting these behaviours prior to initiating therapy, to stratified the population into genetically-defined sub-groups so that the right drug can be given at the right dose to the right patients. As an increasing number of nanotechnology-enabled therapeutics reach the market, there will be growing opportunities to understand how patient germline genetic factors can influence safety and efficacy in clinical practice. The purpose of this presentation is to outline common mechanisms and modalities for small molecule pharmacogenetics, and provide a rational basis to propose potential future applications for nanomedicine posology. Current gaps in knowledge will also be discussed along with nano-specific considerations for future research in this area.

SOME PHYSICOCHEMICAL ASPECTS THAT MAY COMPLICATE ACTIVE TARGETING

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There is a running debate of active versus passive targeting. Three possible complications for active targeting will be discussed. First, the density of targeting ligands seems to play an important role[1]. Second, targeting ligands, or more general, the surface chemistry of the particles to be delivered may be enzymatically degraded[2,3]. Third, the protein corona may partly camuflage the targeting ligands. Thus *in vivo* detection of the protein corona would be needed to investigate this hypothesis.

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OME PHYSICOCHEMICAL ASPECTS THAT MAY COMPLICATE ACTIVE TARGETING

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Hyaluronic acid (HA) is a natural polysaccharide primarily present in the vitreous humor and in cartilages where it plays a key structural role in organizing the cartilage extracellular matrix. HA is used in a wide range of applications including treatment of arthritis (as a viscosupplementation agent for joints) and in a variety of cosmetic injectable products. Its safety profile is thus well established. HA has been investigated as carrier of proteins. HA administration in healthy mice through i.v. injection induces a fast liver accumulation of the polymer and its conjugates. HA can also be recognized by CD44 receptor, which are overexpressed by certain tumour cells. Consequently, systemic administration of HA and its conjugates undergo specific body accumulation owing to the polymer receptor binding. The local use of HA in arthritis treatment might be particularly interesting when this application is combined with the role of carrier of a protein useful for the treatment of the same disease.
OA is a chronic degenerative joint disease that affects >27 million patients in the United States. During disease progression, the normal, delicate balance in the biomechanical status of the joint is impaired leading to cartilage destruction and subchondral bone changes that cause pain, stiffness, and loss of movement in the joints [1]. Salmon calcitonin (sCT) is a drug that has been shown to have therapeutic effects in experimental arthritis by inhibiting both bone turnover and cartilage degradation and reducing the activities of matrix metal proteases (MMPs). An HA conjugate of sCT can overcome the issue of local joint knee injection administration

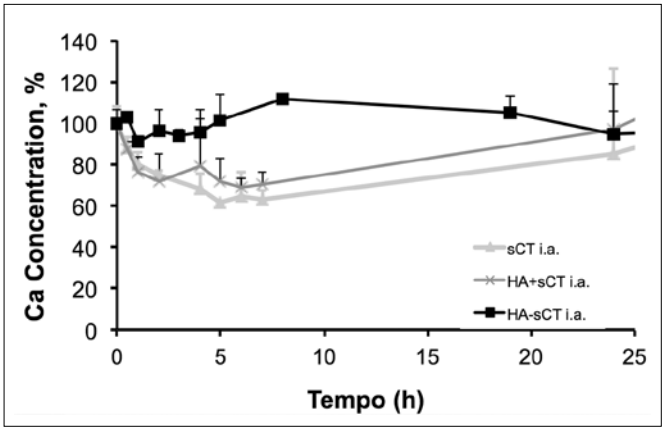
of free sCT and furthermore the rationale of this strategy would be two-fold: i) to retain sCT in the knee joint cavity avoiding the systemic elimination and the fast clearance from the joint, and ii) to combine the sCT and HA activity, HA of about 250 kDa has demonstrated the interesting anti-inflammatory activity in OA by reducing the expression of NR4A1-3, orphan nuclear receptors and MMP-1, -3 and -13 in human chondrocytes [2].
A new HA-aldehyde was synthetized to avoid the formation of heterogeneous and cross-linked products due to the direct activation of carboxylic residues of HA followed by conjugation to the amino groups of calcitonin. This HA-aldehyde was obtained by conjugation of an acetal spacer to the carboxylic groups of HA instead of classic periodate oxidation of the polymer that would lead to ring opening of some HA saccharide units, thus remarkably modifying the polymer backbone. The purified conjugate was evaluated *in vitro* in LLC-PK1 cells to determine the activity retention in comparison to the free peptide (Table 1). The HAylation of sCT did not affect the peptide activity.

Table 1. Activity of free and conjugated sCT measured as capacity to stimulate the increase of intracellular cAMP levels in LLC-PK1 cells.

Samples	% Activity
sCT	100
HA-sCT	98.02
HA	8.51

Since the first aim of the work was to demonstrate a strict local effect of the conjugate without any undesired systemic activity, it was relevant to verify that the polymer was able to keep the drug in the articular space after i.a. injection in the knee.
After the local i.a. administration, the conjugate did not show a systemic reduction of calcium concentration, suggesting that the conjugated sCT was not fast cleared from the knee joint cavity and consequently it did not reach a relevant plasma concentration for a systemic effect. Differently, free sCT or sCT/HA mixtures produced an evident calcium reduction after an i.a. administration in the knee (Fig. 1). This exciting result was further confirmed by directly evaluating the peptide concentration in plasma after the i.a. injection of free and conjugated sCT labelled with fluorescein.

Figure 1. Hypocalcaemic systemic effects of HA-sCT, physic mixture of HA and sCT (HA+sCT) and free sCT after intra-articular (i.a.) administration in Sprague-Dawley rats: HA-sCT or sCT (dosage of 25 µg/kg in sCT equiv. per animal) were intra-articular administered to the left knees (n = 3 per group). Analysis of plasma calcium concentrations showed that HA-sCT did not cause systemic hypocalcaemia that occurred with a physical mixture of HA+sCT or sCT administration.



HA-sCT efficacy was evaluated in a rabbit anterior cruciate ligament transection (ACLT) model. The histopathology studies demonstrated a better, but not statistically significant, performance of HA-sCT with respect to free sCT in the evaluation of articular cartilage morphology (table 2), subchondral bone morphology, cartilage thickness and arrangement of chondrocytes. The improvement of cartilage damages after HA-sCT treatment was significant for two parameters in comparison to the controls (HA with same molecular weight and PBS) while the sCT treatment was significant only for a single parameter and not against HA.

	Damage Score			
	PBS	HA	sCT	HA-sCT
Lateral Femoral Condyle	2.58±0.376	2.17 ± 0.516	1.33±0.683*	0.75±0.689**
Tibial Plateau	2.17±0.683	2.0±0.548	1.5±0.633	0.92±0.74**

*Table 2. Average macroscopic evaluation of the right stifle joint (lateral and medial femoral condyles) expressed as damage score. Score 3: several damage, score 2: moderate damage, score 1: slight damage, score 0: absent. Only sCT and HA-sCT showed protection against OA damage (significance: * = p < 0.05 versus PBS; **= p < 0.05 versus PBS and HA groups).*

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DOCUMENTARY AND MATERIAL STANDARDS IN NANOTECHNOLOGY

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The global increase in applications of nanotechnology in devices, drugs and consumer products designed for early disease detection and improved therapeutic outcome through precision and targeted medicines, and is paving the way for exciting new advances in science, technology and medicine. These advances are providing an opportunity to explore regulatory science research to understand various physical, chemical and biological attributes of novel technologies and collaboratively develop relevant standards with stakeholder involvement. Efforts through the International Organization of Standardization (ISO)[1], the Organization for Economic Co-operation and Development (OECD)[2], ASTM International[3], National Institute of Standards and Technology (NIST)[4], and Joint Research Center (JRC)[5], along with many other Standards development organizations, produced documentary and reference material standards in Nanotechnology.
This presentation will provide an update to the participants on the conclusions from the recent Global Summit on Regulatory Science (GSRS16)[6] on Nanotechnology Standards and Applications. Global regulatory, standards and research agencies, along with stakeholders from industry and academia, developed consensus on the much needed documentary standards and reference materials, critical to advancing the responsible development and safety evaluation of nanotechnology based products at this summit. The topics that will be highlighted include Nanotechnology-derived drug products, medical devices, liposomal drug products, food and food contact material, targeted nanomaterial and personal care products.
Disclaimer: The views expressed in this presentation do not necessarily represent those of the U.S. Food and Drug Administration

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HARNESSING RNA NANOMEDICINE FOR PRECISION THERAPY IN CANCER AND INFLAMMATION

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RNA based approaches have greatly contributed to better understanding of gene expression and function *in vitro*. The capability to apply these strategies *in vivo* in order to validate the role of specific genes in normal or pathological conditions, and to induce therapeutic gene silencing or upregulate a specific protein expression, opened new avenues for utilizing RNA as a novel therapeutic modality. However, the translation of RNA from an effective genomic tool into a novel therapeutic modality has been hindered by the difficulty to deliver RNA molecules into specific target tissues by systemic administration, especially to hematopoietic cells and highly metastatic tumors such as brain tumors and ovarian cancers. Here, I will describe some of the challenges and opportunities in modulating leukocytes response using RNA molecules and discuss adverse effects such as immuno-toxicity. In addition, I will detail the challenges of targeting lipid-based nanoparticles directly into specific cells. Special emphasize will be made on delivery strategies that target glioma cells, ovarian cells and several types of B cell hematological malignancies such as Mantle Cell Lymphoma (MCL) and multiple myeloma (MM) with novel therapeutic targets that were recently identified.
Finally, I will describe the translation of some of these strategies into clinical testing including CMC, process development and scale up.

DIFFERENTIAL UPTAKE OF NANOPARTICLES BY HUMAN M1 AND M2 POLARIZED MACROPHAGES: PROTEIN CORONA AS A CRITICAL DETERMINANT

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BACKGROUND AND AIM

Engulfment of nanoparticles by macrophages is a crucial process of the body's immune response against foreign particles including nanomedicine. Interaction between macrophages and nanoparticles is an interesting area of research for *in vivo* applications of nanotechnologies as well as nano-toxicology. Many factors such as particle shape, charge and size influence uptake by macrophages. However, macrophages exist in different polarization states (M1 and M2), depending on the disease condition. The current understanding of the interaction of particles with these subsets of macrophages is yet limited. The aim of this study was to investigate the interaction behavior of M1- and M2-type macrophages with nanoparticles of different sizes with/without the presence of serum.
Materials/Methods: THP-1 human monocytes were differentiated into M1 and M2 macrophages and the uptake of silica nanoparticle (50-1000 nm) was studied using flow cytometry and different microscopies. Silica nanoparticles were purchased from Micromod GmbH. Phagocytosis gene array was purchased commercially and performed using real-time qPCR.

RESULTS

M1 macrophages show enhanced uptake of smaller particles in the absence of serum. Conversely, human serum tremendously increases the uptake of large particles by M2 macrophages. Increased surface charge diminishes the serum-enhanced M2 uptake. Phagocytosis gene array data suggest that there is induced expression of specific phagocytic receptors in both macrophages. M2-induced phagocytic receptors are found to bind to the known serum proteins that constitute the protein corona around nanoparticles in circulation. This study demonstrates that M1 and M2 macrophages possess different nanoparticle phagocytosis behavior, determined by the nanoparticle size and the presence of serum proteins adsorbed onto their surface.

CONCLUSION

The observed differential uptake by M1 and M2 macrophages will help understand the fate of nanoparticles *in vivo*.

NTHER PROJECT: CLINICAL TRANSLATION ROADMAP FOR IRON OXIDE NANOPARTICLES

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NoCanTher project (H2020-ref#685795) aims at translating iron oxide nanoparticle (IONP) to early clinical development for pancreatic cancer. The treatment approach in this project is based on the use of functionalization of iron oxide nanoparticles together with the effect of hyperthermia generated by an external alternate magnetic field.
Under this project the technological roadmap for nanomedicine translation to patients has been identified in two main critical challenges: the effective pre-clinical screening for clinical trial dossier application and the scaling up at GMP standards. To successfully reach these, the partners' effort will concentrate on the following activities:

- Reaching Clinical trial maturity: NoCanTher consortium will present preclinical testing results to raise a clinical treatment protocol, in line with regulatory requirements for the preparation of the Investigational Medicinal Product Dossier (IMPD). This strategy will allow the project to apply for Clinical Trial Authorisation (CTA) then, will be carried out at Phase I clinical trial. On this matter, NoCanTher multidisciplinary consortium involves the participation of institutions from three different sectors (academia, industry, clinical) and from five different countries (Ireland, France, Germany, Spain and the UK).
- Nanomedicine up-scaling under GMP conditions: NoCanTher will scale up the manufacturing of the proposed nano-formulation from milligram-scale laboratory synthesis up to gram-scale production to generate sufficient material for clinical and regulatory assays. To this aim, BIOPRAXIS will develop a GMP production line, which will be optimised and the relevant quality control will be conducted at the different stages of the up-scaling process.

Assessment and implementation of the proposed strategy and roadmap will be the crucial to get a formulation ready to be tested in a Phase I clinical trial.
In this presentation, the pre-clinical evidence in support of the preclinical maturity will be presented. In parallel, Biopraxis, as industrial partner, will also follow up on the translational roadmap for up-scaling and clinical trial.

THE BIG PICTURE OF NANOMEDICINE CHARACTERIZATION, FROM PCC TO IN VIVO

A. PRINA-MELLO AND THE EU-NCL CORE EXPERT TEAM

The translation of nanomedicine from bench side to bedside is an articulated process that brings a potential nanomed-candidate from the preliminary evaluation of the sterility and contamination level to its *in vivo* toxicity, pharmacokinetics and efficacy assessment through *in vitro* immunotoxicity, heamocompatibility.
Furthermore, the manufacturing process of nanomedicines is a complex and expensive process that must be carefully assessed at the pre-clinical phase before scale-up, and clinical applications are pursued. The physico-chemical characterization can define the fingerprinting and quality of the nanomedicine but cannot be indicative of its safety and efficacy. Conversely, *in vitro* and *in vivo* can provide in depth understanding of the interaction in physiologically relevant models. The purpose of the presentation is to provide a series of case studies where to present the value of each characterization carried out within the overall translational aspect towards the product approval.

ABOUT EU-NCL:

The European Nanomedicine Characterization laboratory (EU-NCL) addresses these issues by providing the critical infrastructure and characterization services required to analyze physical and chemical attributes, *in vitro* biological properties, and *in vivo* characteristics of nanomedicines under development. The EU-NCL services are accessible to all organizations developing candidate nanomedicines, whatever the development maturity. Product developers will benefit from a detailed and confidential characterization data set that supports their decision making for further product development
Please check on line at **www.eu-ncl.eu** for the opportunity to apply for the Characterization access.

NEW RESULTS IN MULTIFUNCTIONAL LIPOSOMES FOR PREVENTION OF ALZHEIMER DISEASE

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The failure of clinical trials largely focused on mild to moderate stages of Alzheimer disease has suggested to the scientific community that the effectiveness of Amyloid-β (Aβ)-centered treatments should be evaluated starting as early as possible, well before irreversible brain damage has occurred. Accordingly, also the preclinical development of new therapies should be carried out taking into account this suggestion. In the present investigation we evaluated the efficacy of a treatment with liposomes multifunctionalized for crossing the blood-brain barrier and targeting Aβ, carried out on young APP/PS1 Tg mice, taken as a model of pre-symptomatic disease stage. Liposomes were administered once a week to Tg mice for 7 months, starting at the age of 5 months and up to the age of 12 when they display AD-like cognitive and brain biochemical/anatomical features. The treatment prevented the onset of the long-term memory impairment and slowed down the deposition of brain Aβ; at anatomical level, prevented both ventricle enlargement and entorhinal cortex thickness reduction, otherwise occurring in untreated mice. Strikingly, these effects were maintained 3 months after treatment discontinuation. An increase of Aβ levels in the liver was detected at the end of the treatment, then followed also by reduction of brain Amyloid Precursor Protein and increase of Aβ-degrading enzymes. These results suggest that the treatment promotes brain Aβ clearance by a peripheral 'sink' effect and ultimately affects Aβ turnover in the brain. Worth of note, the treatment was apparently not toxic for all the organs analyzed, in particular for brain, as suggested by the lower brain TNF-α and MDA levels, and by higher level of SOD activity in treated mice. Together, these findings promote a very early treatment with multi-functional liposomes as a well-tolerated nanomedicine-based approach, potentially suitable for a disease modifying therapy of AD, able to delay or prevent relevant features of the disease.

FLUORESCENT POLYMER NANOPARTICLES FOR LONG-TERM RGB COLOR CODING OF CELLS FOR IN VITRO AND IN VIVO IMAGING

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Fluorescent polymer nanoparticles encapsulating large quantities of dyes, so-called dye-loaded polymer nanoparticles, have emerged recently as attractive alternative to inorganic fluorescent nanoparticles, notably quantum dots.[1] These new nanomaterials, inspired from the fields of polymeric drug delivery vehicles and advanced fluorophores, can combine biodegradability and low toxicity with superior brightness. The latter can drastically improve speed, resolution and sensitivity in fluorescence bioimaging. One of the major challenges in assembling dye-loaded nanoparticles was to overcome aggregation caused quenching, which strongly limited the achievable brightness. We recently introduced a new approach to avoid dye aggregation through the encapsulation of charged fluorophores with bulky hydrophobic counterions (Fig. 1) [2]. Using nanoprecipitation we could obtain nanoparticles from biodegradable and biocompatible polymers like poly(lactic-co-glycolic acid) and poly(methyl methacrylate) with sizes of less than 20 nm that were more than ten times brighter than corresponding quantum dots.[3]

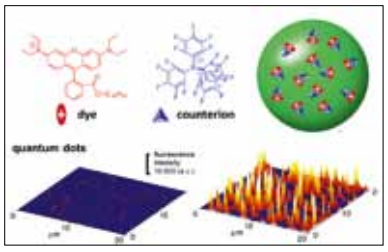


Figure 1. Top: scheme of dye-loaded polymer nanoparticles. Bottom: single molecule microscopy of quantum dots (left) and dye-loaded nanoparticles (right) of similar size.

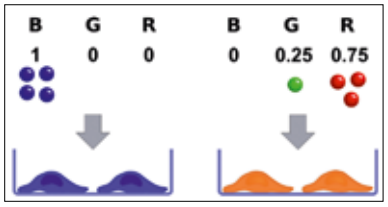
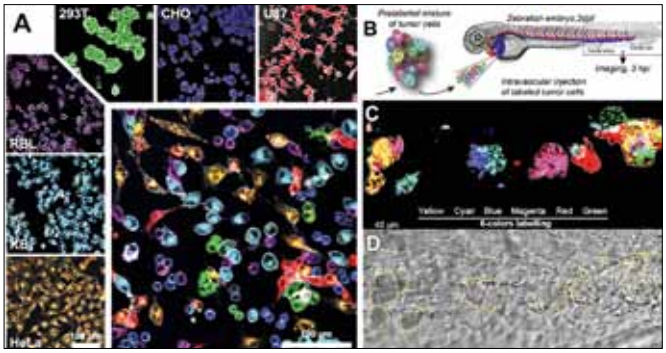


Figure 2. Red-green-blue (RGB) color coding of cells using nanoparticles of three different colors.

Based on these nanoparticles a technology for long-term fluorescence labeling of living cells with programmed color codes is developed (Fig. 2). Extending our approach of counterion controlled encapsulation of dyes to different cyanine dyes allowed creating nanoparticles with three di bands but identical size and surface propertistinct absorption and emissiones that are endocytosed equally well by living cells. Mixing nanoparticles of three colors in different proportions generates any desired color code inside the cells, which is homogeneous within the cell population and trans-

mitted through many generations of daughter cells. This technology is validated on six cell lines (Fig 3 a) and up to 13 color codes, and it enables simultaneous tracking of co-cultured color-coded cell populations for >2 weeks. Cancer cells labelled through this technique could be localized and tracked in living zebrafish upon injection (Fig. 3 b-d). Labeling of up to six different cells in early embryos using this approach allowed imaging and tracking cells and their origin during development of zebra fish embryos.

Figure 3. Nanoparticle based color coding of living cells and its application to tracking of cells injected into zebrafish embryos.



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NEW APPROACHES TO PREVENT ADVERSE COMPLEMENT ACTIVATION: IMPLICATIONS FOR NANOMEDICINE

DANIEL RICKLIN, Associate Professor of Molecular Pharmacy, Department of Pharmaceutical Sciences, University of Basel, Switzerland

In therapeutic medicine, biosurfaces (i.e., biomaterial and cell surfaces) inevitably come in contact with human blood and tissues, where they encounter host defense mechanisms of the human body such the complement system. Evolutionarily tuned to rapidly sense, tag and eliminate microbial intruders, complement may mistakenly recognize biomedical surfaces as potential threat and invoke defensive measures that contribute to inflammatory complications. Such complement-mediated adverse reactions may not only have negative consequences for the clinical outcome and quality-of-life of the patient but also for the functional performance of the biomedical entity. Complement-induced complications have long been described in the case of solid organ transplants, extracorporeal circuits or hemodialysis filters, yet the increasing use of micro- and nanoparticles as drug delivery and/or diagnostic vehicles has added a new dimension to the problem.
Therapeutic control of complement activation during a treatment with such biomedical entities is therefore considered a promising approach that may benefit transplantation- and nanomedicine alike. Fortunately, complement-targeted drug discovery has recently experienced a surge of interest from both the academic and industrial side. Although the arsenal of complement inhibitors in the clinic is still scarce and often high-priced, several new therapeutic concepts are emerging and have advanced to late-stage clinical development. This presentation provides an overview about the various strategies that are currently being explored to keep complement activation in check, and discusses how they may apply to the field of nanomedicine. It also showcases specific examples of our own research, highlighting distinct strategies to broadly block complement activation in circulation, target complement regulators to cell surfaces or create protective coatings that prevent complement activation on biomaterials or foreign cells. The complement inhibitor compstatin, for example, has been successfully evaluated in various preclinical models, including transplant-, hemodialysis- and implant-induced inflammation, and two compstatin analogs are currently in clinical development. Moreover, by taking inspiration from microbial immune evasion strategies, we developed a peptide-based surface coating that recruits the major complement regulator Factor H (FH) from circulation to biomaterial and cell surfaces. When coupled to an appropriate linker, these FH-binding peptides were shown to reduce or even prevent complement activation in model systems.
In view of the diverse modes of how complement can be triggered by nanoparticles and other foreign materials, there likely will not be a “one size fits all” approach but complement inhibitory strategies need to be carefully tuned to the specific clinical problem. In this context, the increasing availability of experimental and/or clinical complement modulators may pave the way for novel therapeutic options to manage complement-mediated adverse effects in nanomedicine and beyond.

STRANGE AND POWERFUL: HOW THE CELL REACTS TO INTRACELLULAR (ARCHAEOLIPID NANOPARTICLES-MEDIATED) PARASITICIDAL DRUG DELIVERYEDER

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The archaeosomes are nanovesicles made of archaeolipids, glycerol ether amphiphile molecules displaying stereochemistry sn2,3 having polyisoprenoid saturated chains. The peculiar chemical structure of archaeolipids is responsible for the structural properties of archaeosomes, that radically differ from the widely known nanovesicles named “liposomes” made of sn1,2 glycerol esters of fatty acids, which is the ordinary structure of phospholipids extracted from animals, plants, bacteria or fungi. Interestingly, the archaeosomes surpass the liposomes, in the following series of critical features that make them valuable for pharmaceutical industry: 1) they display different ligands for endocytic receptors (ruling out the need for covalent derivatization or inclusion) 2) are refractory to oxidative stress, resistant to hydrolytic and enzymatic attack 3) they can be dehydrated, reconstituted and sterilized by heath without losing the colloidal structure 4) their bilayer is quantitatively less permeable to protons and hydroxyls than liposomes in a variable degree depending on the source of the archaeolipids.
The archaeosomes are not immunogenic and different to ordinary liposomes, their intracellular processing leads to an increase in the levels of pERK1/2, suggesting a potential participation in wound healing processes. All those properties make the archaeosomes very well suited candidates for targeted delivery of drugs. Few studies however, have addressed the features of their intracellular pathway after endocytic uptake.
We have recently determined that ~100 nm diameter, -35 mV unilamellar archaeosomes prepared with lipids extracted from the hyperhalophile archaebacteria Halorubrum tebenquichense are captured by multiple endocytic pathways, different according to the cell type. Our results, obtained with the technique of selective inhibition of endocytic routes, indicated that the uptake mechanisms of archaeosomes displayed by J774A1 macrophages (cells with a medium level of scavenger receptor Class A expression) are clathrin and caveolin mediated endocytosis (CME and CavME respectively), plus phagocytosis. On the other hand, Caco 2 cells were used as a model of epithelial cells, and displayed CME, CavME and micropinocytosis to capture archaeosomes. Compared to ordinary liposomes made of hydrogenated soy phosphatidylcholine and

cholesterol, the archaeosomes are captured by multiple endocytic routes that led to an extensive uptake rate, rendering in the order of 15fold higher intracellular lipid accumulation after 5 hours. The interesting consequences of these features will be discussed in the context of their advantageous use as a tool to overcome multidrug resistance, as compared to other types of nanoparticulated carriers.

NANOPARTICLE DELIVERY TO IMMUNE CELLS IN THE LUNG – FROM COMPLEX 3D LUNG MODELS TO IN VIVO EXPERIMENTS

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Engineered nanoparticles (NPs) hold great promise for the treatment of respiratory tract disorders since they allow site-specific delivery and uptake, and were proven to be capable of modulating immune responses. In particular, targeting of dendritic cells (DCs) that are key immune cells to enhance or suppress an immune response in the lung is a promising approach for the treatment of allergic diseases [1, 2].
Since pulmonary immune responses to engineered NPs with varying surface charge are poorly understood the effects of fluorescently-encoded polyvinyl-alcohol-coated gold nanoparticles (AuNPs), functionalized with either negative (-COO^-) or positive (-$NH3^+$) surface charges [3] were investigated *in vitro* as well as *in vivo*. First, a 3D co-culture model consisting of epithelial and immune cells (macrophages and DCs) mimicking the human alveolar epithelial tissue barrier was employed to assess the effects of aerosolized AuNPs. PVA-NH^{3+} AuNPs showed higher uptake compared to their –COO^- counterparts, with the highest uptake recorded in macrophages followed by DCs as shown by flow cytometry. None of the AuNPs induced cytotoxicity, necrosis or increased cytokine secretion, whereas only PVA-NH^{3+} AuNPs induced higher apoptosis levels [4]. Second, for the *in vivo* experiments NH^{3+}-PVA and COO^--PVA AuNPs were intranasally instilled in naïve mice. AuNP uptake by specific immune cell populations in different lung compartments was assessed after 24h by flow cytometry. Following AuNP exposure, the antigen ovalbumin (OVA) uptake by immune cells and OVA-specific CD^{4+} T cell proliferation in lung draining lymph nodes (LDLNs) were examined. The *in vitro* results were confirmed *in vivo*, as macrophages and DC subpopulations preferentially captured NH^{3+}-PVA AuNPs compared to their COO^- counterparts. Although OVA uptake by DCs and macrophages was unaltered following exposure to NH_2-PVA and COO^--PVA AuNPs compared to PBS controls, NH3+-PVA AuNPs, but not COO^--PVA AuNPs, induced enhanced proliferation of OVA-specific $CD4^+$ T cells in LDLNs [5].
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 immune cell populations in the lung and down-stream immune responses. These results also emphasize the reliability of well-defined complex *in vitro* 3D lung models to analyze specific particle-cell interactions, which may be used prior to *in vivo* experiments in order to develop novel innovative NP-based carriers for immune-modulatory treatments in the lung.
This study was supported by the NRP64 program of the Swiss National Science Foundation.

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CHARACTERISING THE ENDOCYTOSIS OF NANOMEDICINES

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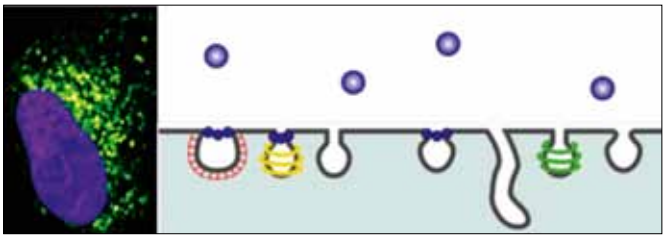
Nanomedicine promises to revolutionise the way we currently deliver drugs to their site of action. Nano-sized materials in fact can enter cells easily by active processes, using cellular pathways rather than freely diffusing according to their solubility and partition coefficient, as many drugs we use.
Nanotechnology nowadays allows us to engineer materials of many different properties such as size, charge, shape etc. and to screen them for their efficacy, while different targeting strategies can be used to try to achieve recognition of the nanocarrier on the cells of interest and increase the specificity of the delivery.
However, even for successful materials, the fraction that arrives to the target is in most cases very small. Furthermore a clear understanding of the reasons why a certain design seems to be successful is often missing. This has led to a growing realization within the drug delivery community that in order to fully exploit nanomedicine potential, the molecular details of the mechanism of nanoparticle uptake and intracellular trafficking need to be characterised.
To this aim, we have combined different methods typically used to study endocytosis and transport mechanisms, such as colocalisation with endocytic markers, the use of transport inhibitors and RNA interference, together with newer methods currently applied in cell biology and not yet used for the characterization of the pathways drug carriers use to enter cells (Fig. 1). The results obtained so far clearly illustrate the complexity of disentangling the pathways involved. When blocking or interfering with a certain transport pathway cells can adapt or respond by activating alternative ones. Furthermore careful controls need to be optimized in order to verify the effect of the different treatments on the pathways under investigation and confirm whether they are blocked effectively or not, and to exclude strong toxicity (due to the treatment) that could affect the outcomes.

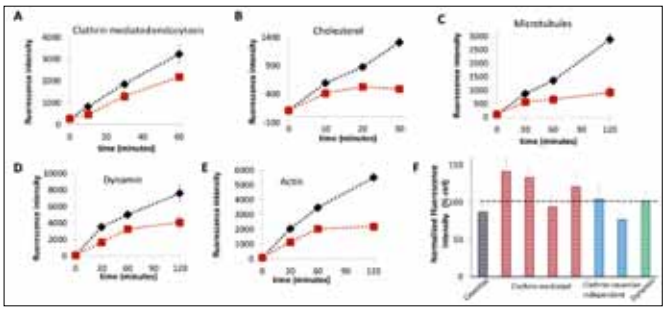
Figure 1. Left: fluorescence image of a cell with internalised green nanoparticles (Blue: nuclei). Right: blue nanoparticles approaching a cell membrane, where different endocytic pathways are illustrated.



We then used a panel of model materials of different size and surface properties, in order to investigate the effect of such parameters on the pathways involved (an example of the results obtained for silica nanoparticles is included in Figure 2). The results clearly illustrate that very different scenarios are obtained, where for some nanoparticles multiple pathways seem to be involved at the same time, while for others it is extremely difficult to block uptake with any of the different treatments applied. The advantages and limits

of these classic methods are discussed and new methods are being optimized to be able to fully understand the nature of the pathways involved in both cases.
We also show that the uptake mechanisms are affected on the one hand by cellular characteristic and on the other by the biological environment in which nanoparticles are applied to cells and the resulting nanoparticle-corona formed.

Figure 2. Transport inhibitors and siRNA panels to characterize nanoparticle uptake mechanisms. Uptake results obtained on cells exposed to fluorescent nanoparticles (in the example: silica) using a panel of transport inhibitors (A-E. Black and red lines are results obtained – respectively - in the absence or presence of a transport inhibitor affecting the structures or pathways indicated in each panel) and a panel of siRNA molecules to block the expression of key proteins involved in different uptake pathways. These panels, together with colocalisation studies by fluorescence microscopy have been used on a series of carriers of different size and surface properties to study how the mechanisms they use to enter cells are affected by such parameters and also by the exposure conditions and resulting corona.



ENTRY OF NANOPARTICLES INTO CELLS: MECHANISMS, CONSEQUENCES AND CHALLENGES IN REACHING THE TARGET

KIRSTEN SANDVIG

Nanoparticles can be used to deliver drugs or other substances both *in vivo* and *in vitro* [1-3], and are commonly used to study basic cell biology. To enter cells the particles exploit the endocytic machinery, and they have been demonstrated to induce changes in cellular uptake and intracellular transport [4,5]. Crosslinking of cell surface molecules may cause signaling in cells [6], and nanoparticles have been found to induce macropinocytosis that facilitates uptake of particles. In several instances this process has been shown to be dependent on the large GTP-binding protein dynamin. To optimize nanoparticle delivery into cells one needs to understand the cellular mechanisms involved in their uptake. Such information may help in deciding the type of particle to use, the size of the particle as well as which components to include at particle surface. Today we know that cells have different types of endocytic mechanisms [7], some giving rise to small vesicles (60-200 nm diameter), whereas other mechanisms such as macropinocytosis are required for uptake of larger particles. One should be aware of that cells growing in a polarized manner are likely to have different endocytic mechanisms which are under differential influence of signaling substances at the two poles [7], and studies of nanoparticle uptake in nonpolarized cells may not give the same results as if uptake in polarized cells is investigated. It may be an advantage to study different cell types as their response to a given nanoparticle may vary. Furthermore, increased cell density may induce changes in membrane lipids and intracellular transport [8], and modification of membrane lipids may change the mechanisms of uptake [9]. 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 [1,7]. Also, robust methods to determine whether a particle is internalized or only at the cell surface are important to provide the investigator with correct data about uptake efficiency, and a challenge is that the drug in the nanoparticle often has to reach the cytosol or the nucleus to exert its action.

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IDENTIFYING MOLECULAR SIGNATURES OF TUMOR DORMANCY AS A BASIS FOR THE RATIONAL DESIGN OF PRECISION NANOMEDICINES

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Tumor progression is dependent on a number of sequential steps, including initial tumor-vascular interactions and recruitment of blood vessels, as well as established interactions of tumor cells with their surrounding microenvironment and its different immune, endothelial and connective cellular and extra-cellular components. Failure of a microscopic tumor, either primary, recurrent or metastatic, to complete one or more of these early stages may lead to delayed clinical manifestation of the cancer. Micrometastasis, dormant tumors, and minimal residual disease, contribute to the occurrence of relapse, and constitute fundamental clinical manifestations of tumor dormancy that are responsible for the majority of cancer deaths. However, although the tumor dormancy phenomenon has critical implications for early detection and treatment of cancer, it is one of the most neglected areas in cancer research and its biological mechanisms are mostly unknown. To that end, we created several models of patient-derived cancer models mimicking pairs of dormant versus fast-growing, primary versus metastatic and drug-sensitive versus drug-resistant cancers using cutting-edge techniques of patient-derived xenografts, 3D printing and genetically-modified mouse models. We investigated the molecular changes in tumor-host interactions that govern the escape from drormancy and contribute to tumor progression. Those led to the discovery of novel targets and provided important tools for the design of novel cancer nano-sized theranostics (therapeutics and diagnostics).[1] Our libraries of precision nanomedicines are synthesized as highly controlled micellar, nanogels, coiled or globular particulated supramolecular structures consisting of linear, hyperbranched and dendritic polymers based on polyglutamic acid, polyethyleneglycol, poly(N-(2-hydroxypropyl)methacrylamide), polyglycerol, and hybrid systems.[2-7] We hypothesize that the acquired knowledge from this multidisciplinary research strategy will revolutionize the way we diagnose and treat cancer.

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safe manner. The system is similar to an 'allergy test', screening the potency of miniscule doses of multiple medicines directly inside the patient's tumor, before beginning a treatment cycle. Based on the screen, a patient-specific drug potency chart is constructed, rating the activity of the different medicines for each individual patient.
In pre-clinical trials we found the system is accurate in predicting the response of triple-negative tumors to medication.
The clinical implications of these approaches will be discussed.
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ENGINEERING EXTRACELLULAR VESICLES FOR DRUG DELIVERY

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Extracellular vesicles (EVs) are submicron membrane particles released by all cells. Mammalian cells can release different EVs, which can be distinguished based on their biogenesis pathway and include exosomes, microvesicles, and apoptotic bodies. Despite these differences in intracellular origin, a uniform EV nomenclature is still lacking. This is due to the difficulties in classifying the vesicles after release since they overlap in size and subtype-specific markers that are unique have not been identified.
The natural cargo of EVs is composed of proteins, coding and non-coding RNAs (like mRNA, miRNA, lncRNA) surrounded by a bilayer of phospholipids and membrane proteins. The cargo reflects the composition of the parental cell although selective enrichment and depletion of certain components has been noted. Interestingly, the biological information packaged in EVs has been shown to be functionally transferred between cells, and as a result may modify the acceptor's cells. EVs can bind acceptor cell surface molecules inducing a response but may also subsequently be internalized by or fused with acceptor cells, resulting in transfer of luminal cargo like mRNAs and miRNAs, and of proteins,
Several applications may require the engineering of vesicles in order to improve interaction with target cells, reduce interaction with non-target tissues or improve loading with therapeutic compounds. Many of these strategies rely on genetically engineering the producing cells to produce (more of) the required RNA or proteins. In addition, techniques form the liposome field, such as post-insertion, allow synethtic modification of vesicle surface and content.
In this presentation, several of the biological and synthetic techniques that we developed in our lab to modify EVs will be discussed and we will contrast our findings to engineering approaches with synthetic liposomes.

PERSONALIZED CANCER NANOMEDICINE

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The field of nanomedicine is taking its first steps towards personalized care. Our research is aimed at addressing a clinical need – predicting how a cancer patient will respond to treatment, before the actual medication program begins. In fact, at least 30% of cancer patients are prescribed a medication that fails to affect the tumor; these numbers greatly increase when dealing with a metastatic or recurrent disease. To address this need, we developed nanoparticles that target tumor and metastasis, where they gauge the activity of medicines in a personalized manner. Specifically, we developed a nanotechnology diagnostic system for predicting the therapeutic potency of anti-cancer drugs inside the patient's tumor in a

ELEPHANT P53 LOADED INTO NANOPARTICLES KILLS CANCER CELLS

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Elephants rarely get cancer. This paradox becomes extremely interesting when considering that elephants have a body mass nearly 100-times greater than that of a human adult, and can live for 80 years. Recently it was discovered that elephant cells have a unique P53 mechanism that protects them against cancer. Specifically, elephant cells express a set of TP53 variants, slight mutations of the human counter-gene, that grant them immunity against cancer-related mutations. Here, we loaded some of these genes into nanoparticles and noticed they induce cancer cell death. The nanoparticle protected the eP53 (elephant P53) gene, or the protein from degradation, and delivered it intracellularly. Using ten different cancer lines, including – breast, ovarian, melanoma, osteosarcoma, pancreatic and others, the cells died after introducing the eP53 nanoparticles.
This new apoptotic mode for treating cancer involves a cancer-protected animal gene nanomedicine, and may prove to be a new anticancer nanomedicine. The clinical implications of this approach will be discussed.

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POLYACETAL POLYMER BASED DELIVERY OF PTX IN PROSTATE CANCER MODELS

SIMO SCHWARTZ

The design of improved biodegradable polymeric carriers which can enhance antitumor and antimetastatic drug efficacy by controlling the concentration and release of the active principle in tumors and metastasis is an ongoing challenge. Paclitaxel (PTX) is a clinically well-established and highly-effective antineoplastic drug used for the treatment of many carcinomas, including prostate. However, the clinical use of PTX is limited by the side effects caused by a poor biodistribution of the drug and by the solvent (Cremophor) in which PTX is dissolved for delivery. Looking for high molecular weight, biodegradable and pH-responsive polymeric carriers, PTX was conjugated to the side-chains of a pH-susceptible biodegradable Poly(ethylene glycol) polymer yielding: tert-Ser-PTX polyacetal. In vitro efficacy of the synthesized conjugate was tested in PCa cells looking at cell viability to determine the half maximal inhibitory concentration (IC_{50}) values. Further, *in vivo* tolerability and therapeutic antitumor and antimetastatic efficacy of the conjugate was evaluated in prostate ortothopic tumors using *in vivo* bioluminescence optical imaging (BLI) and histopathology. In vitro, tert-Ser-PTX conjugate reduced cell viability of PC-3 and LNCaP PCa cells, but with slightly higher IC_{50} values of those for free PTX. In vivo, tert-Ser-PTX significantly reduced the systemic toxicities as-

sociated with free PTX. Regarding the therapeutic efficacy, tert-Ser-PTX polyacetal conjugate was effective in inducing orthotopic LNCaP prostate tumor growth inhibition. Relevantly, tert-Ser-PTX also decreases disease propagation by significantly reducing the distant hematologic, and the locoregional lymphatic and coelomic dissemination patterns as confirmed by *in vivo* and ex vivo BLI and histopathological evaluation. Overall, our results indicate that the tert-Ser-PTX polyacetal could be used as a robust drug delivery system for antitumoral and antimetastatic treatments based on PTX. Conjugation of PTX improves its *in vivo* toxicological and efficacy profiles, over the orthotopic primary tumor and the spontaneous metastatic disease.

OPTIMIZING THEORETICALLY PEPTIDE-PROTEIN INTERACTIONS FOLLOWED BY AN EXPERIMENTAL PROOF THAT IT WORKS

GIACINTO SCOLES, Associated to CNR NANOTEC (Lecce) ITALY and CNR IOM (Basovizza) ITALY and Biology Department, Temple University, Philadelphia, PA (USA)

In his talk I will summarize the state of the art in optimizing peptide – protein interactions using molecular simulations coupled with Monte-Carlo optimization methods and molecular docking calculations. We use these optimizations to design and eventually construct two types of molecular nano devices: 1) Very sensitive protein detection devices and 2) a new kind of molecular drug to, for instance, selectively passivate enzymes.

1) Very sensitive protein detection. To achieve this goal we design nanobodies and we optimize their interactions with the protein of interest (presently the TAU protein). After that, we use DDI or DNA Directed Immobilization (of Protein and Antibodies) to first seed on very flat surface regions of ssDNA and subsequently dock complementary DNA conjugated to an antibody that will recognize the protein. The various phases of the operation are detected by profiling the surface with a stable and sensitive Atomic Force Microscope. Increasing the sensitivity of the last step, by docking a secondary antibody conjugated to a suitable nanoparticle we hope to achieve femtomolar sensitivity that is the sensitivity of the so-called digital ELISA tests.

2) A new kind of molecular drug. By optimizing two nanobodies theoretically and linking them with a not too flexible linker, we are trying to prepare bidentate binders that would be much more selective than their mono counterparts would. For instance, by selecting the molecule active in the VIOXX drug by MERCK instead of one of the two antibodies we can compensate the lack of selectivity that caused the withdrawal of that frg from the market.

The state of the art in our laboratories will be presented at the conference together with the results of experiments that prove the feasibility of at least part of our program.

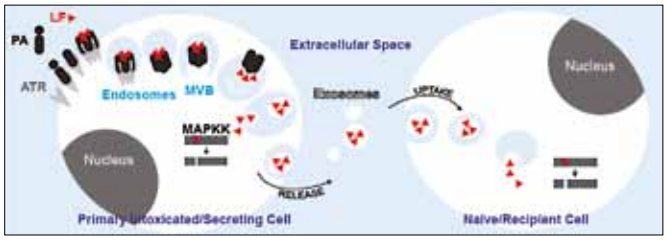
This work was conducted in collaboration with the theoretical group of Sara Fortuna at Sissa and the experimental group of Matteo Castronovo at Leeds.

PATHOGENS IN EXOSOMES

OKSANA SERGEEVA AND GISOU VAN DER GOOT, Global Health Institute, School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

Exosomes are endosomal vesicles that can be trafficked from cell to cell. Recent work on exosomes has exploded, primarily due to the fact that they have been implicated in a variety of diseases and linked to biomarkers. A few groups, including ours, has also found that exosomes can contain pathogens – either bacteria themselves or their pathogenic proteins. We found this out when studying the long-term effect of anthrax toxin. We discovered that lethal factor (LF) of anthrax toxin can be transferred to naïve cells via exosomes. Usually, LF enters the cell using protective antigen (PA) and anthrax receptors and then eventually makes its way into the cytoplasm, where it can cleave MAPKKs and cause apoptosis. By hijacking exosome biogenesis, LF can evade cellular degradation and go on to infect other cells. Other groups have found very similarmechanisms. One benefit of tracking pathogens in exosomes is that their downstream effects are potent. Using anthrax toxin as a tool allows us to observe and probe exosome biogenesis and uptake without purification of the exosomes themselves. We just use the readout of MAPKK cleavage in their naïve cells. This is a large benefit because different laboratories use different exosome purification techniques and work with differing cell types, which has made understanding general exosome characteristics difficult. Furthermore, learning how exosomes are made from cells, let alone from diseased cells, has been a challenge. Our unique pathogen-based system for studying exosomes allows us to understand general mechanisms of exosome release and uptake in any cell type.

Figure: Pathway of anthrax toxin cell entry and release within exosomes; and subsequent exosome uptake by naïve cells.



HEALTH CANADA'S APPROACH TO NANOTECHNOLOGY BASED HEALTH PRODUCTS

HRIPSIME SHAHBAZIAN

Health Canada uses existing legislation and regulations to mitigate potential health risks of nanomaterials and to help realize their benefits. Consistent with other major regulatory bodies around the world, the Department takes a case-by-case approach to assessing the safety of products and substances that may either be or contain nanomaterials. To support the regulation of nanomaterials, Health Canada developed a working definition for Nanomaterial. The working definition is relevant for all products and substances regulated by Health Canada.

Health Canada's Health Products and Food Branch (HPFB) is the national authority that regulates, evaluates and monitors the safety, efficacy, and quality of therapeutic and diagnostic products in Canada. To inform the stakeholders the HPFB created a nanotechnology webpage regarding Health Canada's Working Definition of Nanomaterials and providing general guidance. It advises sponsors and other stakeholders to communicate with responsible regulatory areas early in the development process if their products contain or make use of nanomaterial and provides examples of the type of information that may be required for a nanotechnology-based product's safety assessment.

To facilitate identification and tracking of nanomaterial containing drug submissions Health Canada revised Drug Submission Application Form for Human, Veterinary, Disinfectant Drugs and Clinical Trial Application/Attestation (HC/SC 3011)[2]. Section 56 of the revised form asks the sponsor to self-identify when their application concerns a nanomaterial or 'nano-product'.

Natural health products, such as vitamin and mineral supplements and herbal products for which therapeutic claims are made are regulated under the Natural Health Products Regulations and not as drugs under the Food and Drug Regulations. A similar approach has been adapted for the Natural Health Product License Application Form (PLA-FORM)[3].

To facilitate identification and tracking of nanomaterial containing device submissions Health Canada issued a revised Medical Device License Application form to ask the sponsor to self-identify when their application concerns a nanomaterial and provide size range of nano-scale material particles[4].
Health Canada believes that, in general, its current risk assessment methodologies are applicable for nanomaterials as they allow for sufficient flexibility. To address unique physical, chemical and biological properties of nanomaterials each product is assessed on a case-by-case basis.
Strong relations and dialogue with domestic and international counterparts are also important in achieving program objectives in an increasingly complex regulatory world. Health Canada continues to work closely with domestic and international partners toward consistency with relevant international norms. The Department has developed strong partnerships with other levels of government, academia and stakeholders and ensures that their perspectives are included in its assessment of the health benefits and risks of every therapeutic product it reviews. Health Canada works with international organizations, including regulatory authorities in other countries, to harmonize regulatory standards and processes for therapeutic products.
Health Canada has been a member of the International Regulators on Nanotechnology Working Group since its inception in 2009. As an emerging product category, a nanotechnology-based therapy became a topic for the International Pharmaceutical Regulators Forum (IPRF) in 2014 and in 2015 agreement was reached to establish a Nanomedicines Working Group under IPRF to share non-confidential information. The original members of the International Regulators on Nanotechnology Working Group (Australia – Therapeutic Goods Administration, European Commission, European Medicines Agency, Health Canada, Japan Ministry of Health, Labour and Welfare, U.S. Food and Drug Administration) formed the newly created Nanomedicines Working Group under IPRF. The EU has served as WG Chair since June 2015, and at the recent International Pharmaceuticals Regulators Forum in Osaka, Japan, November 6-7, 2016, HC agreed to serve as WG Chair starting January 2017.
The IPRF Nanomedicines Working Group members will be meeting during the CLINAM 10.
WG. Starting 2017 HC assumed the role of the chair of this WG.

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RNAI AND TUMOR MICROENVIRONMENT: PRE-CLINICAL AND CLINICAL EVIDENCE

AMOTZ SHEMI, CEO, Silenseed LTD, Israel

The distribution of drugs within solid tumors presents a long-standing barrier for efficient cancer therapies. Tumors are highly resistant to diffusion, and the lack of blood and lymphatic flows suppresses convection. Moreover, efficacy in targeting oncogenes by current monoclonal antibodies or small-drug inhibitors is limited. RNAi-based medicine enables effective targeting of 'undrugable' targets' such as KRAS, and, when is based on prolonged and continuous intratumoral drug delivery from a miniature drug source, offers an alternative to both systemic delivery and intratumoral injection. At delivery onset the drug mainly affects the closest surroundings. Such 'priming' enables drug penetration to successive cell layers. Tumor 'void volume' (volume not occupied by cells) increases, facilitating lymphatic perfusion. The drug is then transported by hydraulic convection downstream along interstitial fluid pressure (IFP) gradients, away from the tumor core. After a week tumor cell death occurs throughout the entire tumor and IFP gradients are flattened. Then, the drug is transported mainly by 'mixing', powered by physiological bulk body movements. Steady state is achieved and the drug covers the entire tumor over several months. We present measurements of the LODER™ system, releasing the siG12D (siRNA against mutated KRAS) drug over months in pancreatic cancer in-vivo models. siG12D-LODER was successfully employed in a Phase 1/2a clinical trial with pancreatic cancer patients, and is planned to enter Phase 2 multinational trial in 2016 (Golan et al., 2015, Oncotarget; Shemi et al., 2015, Oncotarget).

BIOGRAPHY

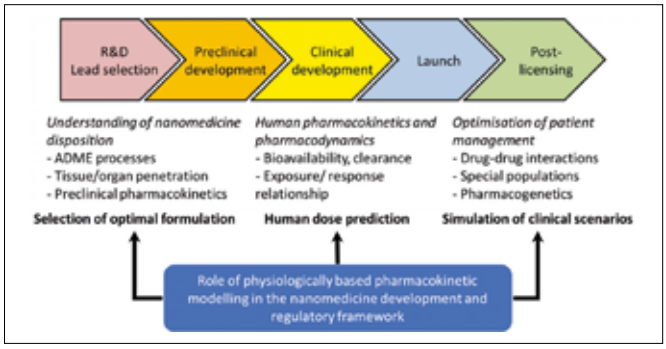
Dr. Amotz Shemi is the Chief Executive Officer and a co-founder in Silenseed. Prior to Silenseed, Dr. Shemi served as a Senior VP Technologies in Medinol LTD, a leading medical-stent company, and beforehand as the CEO of Color Chip, a leader in Ion-exchange based Planar Lightwave Circuits (PLC); Dr.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

INTEGRATING PHYSIOCHEMICAL DESCRIPTORS WITH TISSUE AND CELLULAR DATA TO MAKE QUANTITATIVE PREDICTIONS THROUGH PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELLING

MARCO SICCARDI, Senior Lecturer, Department of Molecular and Clinical Pharmacology, University of Liverpool, Liverpool, UK

The broad variety of materials used for nanoparticle synthesis and their potential combinations define an inexhaustive list of technological platforms, defining numerous scientific and regulatory challenges during the nanomedicine development process. The distribution of nanoparticles through the body represents a pivotal process for the definition of toxicity and efficacy and a successful localised delivery of therapeutic agents at effective and safe concentrations is a critical step to identify successful therapeutic strategies.
Physiologically-based pharmacokinetic (PBPK) modelling is a powerful pharmacological tool, which integrates experimental *in vitro* data into a mathematical framework of anatomical, physiological and molecular processes. This results in an in silico description of absorption, distribution, metabolism and elimination (ADME) and a quantitative *in vivo* estimate of pharmacokinetic behaviour. Thus, PBPK modelling offers opportunities for accelerating rational design of nanomedicines, through progression of candidates with maximum efficacy potential and mitigates risk of subsequent failure. The quantitative prediction of nanomaterial distribution patterns at cellular, organ and whole organism level, is another clear benefit of the approach. Besides describing nanoparticle pharmacokinetics, PBPK modelling has the potential to provide quantitative evaluation of the influence of nanoformulation properties on distribution patterns.
Consequently, PBPK simulations could find valuable applications in multiple steps of the nanomedicine development and regulatory framework, supporting a better understanding of the mechanisms defining distribution and a rational optimisation of dosing in humans. PBPK models have been developed to provide a computational prediction of nanomaterial distribution for multiple technological platforms, carbon nanoparticles, polymeric nanoparticles,

dendrimers, liposomes, quantum dots and solid drug nanoparticles. PBPK modelling has been used to characterise the quantitative relationships between nanoparticle properties and distribution patterns parameters as well as the identification of optimal dosing and nanoparticle characteristics to inform the development of long-acting nanoformulations.
PBPK modelling has a wide spectrum of potential impact for nanopharmacology, with expected benefits for academia to industry, clinician to patient and manufacturer to regulator. The generation of nanoformulations with optimal pharmacokinetics can be achieved only through interdisciplinary research where knowledge from organic/inorganic chemistry, polymer synthesis, nanotoxicology, molecular & clinical pharmacology and mathematical modelling should be integrated. The rational iteration of PBPK modelling with manufacturing, *in vitro* and *in vivo* approaches can accelerate new material platforms towards therapeutic applications and de-risk the development process.



MECHANISMS OF COMPLEMENT CORONA ASSEMBLY ON NANOPARTICLES

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Serum protein corona has been a focus of substantial research in nanomedicine, due to its importance for the biological function of drug delivery and imaging systems. Complement cascade is the critical part of serum immunity responsible for infusion-related toxicity and clearance of nanoparticles, but the mechanisms of assembly of complement factors on nanoparticles and relationship to other components of the corona have been overlooked. Using a MRI contrast agent superparamagnetic iron oxide core (SPIO)–dextran shell nanoworms, we demonstrate that opsonization with human complement C3 takes place via the alternative pathway (AP). The activation was dependent on the binding of the AP factor properdin. Quantitative immunoblotting suggested that on average each nanoparticle bound tens of C3 and properdin molecules, constituting up to 35% (w/w) of absorbed serum protein. In some human subjects the number of complement factors per particle was much higher, suggesting variability of complement activation in general population. Theoretical calculations and high-resolution electron microscopy demonstrated that serum protein corona and complement C3 constitute only a small fraction of nanoparticle volume and likely invade the nanoparticle shell. Further analysis showed that in serum complement C3 bound to the protein corona rather than to the dextran shell. Moreover, surface-bound proteins accelerated the assembly of the alternative pathway of complement on the nanoparticle surface. Significant fraction of C3 was exchangeable and dissociated from nanoparticles along with the soft protein corona, but the particles were efficiently re-opsonized in fresh serum with enhanced immune uptake by leukocytes. Injection of nanoworms into mice showed very rapid loss of C3 and other absorbed proteins. Our experiments are the first demonstration of complement as a dynamic exchangeable entity in the context of soft corona and inform strategies that will improve safety and hemocompatibility of nanomedicines in humans.

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DEVELOPMENT OF NANOPARTICLES FOR CLINICAL USE: IMPORTANCE OF DEGRADATION AND EXCRETION

TORE SKOTLAND, Centre for Cancer Biomedicine, The Norwegian Radium Hospital, Oslo University Hospital and University of Oslo, Norway

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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EDUCATION AND TRAINING FROM THE EUROPEAN AND IMI PERSPECTIVES

PER SPINDLER

This talk will review some of the major European initiatives in training the stakeholder communities of regulatory science and share best practices.

RHEUMATOID ARTHRITIS: UNMET MEDICAL NEEDS AND HOW NANOMEDICINE CAN HELP

STEPHANIE STANFORD AND NUNZIO BOTTINI, Department of Medicine, University of California, San Diego, La Jolla, CA USA

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disease that causes debilitating and painful joint deformation. In the last two decades, multiple immunosuppressant disease-modifying anti-rheumatic drugs were approved by the FDA for RA. While these medications are very effective in some patients, almost 50% of RA patients still fail to achieve remission on the current regimens. Thus there is a clear unmet medical need for novel RA therapies. Nanomedicine holds potential to offer unique solutions for the development of new approaches for treating RA. With the goal of shedding light on possible areas where nanomedicine could be exploited for RA therapy, here we will discuss the key immunological and connective tissue cell types mediating the pathogenesis of this disease.

COLLABORATIVE APPROACHES TO ENHANCE TRAINING IN REGULATORY SCIENCE AND TRANSLATIONAL RESEARCH

SCOTT STEELE, Director, Regulatory Science Programs, Clinical and Translational Science Institute, Director, Rochester Center of Excellence in Data Science, Associate Professor, Public Health Science, University of Rochester, Rochester, NY, USA.

Ensuring emerging technologies are ultimately translated into safe and effective medicines requires a robust research and training infrastructure. This includes preparing for new developments from nanomedicine and 3D printing of medical products to mobile sensors and stem cell therapies. A broad range of international institutions continue to develop formal training programs to advance regulatory science and to accelerate medical product development. Training programs can benefit from further harmonizing core competencies that have been developed to guide training in this area, including sharing educational materials. Formal course work should be further complemented by other training resources, including utilizing case studies, modules and internship opportunities. Finally, research and training in regulatory science and translational research can benefit from an integrated approach, specifically by leveraging global partners from diverse sectors and disciplines, including regulatory agencies, academia, industry, and foundations. This presentation will highlight approaches underway to coordinate regulatory science training and research initiatives, including the development of core competencies to guide and harmonize training. We have leveraged the U.S. Clinical and Translational Science Award Consortium of academic institutions, while building partnerships with the U.S. National Institutes of Health, U.S. Food and Drug Administration (FDA), FDA Centers of Excellence in Regulatory Science and Innovation, pharmaceutical industry, and foundations. Currently, we are exploring how to harmonize efforts with international partners to establish a broader global training network.

NANOPARTICLES INTERACTIONS WITH VIRUSES: FROM STABILIZATION TO VIRUCIDAL DRUGS

FRANCESCO STELLACCI, Institute of Materials, and Interfaculty Bioengineering Institute, Ecole Polytecnique Fédérale de Lausanne (EPFL), Lausanne, 1015, Switzerland, francesco.stellacci@epfl.ch

Viruses kill every day in large numbers especially in low-income countries. Any given day ~1600 children age 1 to 5 die of diarrhea (mostly due to rotavirus). More than 5000 people have died of Ebola in the last outbreak. On the other hand, viruses also save lives as vaccines; unfortunately, because of their thermal instability, vaccines need constant refrigeration. This generates a 'cold chain' need for vaccines that contributes to >50% of the costs of vaccination programs and generates significant logistic problems especially in developing countries. The 'cold chain problem' is considered among the top challenges for global vaccinations. In this talk, these problems will be addressed with the tools of nano-medicine. Results show that nanoparticles (and equivalent small or macro-molecules) can have a wealth of interactions with viruses. Depending on the particles' coating and on the specific virus, particles can (i) thermally stabilize viruses, (ii) inhibit their cell entry, or (iii) be virucidal (i.e. permanently in-activate them outside their host). Each of these interactions can be leveraged to address a key biomedical challenge that viruses pose. A series of systematic studies on virus interactions with nanomaterials will be presented to identify the mechanisms that maximize thermal stabilization or virucidal action. The former will is used to produce additives for vaccines capable of keeping viral vectors stable for more than two months at 37°C, thus directly addressing the 'cold chain problem'. Virucidal efficacy is used to create drugs able to reduce the mortality of infections due to Dengue or Hepes. In all cases the focus used in this research is to find nanoparticles, macro- or small molecules that are safe and inexpensive so that the translation of this research in drugs for low-income countries could be deemed as feasible in the near future.

SCEPTISM IN THE FIELD OF TARGETED NANOMEDICINE: JUSTIFIED OR UNFAIR?

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University Medical Centre Utrecht (UMCU), Division Imaging, Utrecht, The Netherlands, Dept. Biomaterials Science & Technology (BST), MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, Enschede, The Netherlands

One most active sector of research within the field of nanomedicine has been the design of nanoparticulate drug formulations for the targeted delivery and controlled release of chemotherapeutic agents. In fact, novel nanomedicinal drug delivery systems continue to flourish in the research laboratory. However, the number of such nanomedicines that have been approved for the treatment of patients is still limited. Examples are Caelyx/Doxil (doxorubicin), Myocet (doxorubicin), DaunoXome (daunorubicin), Marqibo (vincristine), Onyvide (irinotecan), Onco-TCS (vincristine), and Abraxane (paclitaxel). While these examples illustrate that significant advances have been made over the years in making nanomedicines a clinical reality, there is nevertheless growing sceptism in the scientific literature regarding the future and clinical applicability of targeted nanopharmaceuticals. In this presentation, I will discuss my view on the arguments raised to justify the negative attitude as well as how targeted nanomedicine will face tomorrow.

IN VIVO IMAGING WITH UCNPS (UPCONVERTING NANOPARTICLES) AS AN IDEAL BIO-SAFE IMAGING PROBE

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Fluorescent microscopy techniques have been widely used for diagnosis of diseases for several decades. These techniques have grown in their importance, as more recently the discovery of green fluorescence protein (GFP: 2008 Nobel Chemistry Prize), the development of super-resolution imaging techniques (2014 Nobel Chemistry Prize), and the invention of novel nanoparticle-based probes such as quantum dots (QDs: 2008 Kavli Prize in Nanoscience) has greatly accelerated the use of optical and imaging methods for biomedical applications in clinical fields, for example, disease diagnosis. However, a number of limitations stubbornly persist: photobleaching and photoblinking of organic fluorescence dye molecules and the biocompatibility issue of nanocrystals and QDs remain critical issues to overcome. In addition, imaging platforms working with the near-infrared (NIR) spectral region are required to reduce the noise and background signals in live cell, such as autofluorescence, and increase the signal-to-noise ratio. Within this context, an exponentially growing number of researchers have begun focusing on the development of novel materials that address some aspect of these challenges.

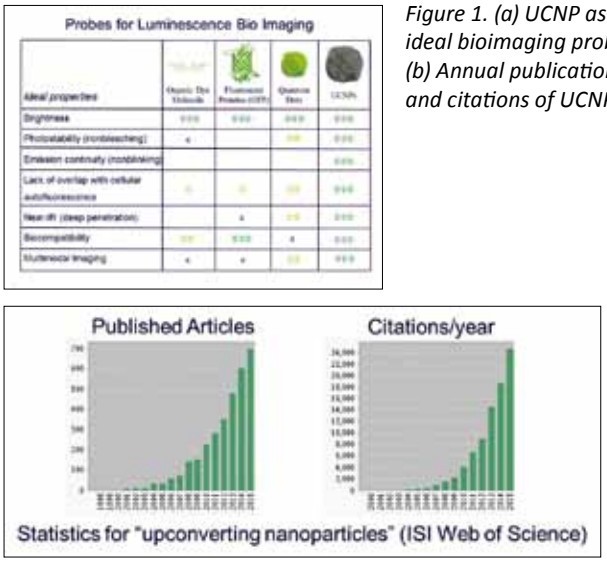
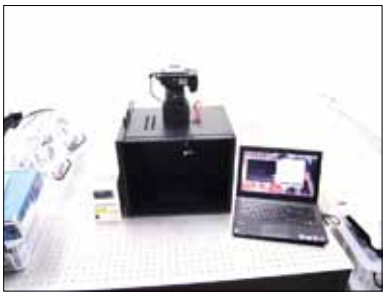


Figure 1. (a) UCNP as an ideal bioimaging probe. (b) Annual publications and citations of UCNP.

Here, we are taking one of the most general and integrated approaches to date for tackling these issues, by adopting upconverting nanoparticles (UCNPs): UCNPs are an ideal single-molecule-(when tethered to anchor into a specific single molecular site) and single-particle-bioprobe of non-bleaching/photostable, non-blinking, non-toxic/bio-safe, and deep penetration depth with no background due to NIR excitation. UCNPs have attracted much attention as novel luminescent nanomaterials (Fig. 1b). UCNPs emit visible photons by absorbing two or more NIR photons. Thanks to such a unique and efficient multiphoton luminescence mechanism, optical imaging with UCNPs has advantages over conventional fluorescence imaging with organic dyes or semiconductor QDs. The NIR excitation increases penetration depth and avoids autofluorescence of biological samples. Moreover, UCNPs show superior photostability exhibiting neither photoblinking nor photobleaching and are much less toxic than heavy metal-containing QDs. In cellular imaging, the absence of autofluorescence and excellent photostability enhance the signal-to-noise ratio, enabling single-particle level imaging and long-term tracking as developed in our research group in KRICT [1]. Several reports on *in vivo* imaging using UCNPs have also been published [2-3].

Multimodal imaging probes based on UCNPs for MRI, PET together with optical luminescence imaging have been developed for more accurate imaging and diagnosis. For extensive applications to *in vivo* imaging probes, however, more optimal nanoparticle properties have to be developed to enhance luminescence intensity. Recently, synthetic methods to obtain small and monodisperse < 10 nm hexagonal-phase UCNPs have been developed [4].

Figure 2 (left) in-vivo sentinel lymph node (SNL) optical imaging of Balb/C mouse after injection of Tm3+-doped UCNPs. (right) Home-made Near-IR in-vivo imaging system for UCNP, developed by RC2NT, KRICT.



In this talk, UCNP-based live-cell & *in vivo* imaging research, obtained by our home-made nano-spectroscopic imaging systems in KRICT, will be presented.

Au-NNP(Au-Nanobridged Nanogap Particles)-based single-particle-tracking Raman imaging results obtained by our home-made nano-spectroscopic imaging systems in KRICT will be also briefly presented [5-7].

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THE RISKY SIDE OF PEGYLATION OF NANO-MEDICINES AND BIOLOGICALS: IMMUNOGENICITY AND IMMUNE REACTIVITY

JÁNOS SZEBENI, MD, PhD, Nanomedicine Research and Education Center, Department of Pathophysiology, Semmelweis University and SeroScience Ltd, Budapest, Hungary

Conjugation of polyethylene glycol (PEG) to liposomes and proteins are widely used today to increase the circulation time, and, hence, therapeutic index of the active pharmaceutical ingredient (API). An unresolved safety issue with PEGylated APIs (PEG-APIs) in the nano size range is their potential recognition by the immune system,

manifested in immunogenicity with subsequent production of anti-drug antibodies (ADAs) and the rise of hypersensitivity (infusion) reactions, referred to as complement (C) activation-related pseudoallergy (CARPA). Known druy<<<<s exemplifying the presence of such problems include PEGylated liposomal doxorubicin (Doxil), liposomal cortisol (Nanocort), PEGylated proteins or enzymes, such as pegfilgrastim (Neulasta), erythropoietin (Mircera), adenosine deaminase (Adagen), L-asparaginase (Pegaspargase) and uricase (Krystexxa), and the PEGylated aptamer (Pegnivacogin). The structural features of these PEG-APIs, as well as the individual factors that make the immune response against them highly variable and thus unpredictable, are poorly understood.

This presentation highlights some recent findings that shed new light on the mechanism of PEG-API-induced immunogenicity and CARPA. One such observation was that i.v. injection of PEG-APIs in pigs led to massive formation of anti-PEG IgM (and much less IgG) antibodies, peaking on day 7-8 and declining to baseline over 4-6 weeks. The phenomenon was shown for PEGylated liposomes (doxorubicin-free placebo Doxil = Doxebo), PEGylated proteins (pegfilgrastim) and PEGylated micelles, all progressing with identical kinetics but different efficacy. Ex vivo FACS studies on isolated spleen cells provided evidence that splenic IgM+ B cells were involved in antibody formation, which could be classified as T-cell independent, „type 2" immunogenicity.

A further novel observations of clinical relevance was that pretreatment of pigs with the human equivalent therapeutic dose of Doxil prevented the immunogenicity of Doxebo via cytotoxicity on splenic IgM+ B cells. This unique collaboration between a nanocarrier (PEGylated liposome) and payload (doxorubicin) in reducing the adverse effects of Doxil provided explanation for the controversy between immunogenicity of PEGylated liposomes and safety of Doxil therapy in patients (Doxil paradox).

In further experiments we measured varying amounts of pre-existing (natural) anti-PEG IgM and IgG in the blood of pigs, which were depleted by PEG-APIs within minutes after their i.v. administration. This is consistent with rapid binding of IgM to PEG underlying C activation, and also explains the reduction or absence of CARPA after repeated administration of the same PEG-API into the same animal (tachyphylaxis).

The concept that natural antibodies play a critical role in PEG-API-induced C activation and CARPA in pigs implies classical pathway C activation, which was strengthened by substantial acceleration of CARPA in immunized animals. The CARPA induced by PEGylated liposomes in Doxebo-immunized animals entailed severe cardiopulmonary distress with many more known symptoms of anaphylaxis, causing cardiac arrest and circulatory collapse in some of the animals. Thus, this protocol represents an unprecedented large animal model of drug-induced lethal anaphylactic shock.

Yet another unexpected finding in the porcine model of CARPA was that immunization with the PEGylated protein (Neulasta), produced anaphylactogenic anti-PEG antibodies that cross reacted with PEGylated liposomes leading to severe CARPA. The potential of such harmful immune conflict between two types of PEG-APIs may deserve attention in the future.

In summary, pigs provide a unique model to study nanoparticulate PEG-API-induced adverse immune effects, their complex inter-relations and approaches of prediction and prevention.

BIOMIMETIC LIPOSOME-LIKE NANOVESICLES ABLE TO TARGET AND MODULATE INFLAMMATION

ENNIO TASCIOTTI

To date, a multitude of micro- and nanocarriers have been developed to improve the systemic delivery of pharmaceuticals. All these carriers are subjected to a number of biological barriers that limit their optimal biodistribution, providing one of the main obstacles to an effective drug delivery. Bio-inspired approaches have been utilized as alternative treatments to evade the mononuclear phagocytic system and facilitate the transport across the endothelial vessel wall. We developed biomimetic nanovesicles called leukosomes, composed of conventional liposomes incorporated with over a 100 plasma membrane proteins derived from leukocytes. Leukosomes conserved the traditional drug delivery capability of liposomes (similar physicochemical characteristics and ability to load hydrophilic, amphiphilic, and hydrophobic payloads), but exhibit longer circulation time, targeted inflamed blood vessels and tissues and permitted a significant delay in the sequestration by the mononuclear phagocyte system compared to liposomes, resulting in an increased therapeutic efficacy in cancer, cardiovascular and autoimmune treatments.

NANO-BIO INTERFACE AND INTRINSIC BIOACTIVITY OF BIOMIMETIC NANOPARTICLES

ENNIO TASCIOTTI

To date, a multitude of micro- and nanocarriers have been devUnderstanding the interactions occurring at the interface between nanoparticles and biological components is an urgent challenge in nanomedicine due to their effect on the biological activity of nanoparticles. After their systemic injection, a protein corona constructed by blood components surrounds nanoparticles' surface and modulates their pharmacokinetics and biodistribution. Biomimicry-based approaches attempt to imitate natural structures and components to transfer specific biological functions to synthetic nanoparticles. We developed several biomimetic formulations based on the use of cell membranes as building blocks and demonstrated superior *in vivo* features resulting from cell-like identity and biological mimicry. Our studies showed that these nanovescicels have an inherent therapeutic activity due not to their payload but rather to the unique protein composition of their surface.

MEETING THE CHALLENGE WITH MICROFLUIDICS: ENABLING NANOMEDICINE DEVELOPMENT FROM BASIC RESEARCH TO THE CLINIC

JAMES TAYLOR, CEO and co-founder of Precision NanoSystems, Inc. (PNI)

Nanomedicines permit access to previously undruggable pathways and to overcome challenges of drug delivery, scientists are optimizing nanoparticles by fine-tuning their size, composition and surface properties. These efforts have yielded substantial results in the laboratory to date, however, a significant need exists for robust manufacturing technologies to transit these discoveries from the bench to the clinic. In this context, we present the microfluidics based NanoAssemblrTM platform that retains consistent quality, efficacy and safety profiles, throughout the drug development process..

Data showing the seamless scale-up from early stage studies using microliter volumes to late stage manufacturing at the scale of tens of litres will be presented.

MANUFACTURING OF NANOMEDICINES – A GMP PERSPECTIVE

STELIYAN TINKOV

Together with gene therapies and personalized medicine, nanotechnology holds the promise to become one of the major drivers for improving human health and quality of life in the next future. However, the gap between genuine patient benefits and cutting-edge scientific research can only be bridged through agile and competent GMP regulations.
The manufacturing of nanomedicines involves a broad range of proprietary technologies, most of which have complex scientific backgrounds and various readiness levels. Likewise, numerous Critical Quality Attributes and Critical Process Parameters are specific to nanomedicines and have to be newly defined for each particular product class. Manufacturing process development by QbD and related GMP regulatory aspects increasingly become a hot topic, especially during the last years as the industrialization of nanomedicine products gains momentum.

REGULATORY CONSIDERATIONS FOR DRUG PRODUCTS CONTAINING NANOMATERIALS

KATHERINE TYNER, Office of Pharmaceutical Quality Science Staff, Center for Drug Evaluation and Research, FDA, Silver Spring, MD.

In recent years there has been an increased focus on developing drug products containing nanomaterials. With this increased focus, there has been a corresponding increase in applications for drug products containing nanomaterials to the United States Food and Drug Administration (FDA) submitted for Agency review. Although subject to the same rigorous regulatory standards as any other drug product, unique properties that arise from the small size and large surface area of nanomaterials may lead to additional scientific considerations when following current FDA guidelines and practices. Such considerations may extend to determining the correct analytical techniques to characterize and control the drug product. This presentation will discuss these scientific considerations and present current regulatory perspectives for drug products containing nanomaterials.

THE IMPACT OF REGULATORY SCIENCE ON THE REVIEW OF DRUG PRODUCTS CONTAINING NANOMATERIALS

KATHERINE TYNER, Office of Pharmaceutical Quality Science Staff, Center for Drug Evaluation and Research, FDA, Silver Spring, MD.

As the number and complexity of drug product containing nanomaterials are submitted to the US FDA increases, there is a commensurate need for both internal and external training, especially in the area of physicochemical characterization, to facilitate the review process. This presentation will highlight FDA/CDER's participation in development of relevant nanotechnology standards as well as training modules to assist the translation of these products to market.

THERANOSTICS IN THE SURGICAL THEATRE

GOOITZEN M. VAN DAM

Since 2007, the field of clinical near-infared fluorescence (NIRF) imaging has taken a huge development from indocyanin green applications towards more disease and in particular tumor-targeted optical contrast agents in various clinical studies. More recently, several groups have reported on phase I and phase II studies of novel NIRF tracers based on antibodies (e.g. bevacizumab, cetuzimab, panitumomab, trastuzumab), nanobodies (e.g. HER2/neu, CAIX targeting), peptides (e.g. c-MET,, folate- receptor alpha) or so-called smart-activatable probe (e.g.[R]ACPPs, MMPs activatable etc). The presentation will give an overview of the tracers currently in clinical evaluation and stages of development combined with clinical applications. Moreover, imaging technology systems for image-guided surgery, image-guided pathology and image-guided endoscopy will be highlighted and compared, illuminating the need for standardization and calibration tools in future clinical studies. New avenues of development, such as targeted photodynamic therapy or photopharmacology in a theranostic fashion will be presented and the future potential for clinical translation.

MEASUREMENT REQUIREMENTS FOR NANO-BIO PARTICLES FOR BIOMEDICAL ADOPTION

HANS VAN DER VOORN

There is an urgent need to develop and adopt better measurement and analysis methods for nano and especially nano-bio particles if these are to become widely adopted for clinical use. The recent interest in the use of Extracellular Vesicles for both therapeutics and diagnostics requires additional capability, sophistication and standardisation due to the complexity and heterogeneity of these nano-bio particles and the quality assurance needs of the medical community. Traditional methods of describing particle concentration, size distribution and particle surface charge have been superseded by the routine availability of much more precise data options than DLS could ever offer for instance.
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 necessary. Dubiously derived unreliable average sizes are of no use in the field. The use of zeta potential as a proxy for particle charge loses meaning when used with complex nanostructures. Particle charge may be better described by electrophoretic mobility and actual aggregation needs to be properly measured. The distribution of charge within the particle set is of high interest in determining the extent to which particle surface functionalisation or particle interactions with biomolecules have occurred.

MIXED MICELLES AN UNDERESTIMATED NANO-FORMULATION FOR PARENTERAL DELIVERY OF POORLY WATER SOLUBLE DRUGS

PETER VAN HOOGEVEST, Lipoid GmbH, Ludwigshafen, Germany, European Summit for Clinical Nanomedicine and Targeted Medicine, Basel, Switzerland, May 7–10, 2017

Mixed micelles comprising phospholipids (soybean phosphatidylcholine) and bile salts, pose, besides oil-in-water emulsions, using phospholipids as emulsifier, and liposomes, alternative phospholipid-based formulation options to solubilize poorly water soluble drug substances for intravenous administration. In these formulations the phospholipid is added to the bile salt to eliminate its hemolytic properties[1]. Minimum weight ratios of bile salt to phospholipid of 0.7-0.8 are needed to obtain clear solutions[2]. Mixed micellar for-

mulations can be prepared by e.g. dissolving the phospholipid component in an aqueous solution of the bile salt, followed by dissolving the drug substance. The aqueous mixed micellar formulation is sterilized by means of sterile filtration and when needed lyophilized. This type of formulation was the first mentioned in the literature in 1909 by B. Moore[3]. In 1916, H. Wieland mentioned the use of mixed micelles to solubilize poorly water soluble drugs[4]. In 1976 Hoffmann La Roche patented a mixed micellar formulation for diazepam[5]. Since then mixed micelles formulations are used in a few injectable and oral products (Konakion MM) to solubilize poorly water soluble drug substances or vitamins for intravenous administration (Cernevit). However, since decades this technology is not being used anymore in new products, which is remarkable, considering the many advantages of this technology: - the excipients are used in marketed products in the EU and USA, indicating adequate stability and acceptance by regulatory authorities,

- the excipients are biocompatible and are natural components present e.g. in blood,
- the excipients are available in parenteral quality,
- they can be produced using relatively simple technologies,
- they have no risk for anaphylactic reactions compared to synthetic solubilizers like e.g. Tween, Cremophor and Solutol; toxicity testing of mixed micelles has been published
- they are suitable for oral as well as parenteral formulation and accepted for pediatric use.

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MECHANOBIOLOGY MATTERS: REASSESSING OUR PROMISES IN NANOMEDICINE

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After 17 years into the National Nanotech Initiative (NNI), it is time to reassess the promises that were made in Nanomedicine and to ask where we have exceeded the expectations, or failed to deliver. Even though billions of US$ went into the development of nanoparticles for cancer therapy, the magic bullet has not been found. The same is true for combating other degenerative tissue diseases, including fibrosis and cartilage regeneration. Only few products for targeted drug delivery to fight cancer are in clinical use today. What might have hampered their successful clinical translation? To improve on future concepts, building awareness regarding misconceptions of the biological mechanisms of nanoparticle delivery processes is required and the case will be made that mechanobiology matters. While highly promising concepts were put forward, mechanical hurdles and constraints under which the nanoparticles have to reach their targets have often been neglected. It is also becoming increasingly evident that the mechanical properties of the tumor microenvironment regulate malignancy, genetic divergence and the response to therapy. Mechanobiology is a rapidly growing field and many novel mechanisms have been deciphered recently how mechanical factors can regulate cell and tissue functions. This was made possible by an increasingly large toolbox of nano- and micro-technologies to quantify forces and mechanical strains at the molecular and cellular levels, yet tools are lacking to map these dependencies at the tissue level.

CELLULAR RESPONSES TO GRAPHENE OXIDE SHEETS: EFFECT OF LATERAL DIMENSION AND THE OXIDATIVE STRESS PARADIGM

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The adverse responses to nanomaterials could be correlated with their structural characteristics and explained by the oxidative stress paradigm. Increased levels of ROS, due to the exposure to nanomaterials, activate cellular anti-oxidative defence and pro-inflammatory pathways, eventually leading to cellular death. In the present study we determined whether the lateral dimensions of endotoxin-free graphene oxide (small GO <1 µm compared to the large GO >1 µm) influenced cellular responses of human lung epithelial cells (Beas-2B). Our second aim was to determine whether the observed response was in agreement with oxidative stress paradigm.
ROS production was assessed using two techniques: hydroethidine oxidation (HE) by flow cytometry and dichloro-dihydro-fluorescein diacetate (DCF-DA) oxidation measured by fluorescence plate reader. We found a dose-dependent increase of ROS levels in correlation with the lateral size of the material. Furthermore, we show the activation of pro-inflammatory pathways involving increased expression of IL-6 and IL-8 genes, analysed by PCR, after treatment with the large GO material. Cellular death was analysed using modified lactate dehydrogenase (LDH) assay and Propidium iodide/Annexin V staining by flow cytometry. A dose-dependent toxicity was observed, with toxic response being more pronounced for the large material. Agglomeration of the GO, modulated by dispersing the material in the presence or absence of serum, was also shown to increase the ROS production, induce pro-inflammatory response and cellular death.
In conclusion, we show that cellular responses to exposure of cells with GO is dependent on the size of the 2-dimensional sheets. Small and well-dispersed GO sheets had no adverse effects on cellular activity. On the other hand, more agglomerated and large GO sheets could induce the production of ROS and subsequently impair cellular activity significantly. The obtained data should assist in “safe-by-design” fabrication of 2D materials in relation to their lateral dimension.

This work was supported by grants from the following projects: EU FP7-ICT-2013-FET-F GRAPHENE Flagship (no. 604391) and “RADDEL” (Marie Curie Initial Training Network (ITN) grant under the EU´s FP7 PEOPLE program).

TRANSLATING REGULATORY REQUIREMENTS INTO CLINICAL PRODUCTS – A CMO PERSPECTIVE

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Over the past few years liposomal drug preparations have been increasingly used in clinical trials. Until now, several liposomal products have reached the market, many other formulations are still in the pipeline. For all these products, simple, economic and GMP-conform production techniques and facilities are necessary.
Here, several points to consider already at the stage of process and product transfer to the CMO should be listed. Product development at early stage should implement the use of high quality raw materials, robust and stable product and process conditions and robust analytical methods. The whole system should be implemented in a robust QA system. Furthermore, the production system should be designed to allow scalable and sterile manufacturing. In addition, it should meet several requirements, such

as simplicity, robustness and easy handling of sterilisation procedures. Furthermore, the modified ethanol injection technique itself is distinguished by mild preparation conditions and the avoidance of hazardous solvents and forces, which may disrupt lipids as well as entrapped substances.
Data will be presented, which describe impact of raw material, process conditions like formulation process and sterilization process as well as optimized storage conditions. In addition, GMP related documentation as well as quality related procedures and documents will be discussed.

OUTCOME-DRIVEN TREATMENT DEVELOPMENT IN COLLABORATION WITH PATIENTS

KAY WARNER

About the development, process for new treatments is expensive due to a number of inefficient steps and its commercial success not sure. Better selection of drugs or products based on an upfront defined, optimal outcome is a recommended strategy. Involving patients into this strategic decision and the development planning and performance is widely debated – but in reality it only works to a very limited degree. There is uncertainty on industry and patient organisation side on how to make this collaboration work. What are the problems and how can these be overcome? This session will present suggestions, experiences and solutions.

Kay Warner: "Creating the framework for efficient industry – patient collaboration".

ABSTRACT:

Better therapies for patients requires a tripartite interaction and It is acknowledged that patients' contribution to the discovery, development and evaluation of medicines enriches quality of research and development, of evidence and opinion and transparency, trust and mutual respect. This can be achieved through close cooperation and partnership between all stakeholders. The EUPATI project has addressed this topic through the publication of a patient involvement roadmap and guidance documents on the interaction of patients in industry-led research. EUPATI program under the leadership of European Patients Forum' continues to drive dialogue in this area, alongside other collaborative projects such as Patients Focused Medicine Development (PFMD). In this presentation you will be provided with an overview of the guidance on operating procedures for patient involvement and recent examples of how through established process GSK has partnered with patients to inform and shape future research.

DRIVING BIOMEDICAL INNOVATION BY ADVANCING REGULATORY SCIENCE AT FDA

FRANK WEICHOLD

Only through applying the best available science can Regulatory Agencies like the US-FDA make sound and responsible regulatory decisions and policies which protect and promote public health. Advancement of the specialized field of regulatory science - i.e. developing and using science based tools, standards and approaches to assess the safety, efficacy, quality and performance of regulated products – offers the opportunity of benefits to all stakeholders in the health industry, particularly when conducted collaboratively with regulatory agency scientists. To mitigate the increasing health care costs and significantly declining R&D venture capital investments in the Bio/Pharma sector (21% decrease in a decade; D. Thomas, C. Wessel 2015) new collaborative and transparent operating models are required where academic institutions are the key interface for regulatory science platforms among government and industry participants to protect patient and consumer interests, and to reduce risks of investments and enhance returns. Federated and independent governance models that foster innovation, broker interests and responsibilities as well as participation and investment into regulatory science among different stakeholders, while protecting scientific integrity and mission of regulatory agencies will be discussed.

ESTHER – A HOLISTIC CONCEPT FOR INCLUSION OF ALL STAKEHOLDERS IN SMART MEDTECH FOR THE BENEFIT OF PATIENTS

KLAUS-MICHAEL WELTRING, Gesellschaft für Bioanalytik Münster e. V., Münster, Germany

The Medtech sector is currently going through a profound transition process from a product based business model of the past towards an integrated care solutions model. The reasons for this transition are the increasing digitization of healthcare and the paradigm shift currently taking place in healthcare from symptomatic treatment of (acute) diseases by blockbusters towards Predictive, Preventive, Personalized, Participatory and Precision medicine. Both will offer new opportunities for patients and the healthcare systems in Europe but also create big challenges for the Medtech industry.
These developments are driven by the convergence of Key Enabling Technologies (KETs) namely nanotechnologies, advanced materials, micro/nano electronics, photonics, biotechnologies, and advanced manufacturing in combination with IT and digital technologies, which will increasingly connect all healthcare sectors and technologies. The marriage of multi-KETs smart medical devices with the Digital Single Market will create new industrial platforms for healthcare characterized by a profound transition in the coming years towards a more collaborative approach of the healthcare industries, namely Pharma, Medtech, IVD, Biotech and Digital Medicine. To establish such new platforms able to master the described complexity of the new smart and connected medical solutions, it is necessary to provide an interface between largely disconnected multibillion euros industries that have very different innovation processes and time frames to work and share technologies together. For example, Medtech is very different from Pharma by the much shorter life cycle of its products, of about 3-5 years vs 10-15 years in Pharma. These differences not only create big challenges for the involved industries but also for the related scientific R&D communities, especially SMEs, which will mainly provide the multi-KETs innovations needed for the digitisation of healthcare.
The Medtech industry has a central role in setting up the new healthcare platforms, since it will not only develop the devices and In-Vitro Diagnostic (IVD) systems ready for integration of digital and IT features of smart and connected medical systems for more personalised diagnostics, but also the delivery systems for the targeted therapeutic approaches developed by the Pharma industry (e. g. Companion products). Due to this central role, the Medtech and IVD industry represented by MedTech Europe has launched an initiative together with the European Commission represented by DG RTD in 2015 called ESTHER.
ESTHER stands for "Emerging and strategic technologies for Healthcare" and represents a European stakeholder platform aiming at:

- Interfacing different science and technology communities to define and agree on cross-KET R&D topics suitable to be integrated with digital components to assemble smart and connected devices and applications
- aligning the R&D topics with industrial strategies and clinical and digital needs
- interfacing different industries to create new business models and value chains (pharma, medtech, IVD, IT and electronics companies)

- supporting SMEs as drivers of innovation in medtech (95% of medtech companies are SMEs)
- sensitising regulators and HTA agencies for new smart and connected applications
- training users (doctors, other care providers) to cope with digital healthcare
- inform patients about the upcoming new smart and connected care solutions to enable them to learn how to benefit from them and to get their feedback to ensure patient approved developments.

The ESTHER stakeholders have already defined a holistic concept from R&D to market access with concrete actions to overpass the silos of technologies (in ETPs), of business models and of industries (Pharma, medtech, Imaging, e-Health), and to interface EU-, national or regional initiatives. By building on the commitment of these stakeholders and by implementing the holistic concept the ESTHER initiative can help to accelerate the implementation of digital and cross-KETs smart and connected medical technologies for the benefit of patients and industries in Europe.

THE HUMAN MACHINE MERGER: ARE WE HEADED FOR THE MMATRIX?

ALON WOLF, Technion I.I.T, Faculty of Mechanical Engineering, Biorobotics and Biomechanics Lab

Modern surgery has developed rapidly with the scientific advances of the past century. From pioneering treatments in the battlefield to the creation of academic surgery, modern advances have ushered in new age for surgical interventions, one that has moved past being regarded as just an accessory to medicine and is instead is so far advanced that it stands in a league of its own. In the old days, surgical procedures were performed by barbers who, between haircuts and bloodletting, also performed invasive procedures that in no way resemble what we consider as surgery today. Those invasive procedures were performed in operating rooms once known as operating theatres. The procedures were often performed in front of a live audience while the patient was awake. Now, just a century later, surgery is performed by trained medical doctors and the old operating theaters have been replaced by sterile rooms where robotic systems often perform part of the operation. Indeed, the operating theater is now referred to as the Operating Room of the Future. In recent years even more advanced and futuristic medical technologies have been developed. These technologies – many of them robotics – enable the surgeon to replace biological parts of the human body with smart machines. This new field of Biomedical engineering research has been named Cybernetics.

We must ask ourselves...

When will the day come where man and machine become one? Or are we there already?

THE MACROPHAGE SWITCH: IMPROVING NANOPARTICLE BIODISTRIBUTION

JOY WOLFRAM, Faculty, Mayo Clinic, Jacksonville, Florida (USA); Affiliate Faculty, Houston Methodist Research Institute, Houston, Texas (USA).

Nanoparticle biodistribution studies have revealed that less than 1% of a systemically injected nanoparticle dose usually ends up in tumors, while up to 99% accumulates in the liver and spleen due to resident macrophages that constitute the mononuclear phagocyte system. An improvement in site-specific delivery could dramatically increase the therapeutic efficacy and safety of nanotherapeutics. The goal of this study was to investigate strategies for pharmacological inhibition of macrophage endocytosis to improve nanoparticle biodistribution. We discovered that the clinically approved antimalarial drug chloroquine suppressed nanoparticle internalization in various macrophage cell lines, while nanoparticle uptake in cancer cells was unaffected in response to treatment. In mice studies, pretreatment with a clinically relevant dose of chloroquine reduced liver and spleen accumulation of soft and hard nanoparticles. Moreover, macrophage preconditioning with chloroquine increased tumor accumulation of liposomes and improved organotropic deposition of silicon particles in the lungs. The novel use of chloroquine as a macrophage-priming agent shows promise as a simple and broadly applicable approach for improving nanoparticle biodistribution. Ultimately, this study defines a paradigm for the combined use of macrophage-preconditioning agents with nanotherapeutics.

COMPUTATIONAL NANOTOXICOLOGY – LESSONS LEARNED FROM THE NANOCOMPUT PROJECT

ANDREW P WORTH, European Commission , Joint Research Centre, Directorate F – Health, Consumers and Reference Materials, Chemical Safety and Alternative Methods Unit incorporating EURL ECVAM, Via Enrico Fermi 2749, 21027 Ispra (Va), Italy

Information on chemical structure can be used to predict the physicochemical and biological properties of chemicals by using quantitative structure-property and structure-activity relationships (i.e. QSPR and QSAR models), or by grouping chemicals based on (structural) similarity and performing read-across between suitable analogues. For “traditional” (soluble) chemicals, these structure-based predictive approaches are well established and are increasingly being used to support the safe design of chemicals and their regulatory assessment. For example, in the European Union, the REACH regulation encourages the use of these predictive approaches as a means of saving resources and avoiding animal testing. However, for particles (including nanoparticles), the development of computational models is still in its infancy, and the regulatory acceptance of such models is hampered by multiple scientific uncertainties. To evaluate the availability and regulatory applicability of computational approaches, and in particular QSPR and QSAR models, the European Commission has carried out a three-year project, Nanocomput, with a view to informing regulatory guidance as well as the direction of future research. This work has been carried out in-house by the Joint Research Centre on behalf of DG Growth. Drawing on the findings of the Nanocomput project, this presentation will reflect on the current status of computational prediction models and will explore the implications for their uptake and acceptance in the safety assessment of nanomaterials.

NEXT GENERATION ANTIBIOTICS

ADA YONATH, Department of Structural Biology, Weizmann Institute, Rehovot 76100, Israel

Resistance to antibiotics and the spread of antibiotics’ metabolites are severe problem in contemporary medicine. In addition to structures of complexes of eubacterial-ribosomes with antibiotics paralyzing them that illuminated common pathways in the modes of antibiotics inhibitions, synergism, differentiation and resistance, recent structures of ribosome from a multi-resistant pathogenic bacteria identified features that can account for species-specific diversity in infectious-diseases susceptibility. Careful analysis and comparisons to ribosomes from benign bacteria indicated novel paths for the design of environmental-friendly degradable, species-specific antibiotics, thus also preserving the microbiome.

CLINAM

European Foundation for Clinical Nanomedicine

10/2017

Basel, May 7–10

CURRICULA VITAE POSTERS

Ibane Abasolo

Ibane Abasolo, obtained the degrees in Biochemistry and Biology from the University of Navarra (Spain) in 1997 and 1998, respectively. During her PhD in the labs of Dr. Alfonso Calvo (CIMA, Pamplona, Spain) and Prof. Zhou Wang (Northwestern University, Chicago, USA), she studied the role of a peptidic hormone, adrenomedullin, in prostate cancer. Afterwards, Dr. Abasolo continued her post-doctoral training in the group of Prof. F.X. Real (IMIM, Barcelona), where she focused on the study of key factors on the progression of pancreatic cancer and cerebellar development. During this time, she gained extensive experience in experimental mouse models, including all the steps from the generation of new transgenic models, to the molecular and cellular characterization of previously existing ones. In 2006 Dr. Abasolo moved to the Insitut d'Alta Tecnologia (PRBB, Barcelona), where she got trained in molecular imaging techniques such as the microPET, SPECT and CT.

Since 2007, Dr. Abasolo is the coordinator of the Area of Functional Validation & Preclinical Research (FVPR) of the CIBBIM-Nanomedicine. Within this area, she is in charge of developing standardized assays for testing the activity and function of candidate genes, target molecules and therapeutic compounds, and providing the industry and other research groups with an optimum technological platform for testing new biomedical applications based on nanotechnology.

Dr. Ibane Abasolo has participated in several international R&D projects (2 Euronanomed projects, 1 ERA-IB, 1 NMP11, 1 Capacitation Program in Nanotechnology) and is currently leading one Euronanomed project (DiamESTar) and is the leader at VHIR of the Smart-4Fabry project (NMBP-10-2016 call) coordinated by the CIBER-BBN. She has also leaded as a PI a project within the national industry-public research cooperation program (INNPACTO-Polysfera) that included the *in vitro* and *in vivo* testing of nanomaterials for cancer treatment. She is also directly dealing with many of the coordinated intramural projects of the Spanish Network for Nanomedicine, Centro de Investigación Biomédica en Red - Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), with the participation of the Drug Delivery and Targeting group. Moreover, as PI, she currently involved in project for targeting breast cancer stem cell using nanotechnology (Marató TV3- Pentri).

RECENT PUBLICATIONS

- Mateo-Lozano S, Bazzocco S, Rodrigues P, Mazzolini R, Andretta E, Dopeso H, Fernández Y, Del Llano E, Bilic J, Suárez-López L, Macaya I, Cartón-García F, Nieto R, Jimenez-Flores LM, de Marcondes PG, Nuñez Y, Afonso E, Cacci K, Hernández-Losa J, Landolfi S, Abasolo I, Ramón Y Cajal S, Mariadason JM, Schwartz S Jr, Matsui T, Arango D. Loss of the EPH receptor B6 contributes to colorectal cancer metastasis. Sci Rep. 2017 Mar 6;7:43702. doi: 10.1038/srep43702. PubMed PMID: 28262839; PubMed Central PMCID: PMC5337985.
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I obtained a BSc in Pharmacology from Al-Quds University, Jerusalem, in 2009. I then worked as a Rheumatology biopharmaceuticals product specialist at Roche Pharmaceuticals in Palestine and I took part in various healthcare projects. I was responsible for sales and marketing of Rheumatology and Virology biological treatments in the Palestinian market. I also worked on establishing pricing strategies and guidelines for new product development. Other responsibilities included managing new product introduction and release activities, I established the Rheumatology biologicals in the Palestinian market and introduced new products to the essential drug list.

In 2013, I completed an MSc in Biopharmaceuticals development at King's College London, United Kingdom. I specialized in the biological aspects of the pharmaceutical Sciences with an emphasis on drug metabolism and biochemical toxicology. My Master degree research focused on studying and investigating delivery systems for gene-targeted therapeutic drugs.

In 2014 I spent six months as a student researcher in bioorganic chemistry Humboldt-Universität zu Berlin, Germany where I worked in both the department of organic chemistry and department of biophysics and I gained experience in the field of DNA modification, synthetic chemistry, and analytical techniques.

For my PhD research at King's College (started at October 2014), I am investigating the use of nanotechnology to develop a smart siRNA delivery systems to aid the treatment of cancer. I'm developing novel particles to overcome the cancer cell barriers for effective gene targeted therapeutics delivery to be used *in vivo*.

AWARDS:

- HESPAL scholarship, British Council scholarships for young Palestinian academics, (UK) 2012
- SALSA research fellowship, German Excellence Initiative, (Germany) 2014
- Faculty for the Future research fellowship, Schlumberger Foundation, (UK) 2014
- Best poster presentation award, British Society of Nanomedicine conference, Swansea (UK 2016

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Leonie Aengenheister studied Forensic Sciences at the University of Applied sciences Bonn-Rhein-Sieg in Rheinbach and Toxicology at the Heinrich-Heine University Düsseldorf in Germany. She gained first research experience in the nanomedical field during her master thesis, where she evaluated the interaction of gold nanoparticles with EGF receptor activity in Caenorhabditis elegans. Currently she is working at the Swiss Federal Laboratories for Materials Science and Technology (Empa) in the Particles-Biology Interaction laboratory and is enrolled as a PhD student at the ETH Zurich. Her aim is the development and use of an advanced *in vitro* placental barrier model to assess nanoparticle translocation in dependence of particle properties and surface modifications.

Wafa T. Al-Jamal

University of East Anglia, School of Pharmacy, Norwich, NR4 7TJ, UK

Dr Wafa Al-Jamal is an overseas and a UK-registered pharmacist. She completed her PhD in Drug Delivery and Nanomedicine in 2008 at UCL School of Pharmacy, London. She is currently a Prostate Cancer Research Fellow at The School of Pharmacy, University of East Anglia (UEA). She joined UEA as a Lecturer in Drug Delivery and Nanomedicine in 2013, after working as a senior research fellow at University College London and King's College London. Wafa's main research interests focus on engineering novel nanomaterials for biomedical applications. Her current research, in Cancer Nanomedicine, aims to design smart vectors to deliver a wide range of therapeutic agents and targeting moieties, and to fabricate multifunctional nanoparticles for combinatory therapy and theranostic applications. Her long-term research career is to facilitate the translation of nanoparticle-based therapeutics from the lab to the clinic. Wafa was the GSK Emerging Scientist Award winner for 2015 for her contribution in the field of Cancer Nanomedicine. She also received Gro Brundtland Award for Women in Sustainable Development for 2017.
Her research group works on developing nanomedicines to target advanced and metastatic prostate cancer. Her multidisciplinary research has been funded by the Royal Society, Prostate Cancer UK, The Engineering and Physical Sciences Research Council (EPSRC), and Rosetrees Trust. She has published over 40 papers in high impact factor journals. Currently, she is a member the Prostate Cancer UK (PCUK) Research Advisory Committee and Visiting Professor at Guizhou Medical School, China.

PUBLICATIONS:

1. Silva V, Al-Jamal WT. Exploiting the Cancer Niche: Tumour-Associated Macrophages and Hypoxia as Promising Synergistic Targets for Cancer Therapy. J Control Release, In press. [IF 7.7]
2. Hodgins NO, Al-Jamal WT, Wang JWT, Parente-Pereira AC, Liu M, Maher J, Al-Jamal KT. 2016. In vitro potency, *in vitro* and *in vivo* efficacy of liposomal alendronate in combination with γδ T cell immunotherapy in mice. J Control Release. 241:229-241 [IF 7.7]
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EDUCATION

- **2010–2016:** 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, Gheybi F, Nikpoor AR, Badiee A, Golmohammadzadeh Sh, Jaafari MR., Therapeutic Efficacy of Cisplatin Thermosensitive Liposomes upon Mild Hyperthermia in C26 Tumor Bearing BALB/c Mice. Molecular pharmaceutics, 2017 Jan (Epub ahead of print).
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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 Pharmaceutical Nanotechnology and Chemical Microsystems (NAMI) Research Unit and Preclinical Drug Formulation and Analysis Group lead by Pro. Hélder Santos, both at the Faculty of Pharmacy, University of Helsinki. In 2013, Patrick Almeida received a grant from the Finnish Cultural Foundation for three years.
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 7 original research articles, 1 review article, 3 book chapters and 1 MSc thesis, as well as 8 conference abstracts, in a total of 20 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.
Additionally, Mr. Almeida was a board member, including vice-chair, of the Finnish Society of Physical Pharmacy committee from 2013-2015, as well as a member of the Finnish Pharmaceutical Society since the year of 2013.

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1 Mohammad-Ali Shahbazi, Patrick Vingadas Almeida, Alexandra Correia, Barbara Herranz-Blanco, Neha Shrestha, Ermei Mäkilä, Jarno Salonen, Jouni Hirvonen, Hélder A. Santos, Taous Khan, "Intracellular Responsive Dual Delivery by Endosomolytic Polyplexes Carrying DNA Anchored Porous Silicon Nanoparticles", J. Control. Release 2017, 249, 111–122.
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3 Mónica P. A. Ferreira, Sanjeev Ranjan, Alexandra M. R. Correia, Ermei M. Mäkilä, Sini M. Kinnunen, Hongbo Zhang, Mohammad-Ali Shahbazi, Patrick V. Almeida, Jarno J. Salonen, Heikki J. Ruskoaho, Anu J. Airaksinen, Jouni T. Hirvonen, Hélder A. Santos, "In Vitro and In Vivo Assessment of Heart-Homing Porous Silicon Nanoparticles", Biomaterials 2016, 94, 93–104.
4 Patrick V. Almeida, Mohammad-Ali Shahbazi, Ermei Mäkilä, Martti Kaasalainen, Jarno Salonen, Jouni Hirvonen, Hélder A. Santos, "Amine-Modified Hyaluronic Acid-Functionalized Porous Silicon Nanoparticles for Breast Cancer Targeting", Nanoscale 2014, 6(11), 10377–10387.
5 Mohammad-Ali Shahbazi, Patrick V. Almeida, Ermei M. Mäkilä, Martti H. Kaasalainen, Jarno J. Salonen, Jouni T. Hirvonen, Hélder A. Santos, "Augmented Cellular Trafficking and Endosomal Escape of Porous Silicon Nanoparticles via Zwitterionic Bilayer Polymer Surface Engineering", Biomaterials 2014, 35(26), 7488–7500.

Aldy Aliyandi

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Aldy Aliyandi is a first year PhD student in Groningen Research Institute of Pharmacy (GRIP) at the University of Groningen, The Netherlands. His scientific research is in the area of nanoparticle-cell interaction and nanomedicine, which mainly focuses on interaction of nanoparticles with endothelial cells.
A native of Indonesia, born in 1991, he obtained his bachelor degree in Pharmaceutical Science and Technology at Bandung Institute of Technology with cum laude predicate (2013, Bandung). His interest in nanomedicine comes primarily from his bachelor thesis, during which he developed nanoemulsion for transdermal vaccine administration. His bachelor project was successfully published in international journal of pharmacy and pharmaceutical science, which was his first international publication. In the same year, he won as 1st winner in Tanoto Student Research Award for a project called "Microemulsion Formulation of Sodium Ascorbil Phosphate as an Anti Ageing Agent". In 2014 he came to The Netherlands and in 2016 he obtained a master degree in Medical and Pharmaceutical Drug Innovation from the University of Groningen. Upon completion of his master study, he performed his master thesis on nanoparticle-cell interaction, during which he started to broaden his knowledge in the biological aspect of nanomedicine. At the end of his master study, he received PhD scholarship from GRIP at the University of Groningen in order to build up his previous work on studying interaction of nanoparticles with endothelial cells.

PUBLICATION:

T. Suciati, A. Aliyandi, and Satrialdi, 2014, Development of Transdermal Nanoemulsion Formulation for Simultaneous Delivery of Protein Vaccine and Artin-M Adjuvant, Int J Pharm Pharm Sci 6(6): 536-546.

Mohamadreza Amin

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The major achievements of my research revolve around liposomes (active and passive tumor targeting, tumor immunotherapy and nasal vaccination). Several years of experience and collaborations with different research groups has provided me a unique knowledge and prospective on different liposomal preparations for different purposes.
Starting in 2004 to present I was involved in variety of projects related to application of liposomes in medical use. I got my Pharm D (2000-2006) after successful defense of my thesis "Formulation and characterization of negatively charged liposomes containing tetanus toxoid and coated with chitosan for nasal immunization" and started my PhD in pharmaceutics (2006-2013). During my PhD, I was working on tumor targeting via passive and active targeting approaches and also immunotherapy of cancer with antigen-loaded liposomes and supervised 5 PharmD thesis. Meanwhile I studied nano materials engineering in college of Ferdowsi University of Mashhad, Iran. Expanded my expertise as a visiting scientist in Laboratory Experimental Surgical Oncology, Erasmus Medical Center, Rotterdam, The Netherlands (2011- 2012). I was awarded as Honored Young Researcher by Iranian Association of Pharmaceutical Scientists in 2012 and finalized my PhD project "Formulation, preparation and characterization of nanoliposomes containing doxorubicin prepared with Disterolphospholipid, DChemsPC,

and targeted with RGD peptides" in collaboration with Francis C. Szoka, from UCSF.
After almost 2 years of mandatory military services I just started my researches in Laboratory Experimental Surgical Oncology in Erasmus MC, as a research scientist and working on strategies to manipulate the protein corona associated with liposomes, immunotherapy of melanoma and modeling of the release kinetics of thermoresponsive liposomes.

PUBLICATIONS:

- Mohamadreza Amin, Mahmoud Reza Jaafari, Mohsen Tafaghodi. Impact of chitosan coating of anionic liposomes on clearance rate, mucosal and systemic immune responses following nasal administration in rabbits Colloids and Surfaces B: Biointerfaces, Volume 74, Issue 1, 1 November 2009, Pages 225-229.
- Mohamadreza Amin, Ali Badiee, Mahmoud Reza Jaafari. Improvement of pharmacokinetic and antitumor activity of PEGylated liposomal doxorubicin by targeting with N-methylated cyclic RGD peptide in mice bearing C-26 colon carcinoma. International journal of pharmaceutics. Volume 458, Issue 2, 31 December 2013, Pages 324–333.
- Mohamadreza Amin, Mercedeh Mansourian, Gerben A. Koning, Ali Badiee, Mahmoud Reza Jaafari, Timo L.M. ten Hagen. Development of a novel cyclic RGD peptide for multiple targeting approaches of liposomes to tumor region. Journal of Controlled Release 220 (2015) 308–315.
- Mohamadreza Amin and Mahmoud Reza Jaafari. Preparation, Characterization, and In Vitro and In Vivo Evaluation of PEGylated Liposomal Doxorubicin Modified with Different cRGD Peptides. Methods in Pharmacology and Toxicology, DOI 10.1007/7653201557 2 (Book chapter)

Anna Balasso

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Since January 2017 Anna Balasso is Post doc at the Dept. of Pharmaceutical and Pharmacological Sciences, University of Padova, working in the group of Drug Delivery. Her research is focused in the development and characterization of novel smart systems for the enhanced delivery of oligonucleotides and small molecules.
She received her Ph.D. in "Pharmaceutical Sciences" in 2017 from the University of Padova where she investigated the use of polysaccharide-based polymer therapeutics for the targeted or controlled drug delivery.
She held the position of associate scientist at University of Helsinki in 2016 where she worked on the development of nanocarriers for drug delivery to the posterior segment of the eye.

PUBLICATIONS

- Balasso A., Salmaso S., Pontisso P., Rosato A., Quarta S., Malfanti A., Mastrotto F., Caliceti P., Re-programming pullulan for targeting and controlled release of doxorubicin to the hepatocellular carcinoma cells, European Journal of Pharmaceutical Sciences, 2017, in press.
- Vila-Caballer M., Codolo G., Munari F., Malfanti A., Fassan M., Rugge M., Balasso A., De Bernard M., Salmaso S., A pH-sensitive stearoyl-PEG-poly(methacryloyl sulfadimethoxine)-decorated liposome system for protein delivery: An application for bladder cancer treatment, Journal of Controlled Release, 238, 2016, 31-42.
- Carta D., Balasso A., Caliceti P, Ferlin M.G., Design, Synthesis, and Photophysical Properties of Pyrroloquinoline-Based Compounds Showing Strong Blue Fluorescence as Potential Dyes for Biomedical Applications, ChemMedChem, 10, 2015, 1846-1862.
- Salmaso S., Bersani S., Scomparin A., Balasso A., Brazzale C., Barattin M., Caliceti P., Journal of Controlled Release, 194, 2014, 168-177
- Salmaso S., Bersani S., Scomparin A., Balasso A., Brazzale C., Barattin M., Caliceti P., A novel soluble supramolecular system for sustained rh-GH delivery, 194, 2014, 168-177.

Marzia Bedoni

Dr. Marzia Bedoni (female) is a senior scientist at the LABION of the FDG (Milan, Italy). She received her MSc degree in Biological Sciences in 2004 (University of Milan). In 2007 she received a PhD degree in Morphological Science at the University of Milan. She carried out her Post-Doctorate at the Fondazione Don Carlo Gnocchi's Nanomedicine and Biophotonics Lab (Milan) granted by the Cariplo Foundation, Seed Capital and europeanFP7. Since 2011 she is permanent researcher in this Foundation. Her research activity is mainly addressed to: biophotonics application (as Raman Spectroscopy) for early diagnosis and therapy monitoring in skin diseases (psoriasis, cancer), leukemia and neurodegenerative diseases; nanoparticles toxicity on human skin; biology of human stratified keratinized epithelia (skin and oral mucosa); biocompatibility in transdermal drug delivery and dry electrodes devices. Lecturer in Human Anatomy for the Faculty of Medicine and Surgery at the University of Milan. She received some awards during international congresses for her research studies. She is author of several peer-reviewed scientific papers, several abstracts for national and international congresses and she is also co-inventor of 4 patents. She is board member of the "Society for Cutaneous Ultrastructure Research (SCUR)", and ordinary member of the "European Society of Dermatological Research" and the "Italian Society of Human Anatomy". She is involved in several national and international projects.

RECENT PUBLICATIONS:

- Morasso, C, Picciolini, S, Mehn, D, Pellacani, P, Frangolho, A, Marchesini, G, Vanna, R, Gualerzi, A, Bedoni, M, Marabelli, F, Gramatica, F. Simultaneous detection of multiple biomarkers by means of SERS on polymer nanopillar gold arrays. Progress in Biomedical Optics and Imaging - Proceedings of SPIE. 2016; 9724, 972404.
- R. Vanna; P. Ronchi; A.T.M. Lenferink; C. Tresoldi; C. Morasso; D. Mehn; M. Bedoni; S. Picciolini; L.W.M.M. Terstappen; F. Ciceri; C. Otto; F. Gramatica

Label-free imaging and identification of typical cells of acute myeloid leukaemia and myelodysplastic syndrome by Raman microspectroscopy. Analyst. 2015;140(4):1054-1064.

- Santini B; Zanoni I; Marzi R; Cigni C; Bedoni M; Gramatica F; Palugan L; Corsi F; Granucci F; Prosperi D; Colombo M. "Cream Formulation Impact on Topical Administration of Engineered Colloidal Nanoparticles". PlosOne.2015;10(5).
- M. Bedoni; C. Morasso; D. Mehn; R. Vanna; C. Pignatari; F. Gramatica. The skin as a spectrum: Raman spectroscopy for in-vivo diagnosis. J Invest Dermatol 2014, 134:4.1176.

Ana Benito

Dr. Ana Benito, Senior Researcher in the Biomaterials Unit at IK4-CIDETEC, obtained her MChem degree in the University of the Basque Country in 1998. She graduated as PhD in Organic Chemistry in the University of the Basque Country in 2004. She worked 9 years in R&D Department of Lilly SA (Alcobendas, Spain) where she specialized in High Throughput Screening (HTS) for hits and leads identification until 2006. Then, she moved to medicinal chemistry group where she specialized in the synthesis of New Chemical Entities (NCE) for lead optimization and candidate selection. Her work was fully dedicated to research transfer into the pharma market. In 2012, she joined IK4-CIDETEC where she worked in the synthesis of single chain polymeric nanoparticles for drug delivery and imaging until 2015, when she started working in NanoPilot project (European Union Framework Programme for Research and Innovation H2020 funded project). Since 2015, she has been fully dedicated to setting-up a pilot plant operating under Good Manufacturing Practice (GMP) for the production of polymer-based nanopharmaceuticals. As a result of her work she has been inventor of 3 patents and is coauthor of 10 **scientific articles**. Some of them:

- "Synthesis and functionalization of dextran-based single-chain nanoparticles in aqueous media". J. Mater. Chem. B, 2017, 5, 1143-1147. DOI: 10.1039/C6TB02773C.
- "Functional Single-Chain Polymer Nanoparticles: Targeting and Imaging Pancreatic Tumors in Vivo". Biomacromolecules, 2016, 17 (10), pp 3213–3221. DOI: 10.1021/acs.biomac.6b00941.
- "Discovery of a Novel Series of Orally Active Nociceptin/Orphanin FQ (NOP) Receptor Antagonists Based on a Dihydrospiro(piperidine-4,7′-thieno[2,3-c]pyran) Scaffold". J. Med. Chem., 2014, 57, 3418−3429. DOI: dx.doi.org/10.1021/jm500117r.
- "Development of LC-MS/MS-Based Receptor Occupancy Tracers and Positron Emission Tomography Radioligands for the Nociceptin/Orphanin FQ (NOP) Receptor". J. Med. Chem., 2012, 55, 4955−4967. DOI: dx.doi.org/10.1021/jm201629q.

Redouane Bouchaala

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Redouane Bouchaala is a Ph.D candidate in Laboratory of Biophotonics and Pharmacology, University of Strasbourg (France). He was born in Algeria; where he studied first optoelectronics engineering in Sétif university, then he got a Master degree in applied optics and photonics. He joined as a PhD candidate the nanochemistry and bio-imaging team led by Andrey Klymchenko in 2013, where he focused in his research work on nanoparticles, by applying a Fluorescence-based approaches to study release of active molecules from lipid nanocarriers in biological media and *in vivo*. During his PhD he managed to master a lot of technics, starting from synthesis and characterization of nanoparticles to fluorescence and correlation spectroscopy. Also microscopy technique like confocal microscopy, STED and small animal microscopy. Along with cell culture and animal experimentation. Now he is working on testing and developing an innovative drug conjugated nanocarriers for tumor xenograft drug delivery.

PUBLICATIONS:

- R. Bouchaala, L. Mercier, B. Andreiuk, Y. Mély, T. F. Vandamme, N. Anton, J. G. Goetz, A. S. Klymchenko, "Integrity of lipid nanocarriers in bloodstream and tumor quantified by near-infrared ratiometric FRET imaging in living mice" J. Controlled Release 2016, 236, 57.
- Mohamed F. Attia, Nicolas Anton, Redouane Bouchaala, Pascal Didier, Youri Arntz, Nadia Messaddeq, Andrey S. Klymchenko, Yves Mély and Thierry F. Vandamme "Functionalization of nanoemulsions with an amino-silica shell at the oil–water interface" RSC Adv., 2015.
- Redouane Bouchaala, Nicolas Anton, Halina Anton, Thierry Vandamme, Yves Mély, Djabi Smail, Andrey S. Klymchenko "Light-triggered release from dye-loaded fluorescent lipid nanocarriers *in vitro* and *in vivo*" submitted

Christos Bikis

Christos Bikis is an MD-PhD student in Experimental Physics at the Biomaterials Science Center of Basel University

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

Tina Bürki-Thurnherr

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Tina Bürki-Thurnherr (1979) studied Biology at the ETH Zurich. During her PhD studies in the lab of Prof. Suter at the ETH Zurich, she contributed to an increased understanding of the molecular processes underlying vertebrate nervous system myelination. After her PhD in neurosciences (2006) she performed her postgraduate studies at the Swiss Federal Laboratories for Materials Science and Technology (Empa) in the field of nanotoxicology. Since January 2015, she holds a group leader position at Empa. Her research interests include the development of advanced barrier models in particular of the human placenta, mechanistic studies on nanoparticle translocation and safety as well as particle-based drug delivery systems.

Marzia Buscema

PhD student at the University of Basel

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

Natalia Calienni

I was born on 10th of June 1991 in Quilmes, Buenos Aires Argentina. I studied Biotechnology at the National University of Quilmes from 2009 to 2014 (Final qualification: 9.07/10.00). I realized a degree thesis in nanotoxicology in zebrafish model. After graduation, I started my PhD. studies in nanomedicine also at the National University of Quilmes (Laboratory of Biomembranes). I am starting my third year of PhD. study "Liposomal formulations of anti-tumoral drugs for topical application: Design and Characterization", with a grant from CONICET (the most important organism of research and science in Argentina). My thesis director is Prof. Jorge Montanari, PhD. and co-director Prof. Silvia Alonso, PhD.

My scientific formation has begun at the Laboratory of Biomembranes in 2012. I have obtained fellowships from Commission for Scientific Research of Buenos Aires (2014 – 2015) for degree student research and from Italian Govern to realize a short stay of three months at the Laboratory of Pharmaceutical Technology, Department of Experimental and Clinical Medicine, University Magna Greece of Catanzaro. Also, I have obtained a financial support from National University of Quilmes to realize this short stay in Italy from March to May 2017.

At the moment, I have three publications in Scientific Journals, two articles recently sent and three in elaboration process (including a review):

-Nanotoxicological and Teratogenic Effects: A Linkage Between Dendrimer Surface Charge and Zebrafish Developmental Stages. Calienni M. N., Feas D. A., Igartúa D. E., Chiaramoni N. S., Alonso S. del V., Prieto M. J. Sent to Small (February 2017).

-Skin penetration and uv-damage prevention by antioxidant nanoberries. Bucci, P.L.; Prieto, M.J.; Milla, L.; Topal, A.E.; Ürel, M.; Calienni, M.N.; Martínez, L.; Alonso, S.; Montanari, J. Sent to Dermatology (January 2017).

-Nutraceutical Emulsion containing Valproic Acid (NE-VPA): A Drug Delivery System for Reversion of Seizures in Zebrafish Larvae Epilepsy Model. Feas D. A., Igartúa D. E., Calienni M. N., Martinez C. S., Pifano M., Chiaramoni N. S., Alonso S. del V., Prieto M. J. Journal of Pharmaceutical Investigation (in press February 2017) DOI: 10.1007/s40005-017-0316-x.

-Development of Nutraceutical Emulsions as Risperidone Delivery Systems: Characterization and Toxicological Studies. Igartúa D., Calienni MN., Feas DA., Chiaramoni N., Alonso S. del V., Prieto M.J. Journal of Pharmaceutical Sciences Online ISSN: 1520-6017, September 2015. DOI: 10.1002/jps.24636.

-Nanoberries: cómo transportar antioxidantes a través de la piel para protegerla de la radiación UV. Bucci, Paula L.; Calienni, Natalia; Alonso, Silvia; Montanari, Jorge A. Revista Cosmética I.S.S.N. 0326-7385, Nº 89 - Vol.30 Nº1 – 2015. Asociación Argentina de Químicos cosméticos.

I participated in fourteen scientific events (national and international) since 2013 and I won the second Carl Zeiss Award with the poster presented in the IILAFeBS, IX IberoAmerican Congress of Biophysics, XLV SAB Annual Meeting (November 2016).

I am student of the Latin American Postgraduate Program of Biophysics (POSLATAM) and the Specialization in university teaching (National University of Quilmes). I realized a total of ten courses related to nanotechnology, nanomedicine, biophysics, ethics, animal histology, microscopy and chromatography. I obtained a fellowship from Argentine-Brazilian Center for Biotechnology to carry out the course "Nanotechnology for innovation in the production of biopharmaceuticals" at the University of São Paulo at Ribeirão Preto, Brazil, in November 2015.

Moreover, I have studied English and Italian at National University of Quilmes and Dante Alighieri of Quilmes, respectively.

I am teacher of Physical-Chemistry in a high school since July 2016 and I am advisor of incoming students of National University of Quilmes since February 2016.

I participated in Scientific Divulgation events and currently I participate in two scientific collaborations with another research groups of Argentina.

Marianna Colasuonno

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I attended the bachelor degree in Medical and Pharmaceutical Biotechnologies in Bari (Italy) from 2009 to 2013. My bachelor thesis was on "Reduction of the effect of incretin drugs after prolonged exposure of pancreatic beta cells to fatty acids: molecular mechanisms".

From 2013 to 2016 I attended the master degree in Medical Biotechnologies and Molecular Medicine in Bari (Italy). During my master degree I attended the course "Optimal Design of Nanoparticles for Biomedical Applications" in which I won a fellowship in Nanomedicine in Houston (USA). I went to Houston Methodist Research Institute in the summer of 2015 and I joined the "Translational Imaging" group of Prof. Paolo Decuzzi. I worked on the synthesis of polymeric nanoparticles as drug (for therapeutic treatment) or contrast agent (for early diagnosis) delivery systems for cardiovascular diseases. At the end of the fellowship I came back to Italy and I went to the Italian Institute on Technology (IIT) in Genoa (Italy) to join the "Laboratory of Nanotechnology for Precision Medicine" of Prof. Paolo Decuzzi. In Genoa I finished my master thesis in "Synthesis and characterization of Discoidal Polymeric Nanoconstructs covered with tissue Plasminogen Activator (tPA-DPNs) for the dissolution of blood clots".

In 2016 I started the PhD course in Sant'Anna School of Advanced Studies in Pisa (Italy) in collaboration with the IIT in Genoa. Now I am working in Genoa with Prof. Paolo Decuzzi on the synthesis of theranostic nanoconstructs for treating and imaging cardiovascular diseases.

Tiziana Di Francesco

Tiziana Di Francesco is a 4th year Ph.D. student at the School of Pharmacy at the University of Geneva, Switzerland. Her research is supervised by Prof. Gerrit Borchard.
Recently she joined the NCTR/ORA Nanotechnology Core Facility at the National Center for Toxicological Research, U.S. Food and Drug Administration in Jefferson (USA) as visiting scientist under the supervision of Dr. Anil K. Patri.
Her research is focused on the development of analytical methods to provide an exhaustive physico-chemical characterization of iron colloid drugs. In particular effectiveness as well as possible differences in terms of efficacy and safety between originator products and so-called similars are evaluated.
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 Biopharmaceutical Sciences Department in Geneva to undertake research for her Masters' thesis, investigating polyelectrolyte nanocomplexes as tools for wound healing.

Daniele Di Mascolo

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In 2010, I graduated in Biological Science (Pathological-molecular curriculum) at the University of Calabria (Arcavacata di Rende, Italy), with an exit mark of 110/100 cum laude. The experimental final Thesis was about Alzheimer's Disease from a human genetics point of view, by the title: "Study of genetic factors affecting the risk to contract cardiovascular diseases in Alzheimer Disease patient. This first laboratory experience trained me in all the basic biological experimental skills.
In 2014, I get a Ph.D. in Biomedical and Computer Engineering at the University of Magna Graecia (Catanzaro, Italy). The scientific subjects concerned polymeric drug delivery system for therapy and imaging, with an experimental final Thesis by the title: "Polymeric Drug Delivery Systems for the Treatment of Pathological Conditions". During the PhD course, I learned also the basic nano- and micro-fabrication processes (Optical lithography, dry and wet etching), as well as optical and electron microscopy, immune histochemistry and flow cytometry.
In 2014 I started a Postdoctoral fellow at The Houston Methodist Research Institute (Houston, Texas, U.S.A.) and currently I hold a Postdoctoral position at the Italian Institute of Technology – Genova – ITALY.
My research activities are mainly focused on nano- and micro-drug delivery systems, mostly polymeric in nature, for the pharmacological treatment of different pathologies, with a particular interest in systems characterization (both for their physico-chemical and for their pharmacological properties) and in their biological efficacy .
Following, some of my more interesting **publications:**

- D. Di Mascolo, C.J. Lyon, S. Aryal, M.R. Ramirez, J. Wang, P. Candeloro, M. Guindani, W.A. Hsueh, P. Decuzzi - "Rosiglitazone-loaded nanospheres for modulating macrophage-specific inflammation in obesity" - Journal of Controlled Release - Volume 170, Issue 3, 28 September 2013, Pages 460–468
- Gizzatov, A., Key, J., Aryal, S., Ananta, J., Cervadoro, A., Palange, A. L., Fasano, M., Stigliano, C., Zhong, M., Di Mascolo, D., Guven, A., Chiavazzo, E., Asinari, P., Liu, X., Ferrari, M., Wilson, L. J. and Decuzzi, P. – "Hierarchically Structured Magnetic Nanoconstructs with Enhanced Relaxivity and Cooperative Tumor Accumulation" - Advanced Functional Materials – 2014
- A.L. Palange, D. Di Mascolo, C. Carallo, A. Gnasso, P. Decuzzi - "Lipid-polymer nanoparticles encapsulating curcumin for modulating the vascular deposition of breast cancer cells" - Nanomedicine: Nanotechnology, Biology, and Medicine - Volume 10, Issue 5, July 2014, Pages 991–1002
- A Lee, D Di Mascolo, M Francardi, F Piccardi, T Bandiera, P Decuzzi – "Spherical polymeric nanoconstructs for combined chemotherapeutic and anti-inflammatory therapies" - Nanomedicine: Nanotechnology, Biology and Medicine 12 (7), 2139-2147
- D Di Mascolo, P Basnett, AL Palange, M Francardi, I Roy, P Decuzzi – "Tuning core hydrophobicity of spherical polymeric nanoconstructs for docetaxel delivery" - Polymer International, 2016/2/1

Simona Dostálová

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Simona Dostalova was born on 2nd of May, 1990. She became a scientific worker in 2011 during her bachelor's studies, focusing on the topic magnetisable nano- and microparticles and their use in isolation of nucleic acids. Her master's education was concluded at Department of Biomedical Engineering, Brno University of Technology in Czech Republic with the thesis focused on Nanocarriers for theranostics. She is currently a postgradual student at Department of Chemistry and Biochemistry, Mendel University in Brno, Czech Republic, focusing on the use of nanocarrier based on ubiquitous protein apoferritin for targeted therapy of cancer.
She has become the receiver of many awards, including 1st instead at The Conference competition XVI. Workshop of Physical Chemists and Electrochemists in Young Scientists session in 2016; The Conference competition MendelNET 2013; or Rector's award for outstanding academic and research results at Brno University of Technology in 2013.
She is the author and co-author of 32 original scientific papers in ISI-indexed journals with a total of 93 citations and h-index H=7 according to Web of Science. Recent publications include:

- DOSTALOVA, S., et al. Apoferritin as an ubiquitous nanocarrier with excellent shelf life. International Journal of Nanomedicine, 2017, vol. 12, in press. ISSN 1178-2013. IF 4.320.
- DOSTALOVA, S., et al., Site-Directed Conjugation of Antibodies to Apoferritin Nanocarrier for Targeted Drug Delivery to Prostate Cancer Cells. ACS Applied Materials & Interfaces, 2016, vol. 8, i. 23, p. 14430-14441. ISSN 1944-8244. IF 7.145.
- BLAZKOVA, I., et al., Apoferritin modified magnetic particles as doxorubicin carriers for anticancer drug delivery. International Journal of Molecular Sciences, 2013, vol. 14, issue. 7, p. 13391-13402. ISSN 1422-0067. IF 2.862.

Andreas Falk

Andreas Falk, MSc (male) is CEO of BNN, studied biomedical sciences, and business administration (University of Graz); was/is part of >15 completed/ongoing national and European projects in the thematic fields of medicine, nanotechnology, nanotoxicology, nano-health, and sensor solutions. He is active within several national and international working groups (member of coordination team of NanoSafety Cluster (NSC), ETP-Nanomedicine (executive board and vice-chair of WG Toxicology&Characterisation) as well as chair of national technology platform NanoMedicine-Austria; member of ETP-SusChem (chair of national technology platform SusChem-AT), NANOfutures (Austrian lighthouse), COST-Actions, chair of industrial innovation liaison-working group of pilot projects/NSC, etc. In the field of nano-safety/nanotoxicology and industrial innovation support, he contributed >70 oral and >20 poster presentations on international scientific conference.

Ralf Friedrich

Dr. Ralf Friedrich is working as a research associate in the Section for Experimental Oncology and Nanomedicine at the Department of Otorhinolaryngology of the University Hospital Erlangen in Germany since 2013 where he is investigating cellular effects of functionalised nanoparticles.

From 2008–2013 he was research associate in the Neuroproteomics group at the Max-Delbrueck-Centrum for Molecular Medicine (MDC) in Berlin, Germany. His research focused on modulators of amyloid formation.

From 2006–2008 he was postdoc in the research group Protein folding and Aggregation at the Leibniz Institute for Age Research in Jena, Germany, where he investigated the mechanism of plaque formation in Alzheimer's disease.

He performed his PhD thesis in 2001–2005 at the Chair for Biochemistry and Pathobiochemistry at the Medical faculty of the Friedrich-Alexander-University of Erlangen-Nuremberg in Germany.

In 2001 he finished his diploma thesis at the Institute for Microbiology, Biochemistry and Genetics at the Friedrich-Alexander-University of Erlangen-Nuremberg in Germany.

From 1996–2001 he studied Biology at the Friedrich-Alexander-University of Erlangen-Nuremberg in Germany.

SELECTION OF PUBLICATIONS

http://www.ncbi.nlm.nih.gov/pubmed/?term=friedrich+rp

1 Friedrich RP, Zaloga J, Schreiber E, Tóth IY, Tombácz E, Lyer S, Alexiou C. Tissue Plasminogen Activator Binding to Superparamagnetic Iron Oxide Nanoparticle-Covalent Versus Adsorptive Approach. Nanoscale Res Lett. 2016 Dec;11(1):297.

2. Hornung A, Pöttler M, Friedrich RP, Weigel B, Duerr S, Zaloga J, Cicha I, Alexiou C., Janko C. Toxicity of mitoxantrone-loaded superparamagnetic iron oxide nanoparticles in a HT-29 tumour spheroid model. Anticancer Res. 36: 3093-3102 (2016).

3. Zaloga J, Pöttler M, Leitinger G, Friedrich RP, Almer G, Lyer S, Baum E, Tietze R, Heimke-Brinck R, Mangge H, Dörje F, Lee G, Alexiou C: Pharmaceutical formulation of HSA hybrid coated iron oxide nanoparticles for magnetic drug targeting. Eur J Pharm Biopharm 101:152-62. doi: 10.1016/j.ejpb.2016.01.017. Epub 2016 Feb 8, 2016

4. Wisotzki EI, Friedrich RP, Weidt A, Alexiou C, Mayr SG, Zink M: Cellular Response to Reagent-Free Electron-Irradiated Gelatin Hydrogels. Macromolecular Bioscience, doi: 10.1002/mabi.201500408, 2016.

5. Friedrich RP, Janko C, Pöttler M, Tripal P, Zaloga J, Cicha I, Dürr S, Nowak J, Odenbach S, Slabu I, Liebl M, Trahms L, Stapf M, Hilger I, Lyer S, Alexiou C: Flow cytometry for intracellular SPION quantification: Specificity and sensitivity in comparison with spectroscopic methods. Int J Nanomedicine, 10:4185-41201, 2015.

Tamás Fülöp

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EDUCATION:

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

TRAININGS:

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

PUBLICATIONS:

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

Iohanan Daniel García Marín

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ohanan Daniel Garcia Marin , native of Mexico City, born in 1994, he obtained his technician degree in clinical laboratory at Centro de Estudios Cientificos y Tecnologicos "Miguel Othon de Mendizabal" (2013, Mexico City) Actually he is a medical student (fifth year) since 2013 and junior researcher since January 2014 at Escuela Superior de Medicina del Instituto Politecnico Nacional" with the support of the program "Beca de Estímulo Institucional de Formación de Investigadores (BEIFI)". He works in the laboratory of Biophysics and Biocatalysis, between his work experience can be mentioned his collaboration in the production of an *in vivo* model for the production of oxidative stress and quantification in rats using scopolamine. This work is published in the article "DETERMINATION OF STRESS OXIDATIVE ON AN ALZHEIMER'S DISEASE LIKE MODEL INDUCED BY SCOPOLAMINE" in the Journal of International Society of Antioxidants in Nutrition & Health (2015) .Most of his experience focuses on the development of in silico studies to evaluate the affinity of new molecules capable of presenting affinity to proteins related to the central pathophysiology of Alzheimer's disease. Their progress can be found published in the article P2-61: IN SILICO EVALUATION AND SYNTHESIS OF NEW MOLECULES AS THERAPEUTIC ALTERNATIVES IN THE ALZHEIMER DISEASE in the Journal of Prevention of Alzheimer Disease (2015) Actually his studies together with his work group focus on the synthesis of the molecules evaluated in silico to be evaluated *in vitro* and *in vivo* in the future, while evaluating new prepositions of molecules that can surpass previous ones.

Hector Garcia Romeu

Hector Garcia Romeu is a first year PhD student at the University of Groningen (Netherlands) studying the internalization and early trafficking of nanoparticles in cells. He obtained his Bachelor's degree in Nanoscience and Nanotechnology at the Universitat Autónoma de Barcelona in 2014. He didhis bachelor's research project at the Institute of Biotechnology and Biomedicine (IBB), Barcelona (Spain), where he studied novel methodologies for the purification and characterization of zebrafish's exosomes (danio rerio).In 2015 he did an internship at IBB where he actively participated in an EXPLORA project: "Development of a novel highly sensitive and interference-free endotoxin detection system using new nanobiomaterials". Finally, in 2015 he was awarded with the MRC Advanced Course Masters Studentship 2015 to follow the Master's Research degree in Translational Medicine at the University of Manchester (UK)where he graduated with distinction in September 2016.

Eduard Gatin

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

Me Eduard Gatin, 1980–1985 Physicist Education, area of research: polymer and materials science, dental materials, calcified tissues and bone tissue regeneration. University of Bucharest, Faculty of Physics. 1990 started as Assist Professor at Faculty of Physics, University of Bucharest.1994– 2000 Doctor in Biology & Physiology (Ph. D degree) and present lecturer. Area of interest, polymer membranes for blood filtration. I continued with research in material science - polymers, advanced Nano materials, ceramics, dental metal alloys, corrosion, dental materials and tissue regeneration. I was integrated for post graduated studies regarding this field, University of Bucharest, Faculty of Physics. Beginning with 2008 I am dedicated to material science related to Medical Field (dentistry, as: dental restoration materials, corrosion, dental enamel quality, tissue regeneration – dentine). From 2010 – present, Lecturer and Associate Professor – Biophyscs Department and Dental Materials from University of Medicine "Carol Davila", Faculty of Dentistry. 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 skills: RAMAN spectroscopy (improved by SERS), SEM, EDX. Between 2010 – 2013, I was postdoctoral student in EU Program PostDoc (to improve research work, EU finance support).
In 2013 it was proposed a method for quality evaluation of dental enamel by Raman method, to be applied " in vivo". On 30th October 2015, patent registration certificate was issued under Nr RO 2013 00043.
On Scopus is available the complete list of publications with Impact Factor, containing 16 publications (largest part of them is focussed on medical dentistry; an important paper was published in 2015, available on Particulate Science and Technology, Vol 33, Issue 4, 2015, pg 429 – 435; another paper entitled "Raman spectroscopy, a sensitive method for bone quality evaluation applied to periodontal patients", Clinical and Experimental Dental Research, 2017 – in press.
Regarding international meetings, I must list EMRS 2012, 2013, 2014, 2015 (invited presentation to Symposium V Bioinspired and biointegrated materials) and CLINAM 2014, 2015, 2016; EMRS 2016, Warsav Meeting (invited presentation).
Is in progress the study " Introducing of RAMAN technique to Periodontology", according bioethical approval dated January 2016, with Semmelweis University Budapest – Faculty of Dentistry, Hungary; the study is improved, with a new step: in vivo evaluation, start up spring 2017.

Gabriela Gerganova

University of Basel, Department of Biomedical Engineering
Biomaterials Science Center
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Gabriela Gerganova, 23 years old, is an Integrated Masters student of Pharmacology at University of Glas-

gow, Scotland. She performs her one-year work placement at the University of Basel, Department of Biomedical Engineering in the group of Prof. Bert Müller. Her research focus is on the formulation, characterization and safety of liposome-based nanocontainers for targeted drug delivery. For the realization of her project, she collaborates with several institutes in Switzerland and abroad: University of Fribourg, Department of Chemistry, group of Prof. Andreas Zumbühl; University of Basel, Pharmazentrum, Pharmaceutical Technology group of Prof. Jörg Huwyler; Semmelweis University, group of Prof. Janos Szebeni. In addition to her main life sciences project, she is working on characterizing the wetting behavior of nanostructured polydimethylsiloxane surfaces by means of contact angle goniometry. During her internship, she has expanded her practical skills in lab experimental work, data analysis and polymer films preparation with the help of on-site laboratory facilities.

Simone E. Hieber

Dr. Simone E. Hieber is a research group leader at the Biomaterials Science Center, University of Basel. She was educated in various disciplines in three countries. She gained a diploma in engineering cybernetics from the University of Stuttgart, Stuttgart, Germany, a master's degree in mathematics from the Michigan Technological University, Houghton, MI, USA, and a PhD in computer science from ETH Zurich, Zurich, Switzerland. During her education she focused on projects in computational modeling and simulations of various systems, ranging from nonlinear turbo charger over metabolic networks and stochastic sprays to large-scale simulations of a swimming fish. After graduation she coordinated the Swiss-wide PhD training network of the Swiss Institute of Bioinformatics. Moreover, she has been supervising the PhD studies in biomedical engineering at the University of Basel.
Since 2012 she has been leading the research group Computational Analysis of Tissues in Health and Disease at the Biomaterial Science Center. The main focus of research includes investigating characteristic parameters of soft and hard tissues based on high-resolution X-ray images. Disease currently considered are arthrosclerosis, epilepsy, stroke and cancer.
She has published more than 30 peer reviewed scientific articles as well as several reviews and book contributions. She received several academic awards including one for the best oral presentation at Bernd Spiessl Symposium in Basel, Switzerland. Dr. Simone E. Hieber is married and has one daughter.

Yang Hu

PhD student
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Translational PKPD research group
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EDUCATION BACKGROUND

- Huazhong University of Science and Technology, Wuhan, China Bachelor's Degree, Pharmacy, 2007.09 – 2011.06
- Huazhong University of Science and Technology, Wuhan, China Master's Degree, Pharmaceutics, 2011.09 – 2014.06
- Uppsala University, Uppsala, Sweden, Translational PKPD research group, 2014.09 – now

RESEARCH EXPERIENCE

- Master thesis: Pharmacokinetics and pharmacodynamics of TJ0711 hydrochloride, a novel antihypertensive drug in beagle dogs. Supervised by: Prof. Gao Li
- Other participated projects during Master period: Clinical bioequivalence study of amlodipine. Pharmacokinetic study of clevidipine in beagle dogs.
- Doctoral project: Quantitative aspects of liposomal drug delivery across the blood-brain barrier (BBB). Supervised by: Prof. Margareta Hammarlund-Udenaes.

FEATURED SKILLS

LC-MS/MS; Bioanalysis; Pharmacokinetics; Blood-Brain Barrier; Microdialysis; Liposomes

PUBLICATION

Hu Y, Rip, J, Gaillard PJ, De Lange EC, M Hammarlund-Udenaes*, The Impact of Liposomal Formulations on the In Vivo Release and Brain Delivery of Methotrexate: A Microdialysis Study, submitted.

Magdalena Janczewska

Laboratory of Biomedical Engineering, Faculty of Chemical and Process Engineering, Warsaw University of Technology, Warsaw, Poland;
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Since 2013 pursuing PhD studies in Laboratory of Biomedical Engineering working on polymeric nanoparticles for theranostics of cancer using radioactive isotopes. In 2013 – 2015 part of the research team of NanoVelos company working on targeted chemotherapy were she gained experience and was responsible for synthesis and characterization of nanoparticles. Since 2015 CEO of NanoThea Inc. – spin off aiming to commercialize application of polymeric nanoparticles with radioisotopes developing diagnostics for PET and PET/MRI.
Magdalena took part in numerous grants such us EuroNanoMed II project "Fluorescent organic nanocrystals for diagnostics of esophageal and colon cancer" and is project manager of projects: Biopolimer Nanoparticles for early cancer diagnostics and Nanoparticles for precise diagnostics and brachytherapy of prostate cancer – currently being carried out in NanoThea company.

PUBLICATIONS:

- I.Wasiak, A.Kulikowska, M.Janczewska, M.Michalak, I.A.Cymerman, A.Nagalski, P.Kallinger, W.W.Szymański, T.Ciach: Dextran Nanoparticle Synthesis and Properties; PLOS ONE 11 (1), 2016
- K.Jabłczyńska, M.Janczewska, A.Kulikowska, T.R.Sosnowski: Preparation and Characterization of Biocompatible Polymer Particles as Potential Nanocarriers for Inhalation Therapy , International Journal of Polymer Science, Vol 2015,

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 and immunological analyses of nanoparticles for medical applications.

Olivier Jordan

Olivier Jordan is Senior Lecturer at the School of Pharmacy, University of Geneva. He graduated in engineering and received his PhD from EPFL working on the micro-encapsulation of insulin-secreting cells for the treatment of diabetes. In the team of Prof Aebischer at the Lausanne University Hospital, he focused on biomaterials engineering for cartilage and nerve prosthesis. Moving to the School of Pharmacy at Geneva University, he developed projects in the field of novel delivery carriers for drugs, protein and therapeutic heat, based on in situ forming implants, nano- or microparticles. He has strong interests in the field of interventional oncology, e.g. using drug-eluting embolization materials to treat liver cancer through anti-angiogenic strategies, or delivering superparamagnetic iron oxide nanoparticles formulations for tumor thermotherapy. He is the author of 55 peer-reviewed publications and holds 8 patents.

RECENT PUBLICATIONS:

- Mylonaki I, Allémann E, Saucy F, Haefliger JA, Delie F, Jordan O. Perivascular medical devices and drug delivery systems: Making the right choices. Biomaterials, in press, 2017
- Kulcsár Z, Karol A, Kronen PW, Svende P, Klein K, Jordan O, Wanke I. A novel, non-adhesive, precipitating liquid embolic implant with intrinsic radiopacity: feasibility and safety animal study. Eur Radiol 27(3): 1248–1256 (2017)
- Mylonaki I, Strano F, Deglise S, Allémann E, Alonso F, Corpataux JM, Dubuis C, Haefliger JA, Jordan O, Saucy F, Delie F. Perivascular sustained release of atorvastatin from a hydrogel-microparticle delivery system decreases intimal hyperplasia. J Control Release 232:93-102 (2016)
- P. Bize, Rafael Duran, Katrin Fuchs, O. Dormond, L. Decosterd, O. Jordan, A. Denys. Antitumor effect of sunitinib-eluting beads in the rabbit VX2 tumor model. Radiology 280(2):425-435 (2016)
- O. Sakr, S. Berndt, G. Carpentier, M. Cuendet, O. Jordan, G. Borchard. Arming Embolic Beads with Anti-VEGF Antibodies and Controlling their Release Using LbL Technology, J Controlled Release, 224:199-207 (2016)

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EDUCATION:

Doctor of Philosophy (Drug Delivery): 2013 – Date
University of Nigeria, Nsukka.
Master of Pharmacy (Pharmaceutics): 2009 – 2012
University of Nigeria, Nsukka.
Bachelor of Pharmacy (Pharmacy): 2002 – 2007
University of Nigeria, Nsukka.

PROFESSIONAL EXPERIENCE:

2015 – Date: Lecturer I, Department of Pharmaceutics, University of Nigeria, Nsukka, Enugu State.
2012 – 2015: Lecturer II, Department of Pharmaceutics, University of Nigeria, Nsukka, Enugu State.
2010 – 2012: Graduate Assistant, Department of Pharmaceutics, University of Nigeria, Nsukka, Nigeria.
2010 –2011: Superintendent Pharmacist, DABAK NIG. LTD., Sagamu, Ogun State, Nigeria
2009 – 2010: National Youths Service Pharmacist, Ngala General Hospital, Borno State, Nigeria.
2008 – 2009: Pupil Pharmacist, Department of Pharmaceutics, University of Nigeria, Nsukka, Nigeria.

AWARDS AND HONOURS:

Overall Best Graduating Student, Department of Clinical Pharmacy & Pharm. Management, UNN: 2006/07 session.
Overall Best Graduating Student, Awlaw High School, Awlaw, Oji-River L.G.A., Enugu State: 1996 Session.
Overall Best Graduating Pupil, Agbada Primary School, Etiti Awlaw, Oji-River L.G.A., Enugu State: 1993 Session.

PUBLICATIONS:

- Attama, A.A., Nnamani, P.O., Kenechukwu, F.C., Odimegwu, D.C.. (2016). General Dispensing Considerations. In: Basic Laboratory Dispensing Techniques (eds. Ibezim, E.C. and Ofokansi, K.C.). University of Nigeria Press Ltd. Nsukka, pp. 1-8.
- Akpa, P.A., Kenechukwu, F.C., Onugwu, A.L., Agbo, C.P. (2016). Dispensing of solid dosage forms. In: Basic Laboratory Dispensing Techniques (eds. Ibezim, E.C. and Ofokansi, K.C.). University of Nigeria Press Ltd. Nsukka, pp. 31-38.
- Ogbonna, J. D. N., Kenechukwu, F. C., Chime, S. A., Attama, A. A. (2016). Cellulose-Based Biopolymers: Formulation and Delivery Applications. In: Encyclopedia of Biomedical Polymers and Polymeric Biomaterials. Handbook of encapsulation and controlled release, Munmaya Mishra (Ed.), Taylor and Francis: New York, USA, Published online: 26 Jan 2016; 1378-1408. http://dx.doi.org/10.1081/E-EBPP-120050066.
- Chime, S. A., Kenechukwu, F. C., Attama, A. A. (2014). 'Nanoemulsions- Advances in formulation, characterization and applications in drug delivery' in: Application of Nanotechnology in Drug Delivery. INTECH Publishers, pp.77-126. http://dx.doi.org/10.5772/15371.
- Okechukwu, D.C., Akpa, P.A., Kenechukwu, F.C., Nnamani, P.O. (2014). Aseptic transfer and processing. In: Laboratory Techniques in Basic Pharmaceutical Microbiology (eds. Okore, V.C. and Attama, A. A.). Praise House Pub. Enugu, pp. 12-17.

Kerda Keevend

Kerda Keevend is currently a PhD student in Swiss Federal Laboratories for Materials Science and Technology (Empa) and ETH Zürich. She obtained her BSc and MSc degree in chemistry from University of Tartu in 2015, focusing on synthesis and characterization of luminescent nanoparticles. In 2016 she joined the group of Dr. Inge Herrmann in Empa. Since then she has been working on correlative bioimaging, including synthesizing imaging probes for cathodoluminescence imaging.

Anna Khimchenko

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

Ida Kokalari

Ida Kokalari was born in 1989. She graduated in Pharmacy in 2015 at the University of Torino, Italy. During her PG thesis she spent six months as a master student in the laboratory of Prof. Claus-Michael Lehr at University of Saarland and Helmholtz Institute for Pharmaceutical Research, Saarbrueken, Germany with a project entitled "Formulation and Characterisation of Poly(D,L–lactide-co-glycolide) nanoparticles containing Gentamicin". This period was supported by the EU Erasmus Programme.
In October 2015, she started her PhD course in Molecular Medicine, Doctoral School in Life and Health Sciences, at the University of Torino under the supervision of Prof. Ivana Fenoglio at the Department of Chemistry. The PhD project is entitled "Evaluating the efficacy of inorganic nanoparticles for photothermal and photodynamic therapies". Her PhD project aims at investigating the photo-thermal, photo-chemical and antioxidant properties of newly synthesized graphitic carbon or carbon-based nanoparticles and at developing them as possible candidate for photo-thermal and photo-dynamic therapy of cancer and other diseases.

PUBLICATIONS

- Riccardo Gassino, Ida Kokalari, Alberto Vallan, Ivana Fenoglio and Guido Perrone; "A compact diode laser based all-fiber delivery system for PDT+PTT with integrated temperature sensing capabilities ", Proc. SPIE 10047, Optical Methods for Tumor Treatment and Detection: Mechanisms and Techniques in Photodynamic Therapy XXVI, 100470G (February 8, 2017); doi:10.1117/12.2254796; http://dx.doi.org/10.1117/12.2254796

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 postdoctoral 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.

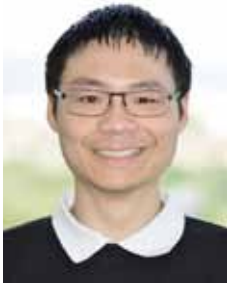
Praneeth Kuninty

Praneeth Kuninty was born in Adilabad, Telangana, India. He obtained his Bachelor of Pharmacy in 2010 from J.S.S College of Pharmacy, Mysore. In 2013, he graduated from the University of Groningen, The Netherlands with a Master in Medical Pharmaceutical Sciences. He did his first master project on the "Characterization of Inulin Nanocrystals". Later he did his second master project on the "SAINT-Lipid Polycation particles, a novel carrier for an improved delivery of siRNA to activated primary endothelial cells". Since July 2013, he is working towards his Ph.D. in the BST group on a novel targets in pancreatic tumor stroma and targeted delivery of small RNAs for pancreatic cancer therapy under the guidance of Dr. Jai Prakash.

LIST OF PUBLICATIONS:

- Kuninty PR, Bojmar L, Tjomsland V, Larsson M, Storm G, Östman A, Sandström P, Prakash J. "MicroRNA-199a and -214 as potential therapeutic targets in pancreatic stellate cells in pancreatic tumor" Oncotarget, 2016 Accepted
- Kuninty PR, Schnittert J, Storm G, Prakash J. "MicroRNA Targeting to Modulate Tumor Microenvironment". Frontiers in Oncology (Invited review) 2016 Accepted
- Kowalski PS, Kuninty PR, Bijlsma KT, Stuart MC, Leus NG, Ruiters MH, Molema G, Kamps JA. "SAINT-liposome-polycation particles, a new carrier for improved delivery of siRNAs to inflamed endothelial cells". Eur J Pharm Biopharm. 2015 Accepted
- Schnittert J, Kuninty PR, Bystry TF, Brock R, Storm G, Prakash J. "Self- assembling peptide nano-complexes to target microRNA to stellate cells in pancreatic tumor stroma". Under review
- Carina Strell, K. Jessica Norberg, Artur Mezheyeuski, Jonas Schnittert, Kuninty PR, Carlos Fernandez Moro, Janna Paulsson, Nicolai Aagaart Schultz, Dan Calatayud, J.-Matthias Löhr, Caroline Sophie Verbeke, Rainer Lothar Heuchel, Jai Prakash, Julia Sidenius

Johansen and Arne Östman. “Stroma-regulated HMGA2 is an independent prognostic marker in PDAC and AAC”. Under review



Willy Kuo

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After being born in Bern, Switzerland on the 14th of February 1988, I attended school and high-school in Zurich. Afterwards, I did my Bachelor’s and Master’s at ETH Zurich in Interdisciplinary Natural Sciences, with the focus on biology and chemistry. This has equipped me to perform biology with an awareness of the properties and reactions of the compounds involved, or chemistry with the needs and constraints of biology in mind. These skills led me to my master’s thesis topic in developing X-ray contrast agents for imaging tissue engineering scaffolds under Dr. Kathryn Stok.
Following on that, I’m working on my PhD thesis at the University of Zurich under Prof. Dr. Vartan Kurtcuoglu and Prof. Dr. Bert Müller. Continuing development of X-ray contrast agents, I’m working on the sample preparation, image acquisition and image processing protocols for multi-modal X-ray micro-CT and 3D light microscopy scanning in whole mouse kidneys. The goal of the project is to build the first atlas of the complete vascular and tubular microstructure of the entire mouse kidney, and combine this with functional data derived from mouse models. These data will be made freely available online in both raw and analyzed forms.

Rachita Lahri

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EDUCATION:

- PhD – King’s College London (2014–2018)
  I am currently a third year PhD student at King’s college London. My project is about understanding the benefits and effects of nanoparticle assisted microwave imaging.
- Master in Research (MRes) in Cancer Biology from Imperial College London, (Merit), (2012–2013)

Before starting my PhD I completed Master in Research (MRes) in Cancer Biology from Imperial College, London – it was a fulltime full-time research-oriented course providing broad training in research in Cancer Biology. During the course, I got an opportunity to experience cutting edge research in the field of cancer. The course gave me good exposure to research and theoretical aspect of cancer biology. For the fulfilment of MRes degree requirement, I worked on two separate 19-week research projects under supervision of experienced clinical researchers and PhD students. My first project was about ‘Describing the role of OPCML in epithelial to mesenchymal transition in epithelial ovarian cancer’. The second project, on which I worked, was titled as ‘Epigenetic changes characterisation of arsenic-induced cellular transformation of the normal urothelial UROtsa cell-line’.

- BSc(Hons) in Chemistry with Biochemistry from Queen Mary, University of London, (2,1), (2009–2012)

WORK EXPERIENCE:

- Worked at Lampton High School in London for 3 weeks under SAS (Student Associate scheme):
- Assisted teachers in taking classes for students of year 7–11.
- Advised student on preparing their university application
- Worked at Primary School with Disabled students at Jaipur, India for 5 weeks – This role left me with a strong sense of responsibility towards other people, and underscored for me the importance of body language in nurturing positive communication between people
- Worked at a Care Home for a week where I Helped disabled people with their daily activities.
- Worked with a GP – This experience proved to be highly valuable and has helped me enhance my communication skills, in particular my listening skills.

PUBLICATIONS:

- Rahman. S, Lahri. R, Dabrowska. A and Hajji, N. miR-372 mediated attenuation of PCAF in arsenic trioxide induced cellular transformation. Molecular Cancer. [Manuscript in preparation]

Eliana M. Lima

Ph.D.
Full Professor or Pharmaceutical Nanotechnology, Universidade Federal de Goias, Brazil.

Eliana M. Lima is a professor of Pharmaceutical Nanotechnology at the School of Pharmacy, Federal University of Goias, in the central region of Brazil. Her work is focused in use of nanostructured drug delivery systems to improve therapeutic outcomes of antitumor, antimicrobial and anti-inflammatory drugs. Her research group has particular interest in the characterization of the nanocarrier systems, including monitoring drug-lipid or drug polymer interactions and nanoparticle-cell interactions. She has been able to show that the mechanism of drug-nanoparticle interaction has an important role in the cellular internalization of the drug, with impact on bioavailability. By investigating cellular behavior, Prof. Lima and her co-workers have demonstrated distinct toxicological profiles attributed to drug nanocarriers and their structural components. During the past decade, several new products have been developed or are in the late stages of development in her laboratory in partnership with pharmaceutical companies.

- SALVADOR, M. A.; COSTA, A. S.; GAETI, M. N.; LIMA, E. M.; A. F. Bakuzis; MIOTTO, R. . Characterization, nanoparticle self-organization, and Monte Carlo simulation of magnetoliposomes. PHYSICAL REVIEW E, v. 93, p. 022609, 2016.
- Gaeti, Marilisa Pedroso Nogueira; Benfica, Polyana Lopes; MENDES, L. P.; VIEIRA, M. S.; ANJOS, J. L. V.; ALONSO, Antônio; REZENDE, K. R.; VALADARES, Marize Campos; Lima, Eliana M. . Liposomal entrapment of 4-nerolidylcatechol: impact on phospholipid dynamics, drug stability and bioactivity. Journal of Nanoscience and Nanotechnology (Print), v. 15, p. 838-847, 2015.
- MENDES, LÍVIA PALMERSTON; DELGADO, JORGE MIGUEL FERREIRA; COSTA, ANGELA DANIELA A; VIEIRA, MARCELO SOUSA; BENFICA, POLIANA LOPES; VALADARES, Marize Campos; Lima, Eliana Martins . Biodegradable nanoparticles designed for drug delivery: the number of nanoparticles impacts on cytotoxicity. Toxicology in Vitro, v. 29, p. 1268-1274, 2015.
- MENDES, LÍVIA PALMERSTON; Gaeti, Marilisa Pedroso Nogueira; DE ÁVILA, PAULO HENRIQUE MARCELINO; de Sousa Vieira, Marcelo; DOS SANTOS RODRIGUES, BRUNA; DE ÁVILA MARCELINO, RENATO IVAN; DOS SANTOS, LÍLIAN CRISTINA ROSA; VALADARES, Marize Campos; Lima, Eliana Martins . Multicompartimental Nanoparticles for Co-Encapsulation and Multimodal Drug Delivery to Tumor Cells and Neovasculature. Pharmaceutical Research, v. 31, p. 1106-1119, 2014.

Jasna Lojk

I'm a postdoc researcher in the fields of nanotechnology and nanomedicine in the Group for nano- and biotechnological applications at the Faculty of Electrical Engineering (University of Ljubljana, Slovenia). In 2015, I finished my PhD in the field of nanoparticle-cell interactions and continued working on analysis of toxicity of different relevant biomedical and industrial nanoparticles, now focusing on the responses of innate immune system to nanoparticle exposure *in vitro*, with some results already published in peer reviewed journals. We also focus on nanoparticle neurotoxicity and the ability of nanoparticles to affect naurodegeneration *in vitro*.

SELECTED PUBLICATION:

- Jasna Lojk, Vladimir Boštjan Bregar, Maruša Rajh, Katarina Miš, Mateja Erdani Kreft, Sergej Pirkmajer, Peter Veranic, Mojca Pavlin. Cell Type Specific Response to High Intracellular Loading of Polyacrylic Acid Coated Magnetic Nanoparticles. International Journal of Nanomedicine, 10 (2015):1449-62.
- Vladimir B. Bregar, Jasna Lojk, Vid Suštar, Peter Veranič, Mojca Pavlin. Visualization of Internalization of Functionalized Cobalt Ferrite Nanoparticles and Their Intracellular Fate. International Journal of Nanomedicine 8 (2013): 919–31.
- Jasna Lojk, David Karlaš, Luka Šajn, Uroš Čibej, Mojca Pavlin. Comparison of two automatic cell-counting solutions for fluorescent microscopic images. Journal of Microscopy, 260 (2015): 107-116.

Valeria Lusi

Current position: PhD student in Bioengineering and Robotics, curriculum Bionanotechnology, at Italian Institute of Technology; dept. of Nanotechnology for Precision Medicine, Genoa.

Research activity: 'Molecules and nanoparticles diffusion studies in different complexity degree extracellular matrix model'.

RESEARCH EXPERIENCE

- MSc Internship at Italian Institute of Technology; dept. of Nanotechnology for Precision Medicine, Genoa- Sept 2015 – Sept 2016. **Research activity:** 'Complete nano and micro fabrication process of silicon template for micro and nanoparticles'.
- Work experience at Italian Institute of Technology, Genoa; dept. of Nanotechnology for Precision Medicine, Genoa- Jul 2015-Aug 2015. **Research activity:** 'Microparticles synthesis and molecules diffusion studies in collagen gel'.

EDUCATION

- Master Degree in Chemical Engineering (University of study of L'Aquila) – Oct 2016 (110/110 cum laude); Title: Multiscale transport of molecules and nanoparticles in cancerous tissues: experimental and modeling.
- Bachelor Degree in Chemical Engineering (University of study of L'Aquila) – Dec 2011 (102/110); Title: Sizing a water purification plant to service of 1500 equivalent inhabitants village.
- Scientific High School Diploma–Jul 2007, Liceo scientifico Marco Vitruvio Pollione Avezzano, (AQ)

CONFERENCE PARTICIPATION

- Nanohealt seminar: focus on oncology; congress center Federico II , Naples- Apr 2014; Main topics: nanotechnology in oncology, treatment of metastatic breast cancer with nab-paclitaxel, new scientific evidence of nab paclitaxel.
- Italian Association for Cancer Research (AIRC) conference, Bologna – Nov 2013

Stefan Lyer

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.

Abhik Mallick

Indian Institute of Science Education and Research (IISER), Pune- 411008 (India)
E-Mail: abhikm019@gmail.com, abhik.malik@students.iiserpune.ac.in

EDUCATIONAL QUALIFICATION:

- Indian Institute of Science Education and Research (IISER), Pune; Ph.D. Chemistry, thesis to be submitted (2017).
- University of Delhi (Hansraj College); M.Sc in Chemistry, 2011.
- University of Calcutta (Scottish Church College); B.Sc. Chemistry (Hons.), 2009.

AWARD/HONOR:

2011 Qualified National Eligibility Test (NET) conducted by Council of Scientific and Industrial Research (CSIR) with rank 70

RESEARCH EXPERIENCE:

- Chemical Synthesis and Characterisation of Nanomedicine for Cancer Therapy
- Biological eperiments (invitro assays) for Cancer therapy

PUBLICATIONS (DURING PH.D):

- Cisplatin-induced self-assembly of graphene oxide sheets into spherical nanoparticles for damaging sub-cellular DNA
- Aqueous phase sensing of cyanide ions using a hydrolytically stable metal–organic framework
- Engineering and In Vitro Evaluation of Acid Labile Cholesterol Tethered MG132 Nanoparticle for Targeting Ubiquitin-Proteasome System in Cancer

- Nanoparticle Mediated Mitochondrial Damage Induces Apoptosis in Cancer
- Dual drug conjugated nanoparticle for simultaneous targeting of mitochondria and nucleus in cancer cells

CONFERENCES:

- International Conference on Nanoscience and Technology (ICONSAT) at IISER Pune, India, February 2016
- Peptide Engineering Meeting (PEM-7) at IISER Pune, India, December 2015
- IUPAC's International Symposium on Bio-Organic Chemistry (ISBOC-10) at IISER Pune, India, January 2015
- International Meeting on Chemical Biology (IMCB-2013) at IISER Pune, India, May 2013
- Workshop on Advances in Biomaterials and Nanobiotechnology at ICT Matunga, India, November 2013

Jasmin Matuszak

IJasmin Matuszak studied Biology at the Friedrich-Alexander University Erlangen-Nürnberg, Germany and obtained her master degree in Cell and Molecular Biology in 2013. In the same year she startet with her doctoral thesis in the lab of Prof. Chrsitoph Alexiou, the Section for Experimental Oncology and Nanomedicine (SEON) at University Hospital Erlangen. Her doctoral thesis deals with the topic of cardiovascular nanomedicine, precisly with the development and testing of possible nanoparticle systems for the treatment or diagnosis of atherosclerotic plaques. During this time she gained research experience in the field of atherosclerosis, nanotechnology and nanomedicine

Sofiya Matviykiv

Jasmin Matuszak studied Biology at the Friedrich-Alexander University Erlangen-Nürnberg, Germany and obtained her master degree in Cell and Molecular Biology in 2013. In the same year she startet with her doctoral thesis in the lab of Prof. Chrsitoph Alexiou, the Section for Experimental Oncology and Nanomedicine (SEON) at University Hospital Erlangen. Her doctoral thesis deals with the topic of cardiovascular nanomedicine, precisly with the development and testing of possible nanoparticle systems for the treatment or diagnosis of atherosclerotic plaques. During this time she gained research experience in the field of atherosclerosis, nanotechnology and nanomedicine.

Tamás Mészáros

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

Gergely Milosevits

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

Hilaria Mollica

E-mail: hilaria.mollica@iit.it/hilaria.mollica@hotmail.it

WORK EXPERIENCE

Nov 2016: PhD student in Bionanotechnology at Italian Institute of Technology, Genova (IT)
Department of Drug Discovery and Development, Laboratory of Nanotechnology for Precision Medicine: Research in cells-to chip and cells-to-cells technologies, dynamics of cells in the chip, microfluidic application and biomaterials
Apr 2016–Oct 2016: Research Fellow at Italian Institute of Technology, Genova (IT);Department of Drug Discovery and Development, Laboratory of Nanotechnology for Precision Medicine: Research in microfluidic application, chip characterization and organ on a chip technology
Sep 2014–Jul 2015: Intern at San Giuseppe Moscati Hospital, Avellino (IT); Department of Hematology and Biochemistry
Mar 2014–May 2014: Short term scientific mission-COST (European cooperation in science and technology) "Institute for Physiological Chemistry and Pathobiochemistry", Münster (DE). Research project: "Investigation of the integrin α5β1 as molecular target of the anti- metastatic metal-based compound NAMI-A in a colorectal cancer (CRC) progression model *in vitro*"
Nov 2012–Giu2014: Intern at Callerio Foundation ONLUS, Trieste (IT);Research project: "Influence of the microenvironment during hepatic metastasis of colorectal cancer"

EDUCATION

Dec 2015: Professional Biologist qualification, University of Benevento (IT)
Mar 2012–Oct 2013: Master in Functional Genomics, University of Trieste (IT); Degree class LM/6
Feb 2012: Bachelor in Biology, University of Napoli "Federico II", Napoli (IT); Degree class L-12

PUBBLICATIONS

- Chiara Pelillo, Alberta Bergamo, Hilaria Mollica, Marco Bestagno, Giovanni Sava. "Colorectal Cancer Metastases Settle in the Hepatic Microenvironment Through α5β1 Integrin", Journal of Cellular Biochemistry, 9 April 2015
- Chiara Pelillo, Hilaria Mollica, Johannes A. Eble, Julius Grosche, Lea Herzog, Barbara Codan, Gianni Sava, Alberta Bergamo; "Inhibition of adhesion, migration and of α5β1 integrin in the HCT-116 colorectal cancer cells treated with the ruthenium drug NAMI-A" Journal of Inorganic Biochemistry; 25 February 2016

Maria Jose Morilla

Maria Jose Morilla is Independent Researcher of the National Council for Scientific and Technological Research (CONICET) and Associate Professor at the National University of Quilmes, Argentina. Morilla is co-director of the Nanomedicine Research Program, she has supervised 2 and co-supervised 4 Ph.D students. Morilla is co-author of 5 book chapters and 46 peer reviewed publications.

Aditi Nandi

I, Aditi Nandi was born on the 11th of March 1991 in Mumbai, India. I completed my schooling from Mumbai and also graduated from Mumbai University obtaining a Bachelors degree in Chemistry. During my graduation I worked as a summer intern at BASF, Mumbai where I was trained in synthesising different paint formulations and polymers and also did a summer internship at NOCIL Limited India, where I was given hands on training on different chromatographic and spectroscopic techniques like gas chromatography, HPLC, ion exchange chromatography, UV-Visible spectroscopy and IR. Next, I joined Indian Institute of Science Education and Research (IISER), Pune for an Integrated PhD programme wherein, I completed my Masters in Chemistry in 2014 with a CGPA of 8.4. During my masters I worked on many research projects ranging from-

- Design and Synthesis of Triazine-Based Tripodal Receptors for Ion Sensing under the guidance of Dr. Pinaki Talukdar
- Study the size of Melanin polymer using Dynamic light scattering under the guidance of Dr. Mrinalini Puranik
- Synthesis of alkynes by Sonogashira cross coupling reaction under the guidance of Dr. M. Jeganmohan.

Currently, I am pursuing my Ph.D. in Chemistry under the guidance of Dr. Sudipta Basu at IISER Pune. My work focuses on the development of novel graphene oxide platforms for targeting the subcellular organelles in cancer cells mainly, the nucleus using anticancer drugs. I have developed novel cisplatin mediated self-assembled graphene oxide nanoparticles from graphene oxide sheets which damage the sub-cellular DNA. I am also trying to modify graphene oxide in order to increase its water solubility and hence make it more biocompatible which will aid in its usefulness for biomedical applications. Further, I am trying to develop drug conjugated nanoparticles (liposomes) encapsulating graphene oxide for targeting subcellular organelles for cancer therapy. I have one publication in my PhD so far titled: Cisplatin-Induced Self-Assembly of Graphene Oxide Sheets into Nanoparticles for Damaging Sub-cellular DNA. Chem.Commun. 2017, 53, 1409-1412. [Impact Factor: 6.567].

I have attended various conferences like 1) International Conference on Nanoscience and Technology (ICONSAT) at IISER Pune, 2) International Conference on Nanoscience and Technology (ICONSAT) at IISER Pune, 3) IUPAC's International Symposium on Bio-Organic Chemistry (ISBOC-10) at IISER Pune.

Hanh Thuy Nguyen

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ACADEMIC BACKGROUND:

2015-now: PhD student, College of Pharmacy, Yeungnam University, Republic of Korea
2010–2012: Master degree, Hanoi University of Pharmacy, Vietnam
2005–2010: Pharmacist degree, Hanoi University of Pharmacy, Vietnam

RESEARCH AREA:

Targeted drug delivery, combined therapy, nanomaterials, anticancer, anti-senescence treatment

PUBLICATIONS:

- Nguyen, H. T.; Tran, T. H.; Thapa, R. K.; Pham, T. T.; Jeong, J.-H.; Youn, Y. S.; Choi, H.-G.; Yong, C. S.; Kim, J. O., Incorporation of chemotherapeutic agent and photosensitizer in a low temperature-sensitive liposome for effective chemo-hyperthermic anticancer activity. Expert Opinion on Drug Delivery 2017;14:155-64.
- Nguyen HT, Thapa RK, Shin BS, Jeong JH, Kim JR, Yong CS, Kim JO. CD9 monoclonal antibody-conjugated PEGylated liposomes for targeted delivery of rapamycin in the treatment of cellular senescence. Nanotechnology. 2017;28:095101.
- Thapa, R. K.; Nguyen, H. T.; Jeong, J.-H.; Shin, B. S.; Ku, S. K.; Choi, H.-G.; Yong, C. S.; Kim, J. O., Synergistic anticancer activity of combined histone deacetylase and proteasomal inhibitor-loaded zein nanoparticles in metastatic prostate cancers. Nanomedicine: Nanotechnology, Biology and Medicine 2016. Epub 2016/12/21..
- Tran, T. H.; Nguyen, H. T.; Pham, T. T.; Choi, J. Y.; Choi, H.-G.; Yong, C. S.; Kim, J. O., Development of a graphene oxide nanocarrier for dual-drug chemo-phototherapy to overcome drug resistance in cancer. ACS applied materials & interfaces 2015, 7 (51), 28647-28655.
- Nguyen, H. T.; Tran, T. H.; Kim, J. O.; Yong, C. S.; Nguyen, C. N., Enhancing the *in vitro* anti-cancer efficacy of artesunate by loading into poly-d,l-lactide-co-glycolide (PLGA) nanoparticles. Archives of Pharmacal Research 2015, 38 (5), 716-724.

Paolo Oliva

My name is Paolo Oliva and I am a scientific researcher. I have 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 Biofarming 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, intrakidneyetc); 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.

Erik Örfi

I joined the Nanomedicine Research Center in 2013 through Scientific Students' Associations as a pharmacy student. I acquired my MSc. and Pharm.D degrees at Semmelweis University Faculty of Pharmacy in 2015. During my industrial practice at Servier Pharmaceuticals I investigated NCE (new chemical entity) structure confirmations by NMR, UV/IR, Raman spectroscopy and X-ray crystallography. My further experiences include pharmacy management, GLP and quality control. I'm currently a PhD student at Nanomedicine Research and Education Center at Semmelweis University. My topic is „Pathophysiology of nanomedicines, especially the cardiovascular and renal effects". I'm performing *in vivo* CARPA (Complement activation related pseudoallergy) experiments on pigs, rat and mice. I'm focusing now on the mice model, where I'm researching novel parameters of CARPA to characterize this phenomenon more precisely.

MY RECENT PUBLICATIONS:

- Cardiovascular Manifestations of Complement Activation-Related Pseudoallergy Following Administation of Liposomal Nanomedicines. László Dézsi, Rudolf Urbanics, Tamás Mészáros, Csenge Vázsonyi, Tamás Fülöp, Erik Őrfi, László Rosivall, János Szebeni, Gábor Szénási. Acta Physiologica. 2014 Aug Volume: 211. Pages: 94-94. Meeting Abstract: P4.27. IF: 4.066
- Features of complement activation-related pseudoallergy to liposomes with different surface charge and PEGylation: comparison of the porcine and rat responses. Dézsi L, Fülöp T, Mészáros T, Szénási G, Urbanics R, Vázsonyi C, Őrfi E, Rosivall L, Nemes R, Kok RJ, Metselaar JM, Storm G, Szebeni J. J Control Release. 2014 Dec 10;195:2-10. doi: 10.1016/j.jconrel.2014.08.009. , IF: 7.633
- The immune system of the gut and potential adverse effects of oral nanocarriers on its function. Őrfi E, Szebeni J. Advanced Drug Delivery Reviews. 2016 Nov 15;106(Pt B):402-409. doi: 10.1016/j.addr.2016.09.009. Review. IF: 17.214

Bekim Osmani

University of Basel, Department of Biomedical Engineering
Biomaterials Science Center
Gewerbestrasse 14, 4123 Allschwil, Switzerland

Bekim Osmani is a PhD student at the Biomaterials Science Center at the University of Basel. He did his B.Sc. in Mechanical Engineering and his M.Sc. in Biomedical Engineering and Robotics at the Swiss Federal Institute of Technology in Zurich (ETHZ) in 2002. After several years of experience in academia and industry, he is currently working towards his PhD degree in Nanosciences. His research interests include biomimetic electrodes, molecular beam deposition and electro-spraying of nanometer thin elastomer films, atomic force microscopy, nanoindentation techniques and mechanical properties of nanometer thin film, polymeric implants and biomedical applications for low-voltage dielectric elastomer actuators.

Myriam Ouberai

Senior Research Associate, Nanoscience Centre, University of Cambridge

Myriam holds a Ph.D. in Chemistry from the Université Joseph Fourier (France) where she developed peptide drug conjugates designed to tackle the aberrant processes involved in Alzheimer's disease. She is working as a senior research associate at the Cambridge Nanoscience Centre (UK) where she has undertaken several multidisciplinary research projects aiming to decipher the molecular processes involved in Parkinson's disease or to improve the efficacy and safety of drugs to treat challenging diseases such as brain cancer. Her research interests include: peptide self-assembly, nanomedicine - drug delivery, biomolecular engineering, protein interaction with lipid membranes and surfaces, Cancer therapy, Diabetes, Parkinson's and Alzheimer's diseases. She also joined the NanoForum strategic network of the University of Cambridge as facilitator to promote interdisciplinary research in nanomedicine. Currently Myriam is working in collaboration with MedImmune-AstraZeneca to apply cutting-edge research in nanoscience to peptide formulation.

PUBLICATIONS

- Galvagnion, C., Brown, J.W.P., Ouberai, M.M., Flagmeier, P., Vendruscolo, M., Buell, A.K., Sparr, E., Dobson, C.M. Chemical properties of lipids strongly affect the kinetics of the membrane-induced aggregation of α-synuclein (2016) Proc. Natl. Acad. Sci. U. S. A., 113 (26), pp. 7065-7070.
- Chen, S.W., Drakulic, S., Deas, E., Ouberai, M., Aprile, F.A., Arranz, R., Ness, S., Roodveldt, C., Guilliams, T., De-Genst, E.J., Klenerman, D., Wood, N.W., Knowles, T.P.J., Alfonso, C., Rivas, G., Abramov, A.Y., Valpuesta, J.M., Dobson, C.M., Cremades, N. Structural characterization of toxic oligomers that are kinetically trapped during α-synuclein fibril formation (2015) Proc. Natl. Acad. Sci. U. S. A., 112 (16), pp. E1994-E2003
- Ouberai, M.M., Xu, K., Welland, M.E. Effect of the interplay between protein and surface on the properties of adsorbed protein layers (2014) Biomaterials, 35 (24), pp. 6157-6163.
- Setua, S., Ouberai, M., Piccirillo, S.G., Watts, C., Welland, M. Cisplatin-tethered gold nanospheres for multimodal chemo-radiotherapy of glioblastoma (2014) Nanoscale, 6 (18), pp. 10865-10873.
- Ouberai, M.M., Wang, J., Swann, M.J., Galvagnion, C., Guilliams, T., Dobson, C.M., Welland, M.E. α-Synuclein senses lipid packing

defects and induces lateral expansion of lipids leading to membrane remodelling (2013) J. Biol. Chem., 288 (29), pp. 20883-20895.

Robert F. Pagels

Princeton University
Dept. of Chemical & Biological Engineering
Princeton, NJ 08544
E-mail: robertfp@princeton.edu
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I studied for my undergraduate degree in Chemical Engineering with a minor in Biochemical Engineering at the University of Delaware on a full academic scholarship. There I worked with Dr. Millicent Sullivan and Dr. Thomas Epps on synthesizing a polymeric micelle system with pH triggered morphological changes. Outside of the lab I was involved in several organizations, including the student chapter of the American Institute of Chemical Engineers (AIChE), of which I was president my senior year. I graduated summa cum laude in 2012 and was recognized as the Outstanding Male Student of the graduating class for both my academic and extracurricular achievements.

I am now at Princeton University on an NSF Graduate Research Fellowship where I received my Master's Degree in 2014 and am currently working towards my PhD in Chemical and Biological Engineering in Professor Robert K. Prud'homme's laboratory. My PhD research has had two major focuses. First, I have worked on converting our Flash NanoPrecipitation (FNP) nanoparticle formulations from model non-degradable polymers to biodegradable and biocompatible polymers. This project has included polymer synthesis, nanoparticle synthesis, and kinetic modeling of the nanoparticle assembly process. My second focus has been to develop Inverse FNP, or IFNP, for the encapsulation and delivery of biologics and other soluble therapeutics. I have successfully used this process to make nanoparticles for circulating delivery applications, as well as microparticles for sustained release applications. During the fall of 2015 I also worked with Dr. Richard Payne at the University of Sydney on an NSF Graduate Research Opportunities Worldwide (GROW) fellowship, where I learned solid phase peptide synthesis and chromatography analysis techniques. In addition to my own research I have mentored undergraduate students for four senior theses and a junior project.

I am a secondary author on several nanoparticle-focused papers, including "Single-Step Assembly of Multimodal Imaging Nanocarriers: MRI and Long-Wavelength Fluorescence Imaging" (Adv. Healthcare Mat., 2015), and "Biodistribution and fate of core-labeled I-125 polymeric nanocarriers prepared by Flash NanoPrecipitation (FNP)" (J. of Mat. Chem. B, 2016). My first primary author paper, "Polymeric nanoparticles and microparticles for the delivery of peptides, biologics, and soluble therapeutics" (J. of Cont. Release, 2015) was published in the special issue titled Drug Delivery Research in North America. I currently have two additional first author papers submitted for publication – one on controlling nanoparticle size with a focus on polymer physics, and the other on the development of the IFNP platform for the delivery of biologics. In addition to academic papers, I also have two patents.

I have presented my research a several conferences including the 2014 Gordon Research Conference on Drug Carriers in Medicine and Biology, the 2014 and 2016 ACS Colloids conferences, and the 2016 ACS National Meeting. My talk, "Assembly of nanoparticles containing biologics and other soluble therapeutics by flash nanoprecipitation" was named the best paper in the ACS National Meeting session: Control of Amphiphile Self-Assembling at the Molecular Level. My work on developing novel delivery systems for biologics also recently received the first place award at the 2017 Princeton Innovation Forum.

Mojca Pavlin

PhD
Head of the Group for nano and biotechnological applications
Faculty of Electrical Engineering,
University of Ljubljana
Senior Research Associate, Institute of Biophysics, Faculty of Medicine,
University of Ljubljana
Trzaska 25, 1000 Ljubljana, Slovenia
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My PhD studies were focused on electroporation, a phenomenon where high-voltage electrical pulses increase cell membrane permeability and thus enable intake of drugs and DNA molecules in cells, e.g. electrochemotherapy of tumors and electrogene therapy. I participated in several national and international research projects, part of my PhD thesis was done within 5.FP Cliniporator project. From 2006–2010 that I was principal investigator of a successful research project (»Mechanisms of DNA transfer in electrogene transfer«) that concentrated at analysis of mechanisms of gene electrotransfer in order to improve understanding of processes and to enhance gene electrotransfer efficiency.

In 2010 I established a young research group Group for nano and biotechnological applications at FE-UL. My current field of work includes different areas of biomedicine, biotechnology and nanotechnology supported by advance numerical modelling. I was PI of several interdisciplinary research projects, the results were published in 40 SCI papers, with over 1000 citations. I am lecturer at postgraduate courses of the PhD program Biosciences UL and I was mentor of several PhD student and master students. The research of our group can be divided in the three segments:

- Nanobiotechnology; development nanoparticles for biomedical applications (urothelial cancer therapy, cell labelling) analysis of NP uptake mechanisms and cytotoxicity in relation with immune response analysis. Development of protocols for assessment of long-term nanotoxicity and immunogenicy of nanoparticles.
- Biotechnology applications with electroporation method – gene electrotransfer/silencing from *in vitro* to 3D *in vitro* tissue models, uptake of small molecules and gene silencing from tumor to primary human cells.
- Research of cancer cells metabolic pathways and related mechanisms *in vitro* on different cancer cell models. We analyze different modulators of cellular metabolism (e.g. metformin) and analyze cell migration, viability and cell signaling.

SELECTED PUBLICATIONS:

- Lojk J, Strojan K Miš K, Bregar VB, Hafner Bratkovič I, Bizjak M, Pirkmajer S, Pavlin M. Cell stress response to two different types of polymer coated cobalt ferrite nanoparticles. Toxicol. Lett, 2017, vol. 270: 108-118
- Strojan K, Leonardi A, Bregar VB, Križaj I, Svete J, Pavlin M. Dispersion of Nanoparticles in Different Media Importantly Determines the Composition of Their Protein Corona, PLOS ONE January 4, 2017
- Lojk J, Bregar VB, Rajh M, Miš K, Erdani-Kreft M, Pirkmajer S, Veranič P, Pavlin M. Cell type-specific response to high intracellular loading of polyacrylic acid-coated magnetic nanoparticles. International journal of nanomedicine, 2015, vol. 10,1449-1462
- Pavlin M and Kandušer M. New insights into the mechanisms of gene electrotransfer - experimental and theoretical analysis. Scientific reports, 2015, 5: 1-11
- Bregar VB, Lojk J, Šuštar V, Veranič P, Pavlin M. Visualization of internalization of functionalized cobalt ferrite nanoparticles and their intracellular fate. International journal of nanomedicine, 2013, vol. 8, 919-931
- Pavlin M, Bregar VB. Stability of nanoparticle suspensions in different biologically relevant media. Digest Journal of Nanomaterials and Biostructures, 2012, vol. 7, 1389-1400

Sara Pereira

My name is Sara Pereira and I just started my third year of PhD at the University of East Anglia, UK.

I was born in 1985 in Lisbon, Portugal and there I obtained my BSc. degree in Biology at the Universidade de Lisboa (2008). Before enrolling in a MSc., I decided that I wanted to put into practice some of the lab techniques that I was taught during the degree and also to experience what scientist's life was. Therefore, I did a 1-year research internship shared between the Gulbenkian Institute for Science (IGC) and the Molecular Medicine Institute (IMM).

There, I worked in Developmental Biology, trying to understand the molecular mechanisms that underlie the left-right asymmetry in vertebrates. Despite having learnt several molecular biology techniques that later on proved to be very useful for my investigation, I quickly realised that I wanted to work in a more applied and translational field of science, where I could feel that my research could have a more straightforward impact on the society. With this objective in mind and having found the Biology degree too theoretical, I chose to proceed to a MSc. in Biotechnology at the Instituto Superior Tecnico (IST), so that I could develop my analytical and critical thinking skills.

After completing my Master's dissertation entitled "Novel Aqueous Two-Phase Systems for the Purification of Antibodies", I was awarded a 2-year grant to work as a Research Assistant in the Nucleic Acid Bioengineering Lab at the same university. Here, I learned molecular cloning, plasmid large-scale purification and mammalian cell culture work. During this period, I started a collaboration with the Nanomedicine Lab at King's College London, where I worked from January 2013 to March 2014. This was a turning point in my personal and professional life - I decided that I wanted to do a PhD in the UK.

In January 2015, I started my PhD in the School of Pharmacy at the University of East Anglia. My project aims at the development of a novel therapy for advanced prostate cancer based on the combination of mild hyperthermia with thermo-responsive liposomes encapsulating doxorubicin-PSA cleavable peptides. During the PhD I have been able to collaborate in several different projects and to publish in peer-reviewed journals. My publications include:

- Fluorinated tranylcypromine analogues as inhibitors of lysine-specific demethylase 1 (LSD1, KDM1A). Borrello MT, Gerwien K, Benelkebir H, Pereira S, Al-Jamal WT, Douglas L, Duriez PJ, Packham G, Haufe G, Ganesan A. Under submission to Bioorganic & Medicinal Chemistry Letters.
- Docetaxel-loaded liposomes: The effect of lipid composition and purification on drug encapsulation and *in vitro* toxicity. Pereira S, Egbu R, Jannati G, Al-Jamal WT. 30 Nov 2016 in: International Journal of Pharmaceutics 514(1):150-159.
- Synthesis of Diagnostic Silicon Nanoparticles for Targeted Delivery of Thiourea to Epidermal Growth Factor Receptor-Expressing Cancer Cells. Behray M, Webster CA, Pereira S, Ghosh P, Krishnamurthy S, Al-Jamal WT, Chao Y. 13 Apr 2016 in: ACS Applied Materials and Interfaces.
- Cationic Liposome- Multi-Walled Carbon Nanotubes Hybrids for Dual siPLK1 and Doxorubicin Delivery In Vitro. Pereira S, Lee J, Rubio N, Hassan HA, Suffian IB, Wang JT, Klippstein R, Ballesteros B, Al-Jamal WT, Al-Jamal KT. Oct 2015 in: Pharmaceutical Research.

Sílvia Pérez-Rafael

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Dr. Sílvia Pérez-Rafael graduated in chemistry (2008) and obtained a Master Degree in Science and Technological chemistry (2008) from the Autonomous University of Barcelona. Her predoctoral research (Chemistry PhD obtained in 2013) focused on recombinant biosynthesised metalloproteins (concretely metallothioneins) characterization and the study of their coordinative properties. The project aims to contribute towards the general knowledge of their structure-function relationship. She applied a wide range of techniques like mass spectrometry, circular dichroism, UV-Vis absorption and fluorescence. During two abroad scientific researches stays, she trained in High-performance liquid chromatography (HPLC) coupled to ESI-MS, ICP-MS and UV-Vis detectors and in ITC (Isothermal Titration Calorimetry) for metal-protein binding studies.

Since 2016, she holds the position of postdoctoral researcher at the Applied Biotechnology team inside the Group of Molecular and Industrial Biotechnology. The goal of the team is the fusion of research, industry and market as to give birth to a new science concept by developing new bio-based or biomimetic technologies, researching the merging of biotechnology, materials science, physicochemistry and polymer engineering. This fusion is achieved by a permanent collaboration with industrial partners as to provide real time solutions to various needs. The research group provide biotech alternatives for material development and industrial processes improvement in terms of efficiency, environmental impact, energy consumption and product performance. The focus on "bio" is an integral part of our research: bio-tools (enzymes), bioprocesses and biomaterials are used to design functional coatings and textiles; cosmetic formulations; nano/micro drug delivery systems; medical and diagnostic devices; antimicrobial/antibiofilm strategies; bioactive and composites materials.

PEER REVIEWED PUBLICATIONS

- Pérez Rafael S, Monteiro F, Dallinger R, Atrian S, Palacios Ò, Capdevila M, "Cantareus aspersus metallothionein metal binding abilities: the unspecific CaCd/CuMT isoform provides hints about the metal preference determinants in metallothioneins', BBA Protein and Proteomics, 2014, 1844 (9), 1694–1707, doi: 10.1016/j.bbapap.2014.06.018.
- Palacios Ò, Pérez-Rafael S, Pagani A, Dallinger R, Atrian S, Capdevila M, "Cognate and noncognate metal ion coordination in metal-specific metallothioneins: the Helix pomatia system as a model", Journal of biological inorganic chemistry, 2014, 19(6), 923-935, doi: 10.1007/s00775-014-1127-4.
- Pérez-Rafael S, Pagani A, Palacios O, Dallinger R, Capdevila M, Atrian S, "The role of histidine in a copper-specific metallothionein", ZAAC Journal of Inorganic and General Chemistry, 2013, 639 (8-9), 1356-1360.
- Guirola M (*), Pérez-Rafael S (*), Capdevila M, Palacios O, Atrian S, "Metal Dealing at the Origin of the Chordata Phylum: The Metallothionein System and Metal Overload Response in Amphioxus"', PLoS One, 2012, 7(8), e43299, doi: 10.1371/journal.pone.0043299. (*both authors contributed equally).
- Pérez Rafael S, Kurz A, Guirola M, Capdevila M, Palacios Ò, Atrian S, "Is MtnE, the fifth Drosophila metallothionein, functionally distinct from the other members of this polymorphic protein family?", Metallomics, 2012, 4(4), 342-349, doi: 10.1039/c2mt00182a.

Marina Pöttler

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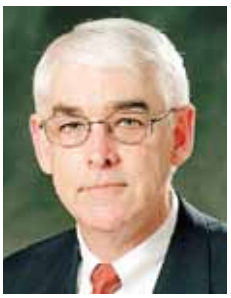
Marina Pöttler is a biologist with the main focus in nano-medicine and cancer research. After she studied biology at the Paris Lodron University in Salzburg (Austria), where she finished her master in zoology/ cell biology and physiology with excellent degree, she stated her PhD studies at the Medical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

Dwi Priwitaningrum

In 1993, Dwi Priwitaningrum started her education at the Faculty of Pharmacy at Institut Teknologi Bandung (ITB), Indonesia. In 1997 she obtained her bachelor's degree and after several years working in practical field, she continued her master study and obtained her Master of Science in 2008. During her master study, she worked with alginate to develop a gastric delivery system in the form of hard alginate capsule to prevent iron-induced gastric side-effects in iron deficiency anemia patients. Since December 2012, she joined Targeted Therapeutics Section of the Department of Biomaterials Science and Technology, MIRA Institute, University of Twente, The Netherlands to undertake her PhD study in therapeutic peptide delivery system and development of 3D model platform to mimic tumor stroma and their interaction with nanoparticles.

PUBLICATION:

Priwitaningrum D, Blonde JP, van Baarlen J, Hennink WE, Storm G, Le Gac S, Prakash J. (2016) Tumor Stroma-containing 3D Spheroid Arrays: A Tool to Study Nanoparticle Penetration. J Control Release. 28;244(Pt B):257-268.



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Robert K. Prud'homme is a professor in the Department of Chemical and Biological Engineering at Princeton University and Director of the Engineering Biology Program. He received his BS at Stanford University and his PhD from the University of Wisconsin at Madison under Professor Bob Bird. He has served on the executive committees of the American Institute of Chemical Engineers Materials Science Division and the U.S. Society of Rheology and was the President of the U.S. Society of Rheology. He has served as the chair of the Technical Advisory Board for Material Science Research for Dow Chemical Company, which directs Dow's materials research programs, and he was on the Board of Directors of Rheometric Scientific Inc., the leading manufacturer of rheological instrumentation. He also served on the Nanotechnology Scientific Advisory Committee for BASF, which provided guidance for future trends in nanotechnology for the company. His awards include the NSF Presidential Young Investigator Award, Princeton School of Engineering and Applied Science Outstanding Teaching Award, the Sydney Ross Lectureship at RPI, the Bird,Stewart and Lightfoot Lecturer at the University of Wisconsin, the Dinesh Shah lectureship at the University of Florida, and the Midland Macromolecular Institute Visiting Professor in Midland Michigan. He has been the organizer and Chair of the Gordon Conference on Ion Containing Polymers, and the Society of Petroleum Engineers Forum on Stimulation Fluid Rheology, in addition to organizing numerous sessions at AIChE, ACS, and SOR meetings. He directed the Princeton-University of Minnesota-Iowa State NSF NIRT Center on nanoparticle formation. His research interests include rheology and self-assembly of complex fluids. Systems of interest are biopolymer solutions and gels, surfactant mesophases, and polymer/surfactant mixtures. The goals of the studies are to understand how weak molecular-level interactions can be used to tune macroscopic bulk properties and phase behavior. Application of the work is directed at nanoparticle formation for the drug delivery, controlled release, targeting, and imaging.

Bita Rasulian

Research Assistant of Nanomedicine
Cellular and Molecular Research Center
Iran University of Medical Science (IUMS),
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2016– Continue
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PROJECTS:

1. Preparation of nano drug carriers and their bone regeneration investigation in a critical sized bone defect model in rat. Supervisour: Dr. Shima Tavakol 2016 (Finished)
2. Bone regeneration investigation of self-assembling peptide nanofiber in a critical sized bone defect model in rat. Supervisour: Dr. Shima Tavakol 2016 (Finished)
3. Preparation of nano drug carriers and their motor neuron recovery investigation in spinal cord injury model of rat. Supervisour: Dr. Shima Tavakol. 2016 to be continue.

Ilaria Francesca Rizzuti

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I was born on 13th of August 1989 in Florence (Italy).
I attended the Bachelor's Degree in Biotechnologies (3 years) at the University of Salento (Lecce-Italy) from October 2008 to October 2011. My bachelor thesis was in Organic Chemistry on: "Determination of the kinetics of enantiomerization of chiral compound through the method of the stopped-flow two-dimensional recirculating (sf-BD- rHPLC)". I did my curricular Internship at Organic Chemistry Department Laboratory at University of Salento from March 2011 to October 2011. From October 2011 to December 2013 I attended the Master's Degree in Medical Biotechnology and Nanobiotechology (2 years), nanobiotechnological curriculum at University of Salento in Lecce (Italy) with mark 110/110 cum laude. My master thesis was in Methods of Nanofabrication for advanced Biotech and Nanoscale analysis, at National Nanotechnology Laboratory of Lecce on "Realization and characterization of microstruttured bioscaffold". I did my curricular Internship at National Nanotechnology Laboratory – CNR Nano (NNL), Lecce from March 2013 to December 2013 and I did one extracurricular Internship at Biochemestry Laboratory Di.Ste.Ba, University of Salento from October 2012 to March 2013. From January 2014 to January 2015 | at Imast Scarl - technology district on engineering of polymeric and composite materials and structures - Piazza Bovio, 22; Naples (http://www.imast.biz) - I worked as Junior Researcher in the project "Researchers in the field of polymer micro and nano-particle systems for administration of pharmacologically active molecules" (POLIfarmaform - Code PON02_00029_3203241 / F1). I did my Practical training of the program in Dompè Farmaceutici S.p.A. (R & D Technology).
In November 2015 I started the PhD course at the University of Genoa (Italy) in collaboration with the IIT in Genoa on the "Laboratory of Nanotechnology for Precision Medicine" of Prof. Paolo Decuzzi. During the last year I was working on the optimization of the Nanofabrication process of Discoidal Polymeric Nanoconstructs (DPNs) and now I am working on the controlled drug release from (DPNs) the using ultra-sounds.

Hima Bindu Ruttala

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I have completed my Bachelors in PYDAH college of Pharmacy, Andhra University. India. 2009–2013.
Then I have graduated my master degree in drug delivery from Gachon University in South Korea. 2013–2015.
Currently I am doing PhD course (5th semester) in Department of Pharmaceutics from Yeungnam university in South Korea. 2015–2018

PUBLISHED PAPERS

- Hima Bindu Ruttala, Ramasamy T, Shin BS, Choi HG, Yong CS, Kim JO. Layer-by-Layer Assembly of Hierarchical Nanoarchitectures to Enhance the Systemic Performance of Nanoparticle Albumin-bound Paclitaxel. Int J Pharm. S0378-5173(17)30011-X.2017
- Hima Bindu Ruttala, Ramasamy T, Poudal BK, Choi YJ, Choi JY, Kim J, Ku SK, Choi HG, Yong CS, Kim JO. Molecularly targeted co-delivery of a histone deacetylase inhibitor and paclitaxel by lipid-protein hybrid nanoparticles for synergistic combinational chemotherapy. Oncotarget, In Press. 2017
- Ramasamy T, Ruttala HB, Chitrapriya N, Poudal BK, Choi JY, Kim ST, Youn YS, Ku SK, Choi HG, Yong CS, Kim JO. Engineering of cell microenvironment-responsive polypeptide nanovehicle co-encapsulating a synergistic combination of small molecules for effective chemotherapy in solid tumors. Acta Biomater. 2016; S1742-7061(16)30562-1. 2016
- Hima Bindu Ruttala, YT Ko. Liposomal co-delivery of curcumin and albumin / paclitaxel nanoparticle for enhanced synergistic antitumor efficacy. Colloids Surf B Biointerfaces . (2015) 128 (419-426). 2015
- Hima Bindu Ruttala, YT Ko. Liposome Encapsulated Albumin-Paclitaxel Nanoparticle for Enhanced Antitumor Efficacy. Pharmaceutical Research. (2015) 32 (3). 2015

Jonas Schnittert

In 2009, Jonas Schnittert started his study in Biomedical Engineering at the University of Twente. In 2013 he obtained his bachelor's degree and continued with his masters in Biomedical Engineering. During his master's thesis he investigated novel miRNA delivery strategies in the section Targeted Therapeutics of the Department of Biomaterials Science and Technology, University of Twente. After completing his master thesis in 2015, he started his PhD with focus on novel targets in tumor stroma and gene delivery system in the section Targeted Therapeutics of the Department Biomaterials Science and Technology, University of Twente.

Georg Schulz

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2008: Diploma in theoretical physics at the University of Freiburg, Germany.
Diploma thesis on Exciton dynamics in circular and elliptical aggregates.
2008: scientific collaborator at the group of Theoretical Quantum Dynamics (University of Freiburg, Germany).
2012: PhD at Biomaterials Science Center (University of Basel, Switzerland) on the topic of human brain imaging, in particular magnetic resonance imaging, phase contrast X-Ray computed tomography and small-angle X-ray scattering.
2012–2015: PostDoc at Biomaterials Science Center, University of Basel, Switzerland
since 2015: Group leader of the high-resolution X-ray imaging group, Biomaterials
Science Center, Department of Biomedical Engineering, University of Basel, Switzerland
since 2017: Manager of the Core Facility 'Micro- and Nanotomography', Department of Biomedical Engineering, University of Basel, Switzerland

Matej Siketanc

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Matej Siketanc is a project student at the Biomaterials Science Center. He did his B.Sc. in Nanoscience at the University of Basel in 2016 and started with his Master studies in Nanoscience also at the University of Basel with specialization in molecular biology at the same year. As project student, he is working on the improvement of electrospray deposition techniques with the aim to fabricate nanometer thin polymer layers. Since 2016 he is helping assistant at the mathematical institute of the University Basel.

Katerina Spyridopoulou

Katerina Spyridopoulou was born in 1982 in Alexandroupolis, a town in the north-eastern Greece. She is in the last year of her PhD studies in the Department of Molecular Biology and Genetics at the Democritus University of Thrace (DUTh), Greece. Katerina, after completing her undergraduate degree at the same department in 2011, she continued her studies by doing a Master's degree. Her research interest in nanotechnology, led her to pursue and engage in an interdisciplinary collaboration between the Department of Physics (Aristotle University of Thessaloniki-AUTh), and the Department of Medicine (DUTh) for the completion of her Master thesis entitled "Study on the potential of magnetic nanoparticles as agents for biomedical applications and novel therapeutic strategies". She was awarded (with Distinction) the MSc in Clinical Pharmacology and Therapeutics in 2013. She decided to continue her research in the same field by applying for a PhD. She is a PhD student since October 2013. Her supervisors are Dr. Katerina Chlichlia (Assoc. Prof. of Molecular Immunology, DUTh), and Dr. Orestis Kalogirou (Prof. of Applied Physics, AUth). The title of her PhD thesis is «Design and application of bioactive nanoparticles in cancer prevention and therapy». In parallel, from November 2013 to November 2015 she worked as a Research Fellow for the GSRT funded (EU co-funded) project «SYNERGASIA II: 11SYN_2_566: "Novel functional foods containing bioactive essential oils from Greek endemic species with health promoting properties"». Among her scientific interests are synthesis and study of the structural and physicochemical properties of nanoparticles and the biomedical applications of magnetic and selenium nanoparticles in particular for cancer prevention and therapy. She has investigated the biosafety profile of metal nanoparticles both *in vitro* and *in vivo*. Much of her work has been on the development of novel therapeutic approaches against colon cancer, based on externally stimulated magnetic nanoparticles. Moreover, by employing green synthesis, she has developed a protocol for the production of biogenic selenium nanoparticles with antitumor potential, by Lactobacillus casei, a bacterial strain whose anticancer activity she has previously studied. Katerina is co-author of four papers published in peer reviewed international scientific journals. Also, her work has been presented in several International Conferences.

PUBLISHED SCIENTIFIC PAPERS

- Tiptiri-Kourpeti A*, Spyridopoulou K*, Santarmaki V, Aindelis G, Tompoulidou E, Lamprianidou E, Saxami G, Ypsilantis P, Lampri E, Simopoulos C, Kotsianidis I, Galanis A, Kourkoutas Y, Dimitrellou D, Chlichlia K. Lactobacillus casei Exerts Anti-Proliferative Effects Accompanied by Apoptotic Cell Death and Up-Regulation of TRAIL in Colon Carcinoma Cells, to PLoS One. 2016 Feb 5;11(2):e0147960, *Equal contributing authors
- Tiptiri-Kourpeti A, Spyridopoulou K, Pappa A, Chlichlia K. DNA vaccines to attack cancer: Strategies for improving immunogenicity and efficacy, to Pharmacol Ther. 2016 Sep;165:32-49
- Fitsiou E, Mitropoulou G, Spyridopoulou K, Tiptiri-Kourpeti A, Vamvakias M, Bardouki H, Panayiotidis M, Galanis A, Kourkoutas Y, Chlichlia K, Pappa A. Phytochemical Profile and Evaluation of the Biological Activities of Essential Oils Derived from the Greek Aromatic Plant Species Ocimum basilicum, Mentha spicata, Pimpinella anisum and Fortunella margarita, to Molecules 21(8):1069 • August 2016
- Spyridopoulou K, Tiptiri-Kourpeti A, Lampri E, Fitsiou E, Vasileiadis S, Vamvakias M, Bardouki H, Gousia A, Malamou-Mitsi V, Panayiotidis M, Galanis A, Pappa A, Chlichlia K. Dietary mastic oil extracted from Pistacia lentiscus var. chia suppresses tumor growth in experimental colon cancer models. Scientific Reports. 2016, In press

Klemen Strojan

Klemen Strojan obtained his master's degree in Biotechnology at University of Ljubljana, Slovenia. He is currently employed as a young researcher and working toward PhD in Nanoscience at University of Ljubljana.

His main research interest is nanoparticle-protein interaction in biological systems and nanoparticle interaction with innate immune system. As a member of an interdisciplinary Group for nano and biotechnological applications he is responsible for developing nanoparticle-based applications.

As a researcher he is included in three national research projects funded by Slovenian Research Agency (No. J7-7424, J3-6794, J2-6758).

- Strojan, K.; Leonardi, A.; Bregar, V. B.; Križaj, I.; Svete, J.; Pavlin, M. Dispersion of Nanoparticles in Different Media Importantly Determines the Composition of Their Protein Corona. PLOS ONE 2017, 12, e0169552.

Yuki Takechi-Haraya

Yuki Takechi-Haraya received B.S. degree (pharmacy) in 2009 and M.S. degree (pharmaceutical sciences) in 2011 from the University of Tokushima. After working as a health insurance pharmacist for 2011, He became an assistant professor at Himeji Dokkyo University in 2012. He received his Ph.D. degree (pharmaceutical chemistry) in 2015 from the University of Tokushima. He then started to work as a research resident for Japan Agency for Medical Research and Development, and participated in research at National Institute of Health Sciences in Japan (NIHS) for development of method to analyze mechanical property of nano-sized liposomes as drug carriers. After the program ending, He became a research scientist of Division of Drugs at NIHS in 2016. His current interest covers atomic force microscopic imaging, analytical science for drug evaluation, drug delivery system based on nanotechnology.

REPRESENTATIVE PUBLICATIONS

- Takechi-Haraya, Y., Sakai-Kato, K., Goda, Y. "Membrane Rigidity Determined by Atomic Force Microscopy Is a Parameter of the Permeability of Liposomal Membranes to the Hydrophilic Compound Calcein" AAPS PharmSciTech 2016, American Association

of Pharmaceutical Scientists, DOI: 10.1208/s12249-016-0624-x
- Takechi-Haraya, Y., Sakai-Kato, K., Abe, Y., Kawanishi, T., Okuda, H., Goda, Y. "Atomic Force Microscopic Analysis of the Effect of Lipid Composition" Langmuir, American Chemical Society, 32, 6074-6082, 2016.
- Takechi-Haraya, Y., Nadai, R., Kimura, H., Nishitsuji, K., Uchimura, K., Sakai-Kato, K., Kawakami, K., Shigenaga, A., Kawakami, T., Otaka, A., Hojo, H., Sakashita, N., Saito, H. "Enthalpy-driven interactions with sulfated glycosaminoglycans promote cell membrane penetration of arginine peptides" Biochimica et Biophysica Acta, Elsevier B.V., 1858, 1339-1349, 2016.
- Takechi (Haraya), Y., Shintani, Y., Kimoto, D., Okamura, E. "Regulation of Phospholipid Protrusion in the Cell Sized Vesicle by Hydrophobic Bisphenol A" Membrane, The Membrane Society of Japan, 40, 38-45, 2015 (Award by the Membrane Society of Japan for outstanding Membrane paper).
- Takechi (Haraya), Y., Yoshii, H., Tanaka, M., Kawakami, T., Aimoto, S., Saito, H., "Physicochemical Mechanism for the Enhanced Ability of Lipid Membrane Penetration of Polyarginine" Langmuir, American Chemical Society, 27, 7099-7107, 2011.

Behnaz Tavakol

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Behnaz Tavakol is a student of medicine in Kashan University of Medical Sciences, Iran. She was born on 1990 and choose as a talent student in high school with score of A by the ministry of education. She is interested in the field of nanomedicine and has some paper publications and patents in this field.

SOME ARTICLES PUBLISHED TO REFEREED JOURNALS:

- Noggin along with a self-assembling peptide nanofiber containing long motif of laminin induces tyrosine hydroxylase gene expression. Shima Tavakol, Sayed Mostafa Modarres Mousavi, Behnaz Tavakol, Elham Hoveizi, Jafar Ai, Seyed Mahdi Rezayat. Molecular Neurobiology (2016) DOI: 10.1007/s12035-016-0006-0.
- Mechano-transduction signals derived from self-assembling peptide nanofibers containing long motif of laminin influences neurogenesis in in-vitro and in-vivo. Shima Tavakol*, Sayed Mostafa Modarres Mousavi, Behnaz Tavakol, Elham Hoveizi, Jafar Ai, Seyed Mahdi Rezayat. Molecular Neurobiology (2016) DOI 10.1007/s12035-016-9836-z.
- 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.
- 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 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.

PATENT

- An osteogenic and angiogenic cocktail. Shima Tavakol, Amin Almasi, Seyed Mahdi Rezayat. Under filling of United States Patent, US 62/347, 928.
- Hydrogel based peptide nanofiber containing long motif of laminin for application in medical studies; International category A61, Patent no 82433.

Shima Tavakol

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Dr. Shima Tavakol is assistant professor at Cellular and Molecular Research Center, Iran University of Medical Sciences and the first post-Doc of nanomedicne in Iran who was born on 1982 in Kashan, Iran. She has the responsibility as a secretary of Education and Research Committee at Iranian Society of Nanomedicine and is a member of Nano-Tissue Engineering Committee, Presidency of the Islamic Republic of Iran, Vice Presidency for science and Technology. She was awarded Young Faculty Award by Ministry of Health and Medical Education and the Young Faculty investigator in Iran University of Medical Sciences by Iran University of Medical Sciences in 2016. However. in a graduated level, she was awarded as the best Ph.D graduate of Nanotechnology in Iran by Iranian Nanotechnology society and as the best Ph.D graduate of School of Advanced Technologies in Medicine by Tehran University of Medical Sciences in 2014 and ranked First, among Ph.D students in the Board exam in 2012. Besides of her awards, she has some publications, book compliations and patents in the field of nanomedicine. She is especially interested to the field of nano-tissue engineering via self-assembling peptide nanofibers and drug nanocarriers. her greatest wish in life is to cure the spinal cord injury patients and she thinks that there is no higher joy than to see the smile of a child with parents who are able to walk rather than sit on wheelchairs.

AWARDS AND HONORS

- 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

PATENT

- An osteogenic and angiogenic cocktail. Shima Tavakol, Amin Almasi, Seyed Mahdi Rezayat. Under filling of United States Patent, US 62/347, 928.
- 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)

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- Nanotechnology - based approaches for targeting and delivery of drugs and genes; Toxicology concerns of nanocarriers, 1 chapter, Elsevier (English).
- New Developments in Gold Nanoparticles and Nanoshells Research, 1 chapter, Nova publication (English) in-print
- Nanomedicine. 2 chapters, Jahad Daneshgahi, Tehran, Iran.
- Introduction of Physiology (Persian) Publisher; Taaliye Andishe, Tehran, Iran.
- Embryology summery (Persian) 2014 Publisher; Taaliye Andishe, Tehran.

Tino Töpper

ITino Töpper studied at the Albert-Ludwigs University of Freiburg i. Br. and received his diploma degree in physics in 2011. From 2010 to 2011 he prepared his diploma thesis specialized on semiconductor lasers at the Fraunhofer Institute of solid state physics (IAF) in Freiburg where he continued his work after graduation as a research assistant till 2012. In December 2016, he finished his Ph.D. thesis and received his doctoral degree in experimental physics with summa cum laude at the Biomaterials Science Center (University of Basel). As a member of the former Nanotera.ch founded project "Smartsphincter" he works on implantable low-voltage dielectric transducers based on nanometer-thin polymer membranes.

Harald Unterweger

In 2008, I started my academic career with the Bachelor degree course Nanotechnology at the Friedrich-Alexander-University Erlangen-Nuremberg (Erlangen, Germany) and graduated in Bachelor of Science in 2011. Being among the top 10 of my year, I was awarded with the "SEMIKRON Foerderpreis" (engl: SEMIKRON advancement award) for emerging students. I continued my studies with the Master degree course Nanotechnology at the same university and graduated with honors in 2013. Since 2014, I am a PhD student at the Section of Experimental Oncology and Nanomedicine (SEON) in cooperation with the Department of Materials and Science, under the supervision of Prof. Dr. Christoph Alexiou and Prof. Dr. Aldo R. Boccaccini. My research topic is the fabrication of highly biocompatible superparamagnetic iron oxide nanoparticles (SPIONs) for drug delivery and imaging purposes. For the presentation of my results, I was awarded with a poster prize at the international MAGMEET conference 2014 in Dresden, Germany, and at the CLINAM in 2016.

Alexandra Vaideanu

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I am a trained chemist, passionate about interdisciplinary research, with great enthusiasm and experience in the field of nanotechnology, nearing completion of postgraduate degree in Engineering and Clinical Neuroscience.
I am originally from Romania which is where I studied in high school at Colegiul National Petru Rares. I have always been found of chemistry (and the other sciences too). I discovered nanoscience and nanotechnology whilst preparing for a national competition in chemistry. I was fascinated by shape memory alloys, ferromagnetic liquids and self-assembling motors which is why I decided that I was going to study these materials at university. Very little in this area was available for me in Romania so I started applying for programmes in the US and UK. This led to me going to Liverpool to study Chemistry with Nanotechnology. After finishing my chemistry degree it was clear to me that I wanted to follow an academic career which is why I applied to do my PhD at Cambridge. I came here to work on a project focused on tailoring a gold nanostructure based technology which would cross the blood brain barrier by targeting specifically the tumour whilst also transporting chemotherapy to the site; radiation therapy is also enhanced which conveys the efficacy potential of this multimodal therapy.

RESEARCH EXPERIENCE

In order to gain hands on experience of research in academia I applied for two summer research projects which I thoroughly enjoyed and confirmed my decision to embark onto a PhD.
Jun–Jul 2012 Research assistant, Department of Chemistry, University of Liverpool Photocatalysis of dyes using titanium oxide nanoparticles
Jul–Sep 2012 Research assistant, Institute of Integrative Biology, University of Liverpool Photo–thermal imaging of gold and iron nanoparticles in cells (HeLa and kidney stem cells)

PUBLICATIONS

- Văideanu A.G. et al. "Self-assembled porous like gold nanostructures deliver cis-platinum to neuropilin-1 overexpressing glioblastoma multiforme cells" (in preparation)
- Văideanu A.G. et al. "Synergistic effect of radiotherapy and cisplatinum chemotherapy delivered via gold nanoparticles in glioblastoma multiforme", Journal of Interdisciplinary Nanomedicine, 2016

PROFESSIONAL MEMBERSHIP

- AMRSC Associate Member of the Royal Society of Chemistry
- British Society for Nanomedicine and European Society of Nanomedicine member
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Danillo Fabrini M. C. Veloso, Ph.D. candidate in the field of Pharmaceutical Nanotechnology at the School of Pharmacy, Federal University of Goias, Brasil. He works developing nanostructured drug delivery systems and new pharmaceutical devices, also with analytical/bioanalytical research, developing and validating methodologies, toxicological analyses and *in vivo* experimentation. Along with other researchers of Federal University of Goias, he develops and characterizes nanoparticles used in bone tissue engineering and treatment of infectious diseases. He has worked in national pharmaceutical industries, multinational and clinical research centers.

PUBLICATIONS:

- CLERES, L. M.; Ávila, R.I.; VELOSO, D. F. M. C.; PEDROSA, T. N.; LIMA, E. S.; COUTO, R. O.; LIMA, E. M.; Batista A C; PAULA, J. R.; VALADARES, M. In vitro safety and efficacy evaluations of a complex botanical mixture of Eugenia dysenterica DC. (Myrtaceae): Prospects for developing a new dermocosmetic product. Toxicology in Vitro, 2017.
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WORK EXPERIENCE:

2003/8 – so far, Nanjing Medical University, college of pharmacy, professor
1984/9-2003/7, Drug research institute of Jiangsu province and Jiangsu province research key laboratory of drug delivery system, director

EDUCATION:

1999/9-2000/6, China pharmaceutical university, pharmacy, master's degree
1977/10-1982/2, Shenyang pharmaceutical university, pharmacy, undergraduate

ACHIEVEMENTS & ACTIVITIES:

Drug evaluation experts in Jiangsu province Society of Jiangsu pharmaceutical association, deputy director of professional committee members Jiangsu province science and technology project/ achievement evaluation experts

PUBLICATIONS:

(1) Wang, Baoyan,Lv, Lingyan,Wang, Zhongyuan,Zhao, Yue,Wu, Lin,Fang, Xiaoling,Xu, Qunwei ,Xin, Hongliang ,Nanoparticles functionalized with (*) (*) Pep-1 as potential glioma targeting delivery system via interleukin 13 receptor alpha 2-mediated endocytosis,Biomaterials,2014,35(22) 5897-5907
(2) Wang, Zhongyuan,Zhao,Yue,Jiang,Yan,Lv,Wei,Wu, Lin,Wang, Baoyan,Lv, Lingyan,Xu, Qunwei ,Xin, Hongliang ,Enhanced (*) (*) anti-ischemicstroke of ZL006 by T7-conjugated PEGylated liposomes drug delivery system,Scientific Reports,2015,5(12651) 1-15
(3) Wang, Baoyan,Lv, Lingyan,Wang, Zhi,Jiang,Yan,Lv,Wei,Liu, Xin,Wang, Zhongyuan,Zhao,Yue,Xin, Hongliang , ,Improved (*) Xu, Qunwei(*) anti-glioblastomaefficacy by IL-13Rα2 mediated copolymer nanoparticles loaded with paclitaxel,Scientific Reports,2015,5(16589) 1-13 (4) Li, Jing,Wu, Lin,Wu, Weijun,Wang, Baoyan,Wang, Zhongyuan,Xin, Hongliang, ,A potential carrier based on liquid crystal (*) Xu, Qunwei(*) nanoparticles for ophthalmic delivery of pilocarpine nitrate,International Journal of Pharmaceutics,2013,455(1-2) 75-84
(5) Wu, Weijun,Li, Jing,Wu, Lin,Wang, Baoyan,Wang, Zhongyuan,Xu, Qunwei ,Xin, Hongliang ,Ophthalmic Delivery of Brinzolamide by Liquid (*) (*) Crystalline Nanoparticles: In Vitro and In Vivo Evaluation,AAPS PharmSciTech,2013,14(3) 1063-1071

Keni Yang

Keni Yang got her bachelor and master in pharmaceutics from China Pharmaceutical University. During master in 2013–2015, she joined Prof. Xing-Jie Liang's lab in National Center for Nanoscience And Technology, CAS, China as a visiting student, focusing on the development of nano-drug delivery system and theranostics. She has been then awarded a PhD scholarship from China Scholarship Council and started her PhD with the supervision of Dr. Anna Salveti in Groningen Research Institute of Pharmacy, University of Groningen since 2015. Her PhD project is exploiting the nanoparticle corona for targeting nanomedicines

PUBLICATIONS

• Yang K#, Li S#, Jin S, Xue X, Zhang T, Zhang C, Xu J, Liang XJ. Micelle-like luminescent nanoparticles as a visible gene delivery system with reduced toxicity. Journal of Materials Chemistry B. 2015 Jan 1;3(42):8394-400. (#contributed equally)
• Yang K, Zhang CQ, Wang W, Wang PC, Zhou JP, Liang XJ. pH-responsive mesoporous silica nanoparticles employed in controlled drug delivery systems for cancer treatment. Cancer biology & medicine. 2014;11(1):34-43.
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CLINAM

European Foundation for Clinical Nanomedicine

10/2017

Basel, May 7–10

ABSTRACTS POSTERS

MULTIFUNCTIONAL NANOLIPOSOMES FOR THE TREATMENT OF FABRY DISEASE

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Fabry disease is an inherited X-linked lysosomal storage disease, caused by the deficiency of α- galactosidase (GLA), an enzyme required for the metabolism of several glycoesphingolipids. The absence of this GLA, leads to a predominant accumulation of glibotriaosylceramide (Gb3) in the vascular endothelium and smooth muscle cells of different organs, eventually leading to an early death patient at the fourth or fifth life decade. Current treatment of Fabry disease consists in the bi-weekly injection of recombinant GLA, but its effectiveness is known to be limited due to the short half-life and poor biodistribution of the protein.

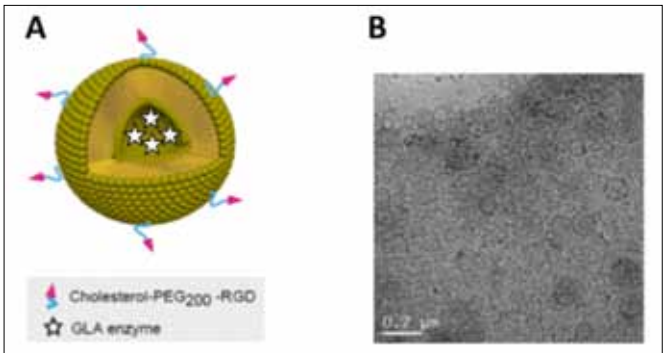

Figure 1: (A) Representative structure of the nanoparticle where the free enzyme is encapsulated within a lipid bilayer of DPPC and cholesterol. (B) CryoTEM images of nanoliposomes showing its homogeneity in size and structure.

In order to improve the pharmacokinetics and the efficacy of the GLA enzyme, nanoliposomes containing the recombinant enzyme were prepared following the DELOS-SUSP methodology, based on the use of compressed CO_2 [(1,2)]. Moreover, a c(RGDfK) peptide ligand was incorporated in the membrane bilayer of the vesicles to enhance the targeting and the uptake efficiency of the GLA-loaded conjugates to the endothelial cells (Figure 1). This targeted nanoliposomes have been patented and licensed to Biopraxis Research AIE [(2)], company that is currently working on the scale-up of the nanosystem within the Smart4Fabry H2020 project.

Previous results have shown that GLA-nanoformulations were able to reduce lysosomal Gb3 deposits more efficiently than the free enzyme, thanks to the stabilization of the GLA enzyme and the enhanced internalization offered by the RGD-targeted nanoliposomes [(4)]. Here, we report that *in vivo* pharmacokinetics and biodistribution of the commercially available GLA enzyme (Replagal) is also significantly improved by the use of nanoliposomal carriers. In detail, administration of nanoliposomal GLA increased the half-life of the enzyme from 8.5 to 12.1 min (Figure 2). Moreover, 30 min post-adminstration only 0.002% of the naked GLA (Replagal) administered to GLA knock-out mice was still in circulation, whereas ten times more enzyme was available in plasma at the same time point in mice receiving the nanoliposomal formulation (Figure 3A). Interestingly, liver accumulation of the GLA enzyme was reduced in a 96% in mice treated with nanoliposomal GLA compared to animals receiving naked GLA (Figure 3B).

In vivo efficacy assays measuring the Gb3 levels in Fabry mice treated with GLA nanoliposomes are currently ongoing, and will finally demonstrated whether the use of such nanoliposomes could eventually ease the treatment of Fabry patients.

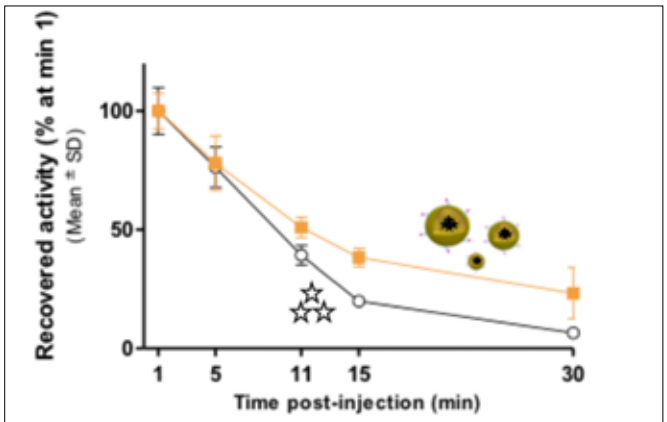

Figure 2 : Plasma stability of the free drug and nanoliposomes admnistered intravenously to Fabry mice at 1 mg/Kg. Half life times of 8.5 and 12.11 min were obtained for the free drug and the nanoliposome, repectively (p-value = 0.0018).

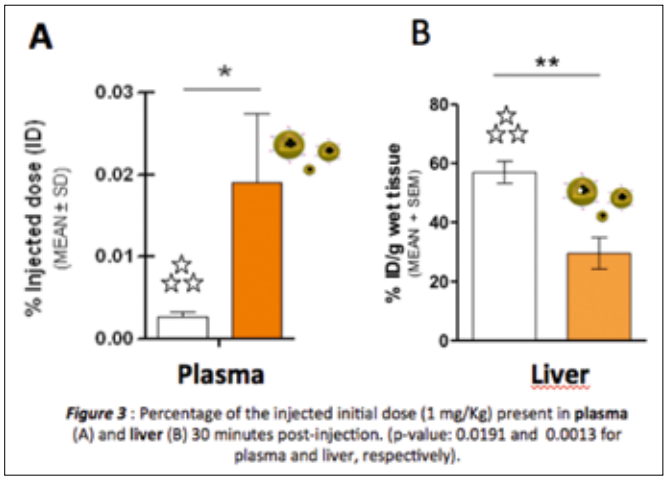

Figure 3 : Percentage of the injected initial dose (1 mg/Kg) present in **plasma** (A) and **liver** (B) 30 minutes post-injection. (p-value: 0.0191 and 0.0013 for plasma and liver, respectively).

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Acknowledgements
This work has been supported by the LIPOCELL project financed by CIBER-BBN and TERARMET project financed by the Spanish Ministry of Economy (RTC-2014-2207-1). Authors also acknowledge the recently granted funding by the European Commission through the H2020 initiative for the SMART4FABRY project (720942).

THE COMBINATION OF PH-RESPONSIVE PEPTIDE AND CATIONIC LIPOSOMES CAN IMPROVE SIRNA TRANSFECTION EFFICIENCY IN CANCER CELLS

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BACKGROUND

The presence of small interfering RNA (siRNA) in a cell leads to silencing of protein expression, offering the potential of a powerful therapeutic option for treatment of many conventionally intractable diseases. However, due to the excessive negative charge and sensitivity against RNase, siRNA has a short half-life in the blood

and struggles to penetrate within the target cells. Successful development of siRNA therapies depends on the ability for it to be efficiently delivered inside the target cells efficiently while avoiding enzymatic degradation and/or aggregation. Here, we present the design and optimisation of a cationic liposomal/pH responsive histidine rich cationic peptide based nano-carrier for siRNA delivery (lipid/peptide ternary complex). The combination of liposome and peptide generates a stable complex with a suitable particle size, good encapsulation and cellular uptake. Improved silencing abilities in comparison to commercial positive control (Lipofectamine RNAiMAX2000), aided by enhanced endosomal escape, suggest application both *in vitro* and *in vivo* to silence therapeutic targets in a variety of cancer cells.

METHODS

The two-major used components in the formulation are 1) DODAG (1 N', N'-dioctadecyl-N-4,8-diaza-10 aminodecanoylglycine amide) (Figure 1), which is an innovative cationic lipid which was designed to be a structural chimera involving the N(1)-cholesteryloxycarbonyl-3-7-diazanonane-1,9-diamine (CDAN) polar head group and the dialkylglycine amide moiety of dioctadecylamido glycylspermine (DOGS) to improve positively charged lipid interaction with siRNA. DODAG has been demonstrated to improve interaction with siRNA. 2) LAH4-L1 (Figure 2) is a 26-amino acid, histidine rich, amphipathic helical peptide (KKALLAHALH LLALLALHLA HALKKA). It is one of several membrane-active peptides that display increased affinity toward anionic lipids and possess DNA delivery capabilities. Liposomes (20% DODAG, DOPC/ cholesterol/DSPE-PEG2000)/siRNA, LAH4-L1/siRNA (Figure 3) and their combination with siRNA (Figure 4) have been investigated as delivery systems. Their physicochemical characteristics such as size, zeta potential, siRNA retention (PicoGreen assay and gel electrophoresis studies), release behaviour and aggregation behaviour of formulations were tested. *In vitro* cell uptake and luciferase knock-down studies were also tested in MDA-MB231 and A549 cells. Complexes were administered intravenously in mice to test their *in vivo* biodistribution.

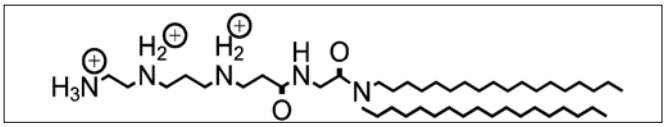


Figure 1: Structure of (DODAG) N',N'-dioctadecyl-N-4,8-diaza-10-aminodecanoylglycine amide.

Figure 2: Cartoon representing the amphipathic secondary structure of the LAH4-L1 peptide (Red: hydrophobic, Blue: Hydrophilic).

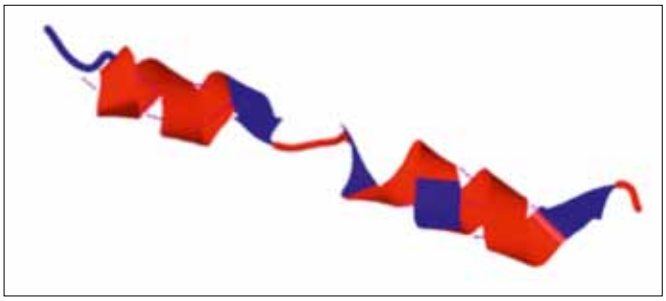
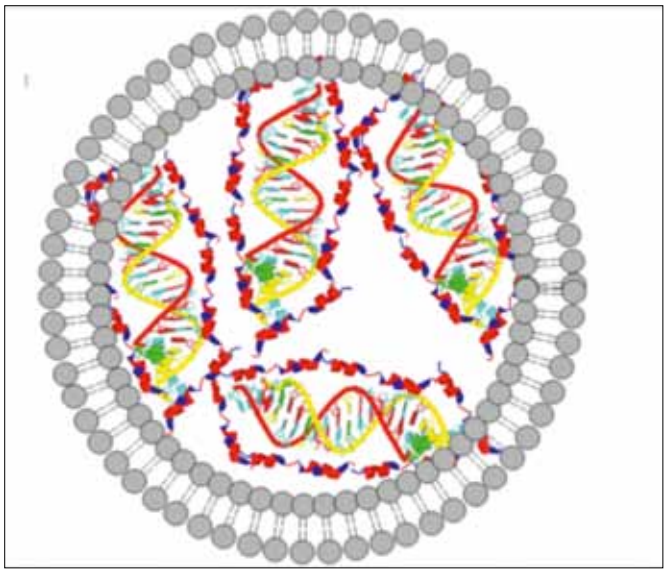


Figure 3: schematic representation of the expected shape of LAH4-L1/siRNA complex

Figure 4: Schematic representation of the expected shape of the Lipid/Peptide/siRNA complex (ternary complex)



RESULTS

The hydrodynamic diameter of the particles was characterised; (siRNA/liposomes, peptide/siRNA and ternary complex; 100 - 115 nm, 130 - 180 nm, 125 - 150 nm, respectively) and zeta potential (siRNA/liposomes, peptide/siRNA and ternary complex, +10 to +20 mV +25 to +35mV, +20 to +25 mV, respectively). All complexes associated with siRNA have more than 80% complexation efficiency. Although lipoplexes showed better encapsulation, they were less stable as partial release was observed after co-incubation in serum. Ternary complexes showed a better protection for the siRNA as they could retain siRNA when tested in different serum conditions for stability. Improved cell uptake was seen with ternary complex in comparison with liposomal and Peptide siRNA complexes (A549 percentage cells uptake of FAM-labelled siRNA increased from 1.4% for siRNA/Liposomes to 32.9% for the Ternary complex). *In vitro* studies suggest improvement in gene silencing with ternary complex and *in vivo* studies are ongoing to understand the pharmacokinetic behaviour of the particles.

CONCLUSIONS

A stable lipid/peptide ternary complex for siRNA delivery with defined physicochemical properties was designed and evaluated and shown to be a promising delivery system for siRNA for both *in vitro* and *in vivo* applications. Current and future work is focused, *in vitro*, on the improvement of cell targeting and *in vivo* on studying the PK/PD properties of the new system.

Acknowledgment:
This work has been funded by Schlumberger Foundation- Faculty for the Future theme.

INVESTIGATION OF SURFACE MODIFICATION DEPENDENT TRANSLOCATION OF GOLD NANOPARTICLES ACROSS THE HUMAN PLACENTA USING IN VITRO AND EX VIVO APPROACHES

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Pharmacotherapies for pregnant women mostly rely on postmarketing surveillance, since these women are radically excluded from

many clinical trials. For pharmaceutical industries, investigation is risky, cost-intensive and another thalidomide debacle would be highly destructive for the whole company, in addition to the potential harm that could be inflicted to the pregnant woman and her unborn child. However, considering that many women are taking drugs during pregnancy, novel safe therapeutic strategies to specifically treat maternal, fetal or placental diseases are urgently needed. One promising strategy is the use of nanoparticles (NP) as drug carriers, and numerous applications especially target-ing cancer are currently under clinical investigation [1, 2]. However, only a few studies have explored the applicability of NP-based approaches for pregnant women. For example, liposomes decorated with tumor-homing peptides were able to deliver insulin-like growth factor 2 to murine placenta, improving fetal weight of growth restricted pups without affecting wild-type ones [3]. In another study, drug-conjugated polyamidoamine (PAMAM) dendrimers were found to barely cross the human placenta in ex vivo perfusions, thus providing the first promising strategy for NP-based treatment of maternal diseases [4]. Placental complications such as choriocarcinoma could be potentially treated with nanocells containing doxorubicin, as demonstrated by *in vitro* and ex vivo studies [5]. However, the impact of NP properties and surface modifications on placental uptake, accumulation and translocation as well as the underlying transport mechanisms are not yet fully understood [6].

Here, we investigated the influence of particle surface modifications on placental transfer using 3 nm PEGylated and 4 nm carboxylate spheric gold NPs (Au-3-PEG and Au-4-COONa NPs). Au is often used as a core matrix in NP-based drug delivery or diagnostic studies due to its unique characteristics [7]. Translocation of Au NPs was determined in ex vivo perfusions of human term placentas and in a newly developed *in vitro* co-culture model. Interestingly *in vitro* data demonstrated that Au-3-PEG NPs did not penetrate the trophoblast cells, which constitute the first cell barrier for compounds deliv-ered via the maternal blood (Figure 1). In contrast, Au-4-COONa NPs were internalized and accumu-lated in the trophoblast layer but did not cross to the basolateral side. Human ex vivo perfusion studies confirmed the absence of fetal transfer of Au-3-PEG and Au-4-COONa NPs. These findings indicate that it is possible to steer placental uptake and tissue accumulation of Au NPs by tailoring their surface modification. The absence of fetal transfer suggests that such Au NPs could be further explored as a drug carrier to treat pregnant women or placental complications with reduced off-target effects.

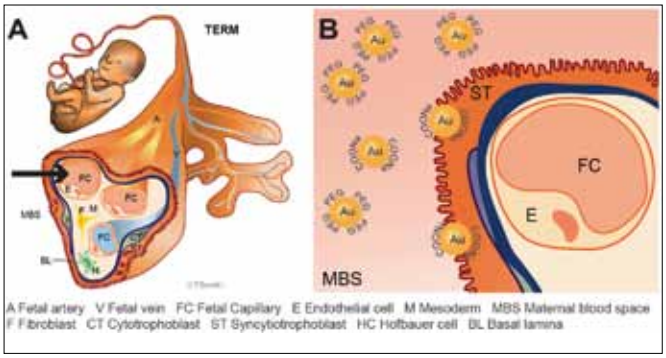

Figure 1: Nanoparticle translocation at the human placental barrier. (A) Scheme of the human placental barrier structure at the end of pregnancy and potential uptake pathway for NPs present in the maternal blood stream (black arrow). (B) Uptake and translocation studies using human in vitro and ex vivo placenta models demonstrate the absence of placental transfer of Au-3-PEG and Au-4-COONa NPs. However, Au-4-COONa NPs accumulated in the trophoblast cells whereas Au-3-PEG NPs were not internalized by trophoblast cells. Figure was reproduced and modified with permission of T. Buerki [8].

Acknowledgments: This research is supported by funding from the 7th Framework Program of the European Comission (EC-FP7-NANOSOLUTIONS-309329) and the BMBF-project NanoUmwelt (03X0150).

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TARGETING HYPOXIA IN 3D TUMOUR SPHEROIDS USING NOVEL COPPER-TIRAPAZAMINE NANOPARTICLES

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INTRODUCTION:

Hypoxia plays a key role in promoting angiogenesis, metastasis, and drug resistance. Tirapazamine (TPZ) is the most advanced hypoxia-activated prodrug and has shown great specificity and potency in inhibiting tumour growth. It is currently in phase III clinical trials to treat non-small cell lung cancer and cervical cancer, and its efficacy *in vivo* has been limited due to its rapid metabolism, and inadequate diffusion in the tumour mass. This project offers a new strategy to enhance the therapeutic efficacy of TPZ by developing a novel nanoparticlel-based delivery system, that efficiently encapsulates TPZ as a cupric-complex [$Cu(TPZ)_2$]. The system developed herein could offer an enhanced penetration in tumour tissues, leading to a higher therapeutic efficacy in cancer patients.

METHODS:

$Cu(TPZ)_2$ complexes were prepared to improve the encapsulation of TPZ in liposomes. Next, a remote loading method was developed to stably encapsulate $Cu(TPZ)_2$ in different liposomal formulations. Liposome physicochemical properties (size, surface charge, and stability) and morphology were determined using dynamic light scattering (DLS), and transmission electron microscopy (TEM), respectively. The cytotoxicity of TPZ, $Cu(TPZ)_2$ and $Cu(TPZ)_2$–loaded liposomes were assessed *in vitro* using 2D and 3D prostate tumour models. The development of hypoxia was validated in both models, using the CYTO-ID® Hypoxia/Oxidative Stress Detection kit. Cytotoxicity was assessed using resazurin cell viability assay, and spheroid growth delay assay.

RESULTS:

In this work we systematically evaluated the effect of buffer pH, incubation temperature, time, and lipid content on $Cu(TPZ)_2$ complexes loading into liposomes. Our results showed high encapsulation (>70%, determined by HPLC) for all lipid formulations prepared. Temperature, buffers and the incubation time dramatically

affected the final encapsulation efficiency. We also observed that drug loading increased at higher drug:lipid ratios, and good drug retention was observed over-time (4ºC up to 1 month). $Cu(TPZ)_2$ complexes and liposomal formulations maintained their selectivity under hypoxia. More interestingly, higher toxicity was observed with our liposomal formulations, compared to the free drug. Furthermore, their toxicity was dependent on the cell line, drug concentration, and the incubation time used.

CONCLUSION:

This is the first study showing $Cu(TPZ)_2$ loading into a wide range of liposomal formulations, with enhanced toxicity in 3D tumour spheroids. These results are encouraging to assess the therapeutic efficacy of these novel liposomal formulations *in vivo* models, which could offer a promising approach to target hypoxia in advanced prostate cancer patients.

Acknowledgements: This work was supported by Prostate Cancer UK (Grant CDF-12-002), the Engineering and Physical Sciences Research Council (EPSRC) (EP/M008657/1), and University of East Anglia.

OPTIMIZING CISPLATIN THERMOSENSITIVE LIPOSOMES TO IMPROVE THERAPEUTIC EFFICACY AT MILD HYPERTHERMIA IN C26 TUMOR BEARING BALB/C MICE

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Two of the greatest hurdles of the current traditional chemotherapy are reduced bioavailability at tumor site and undesirable side effects. SPI077™, the stealth cisplatin liposomes failed to show improved therapeutic outcomes in clinical trials due to the poor cisplatin encapsulation and insufficient spontaneous drug release at the tumor site. Local hyperthermia (HT) is an external trigger that can be applied to induce controlled drug release at the tumor site. Since then, various thermo-responsive liposomes were developed to improve the pharmacokinetics features of lipid vehicles. A lipid membrane such as SPI077™ demonstrates an almost negligible release *in vivo*, likely due to the high content of HSPC. On the other hand, cisplatin thermo-responsive liposomes (TSLs) developed based on Thermodox® formulation showed instability and premature drug leakage following injection even at normal physiological temperatures. These two strategies represent two extreme situations of release kinetics that depend on the exact lipid composition of liposomes, resulting in quite different therapeutic outcomes. This study reports on the activity of cisplatin thermosensitive liposomes incorporating different HSPC ratios in DPPC/MSPC/PEG2000-DSPE matrix (90/10/4) plus mild HT (42 °C).

METHODS

For the first time, cisplatin TSLs with different molar ratios of HSPC (90, 60, 45, and 30) in a DPPC/MSPC/PEG2000-DSPE matrix were prepared and analyzed *in vitro* and *in vivo*. TSLs were loaded with the poorly membrane permeable anticancer drug, cisplatin, through the passive equilibration method. Photon correlation spectroscopy and DSC measurements were performed to assess size, zeta potential and Tm of different formulations. Drug release profiles, *in vitro* cytotoxicity under different thermal conditions (37 and 42 ºC) and *in vivo* plasma stability under normothermia were studied. For the comparative tumor efficacy and survival study, selected formulations were compared under one- and two-step HT treatments and normothermia condition in mice model bearing C26 colon carcinoma. In one-step hyperthermia approach, HT was initiated 15 min before liposomes administration and continued for up to 1 h. In a two-step HT, one HT session was applied before the injection of liposomes for 1 h and the second session was 24 h after the liposome injection for 45 min to trigger cisplatin release. Local HT was applied by immersing the tumor-bearing leg into a water bath to achieve a more uniform temperature and drug distribution throughout the tumor.

RESULTS

The incorporation of HSPC into the DPPC bilayers altered the thermotropic phase behavior of the membrane and changed the rigidity of bilayer structure. The addition of HSPC to the corresponding DPPC lipid matrix increased the transition temperature of liposomes (90 nm). *In vitro* data demonstrated >90% cisplatin leakage from nano-sized DPPC 90-lyso-TSL (LTSL) within 10 min at 42 °C, while other TSLs bearing HSPC showed greater stability (Fig. 1).

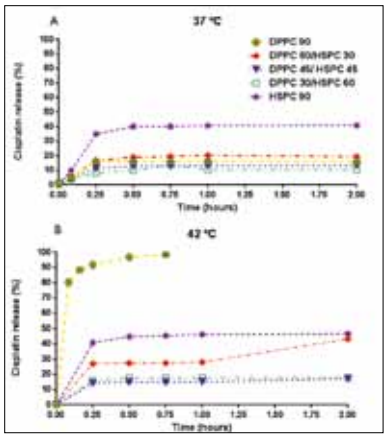

Figure 1. Temperature dependent in vitro release of different cisplatin thermosensitive liposomes

The plasma kinetic of cisplatin demonstrated higher cisplatin leakage from DPPC 90-LTSL in the first 4 h (from 17.4 to 0.4 µg/ml) compared to other formulations. Indeed, increasing HSPC fraction in liposome bilayers significantly improved drug retention in blood (Fig. 2).

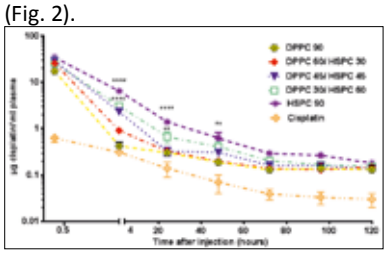

Figure 2. Plasma kinetics for the liposomal carriers and cisplatin after iv administration in non-tumor bearing BALB/c mice under normothermia condition

Though DPPC 90-LTSL plus one-step hyperthermia was expected to provide a unique drug release, the premature drug leakage as well as the likely wash-back of a great portion of drug into the blood circulation resulted in reduced survival. On the other hand, stabilized DPPC 30/HSPC 60 /MSPC 10/PEG2000-DSPE 4 liposomes plus two-step hyperthermia greatly enhanced the survival of animals (Fig.3).

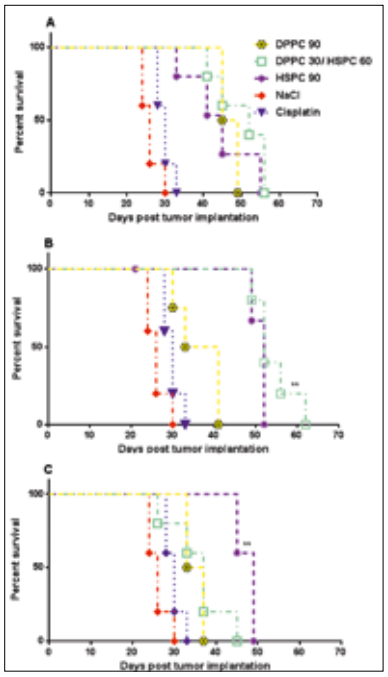

Figure 3. In vivo survival experiments in female BALB/c mice bearing C26 colon carcinoma tumor. Local HT was applied by immersing the tumor-bearing leg into a 43 °C water bath. Therapeutic efficacy was evaluated under (A) one-step HT (B) the 2-step HT protocol, and (C) normothermia condition

We also observed that simultaneous administration of DPPC 90-LTSL with HT did not prolong the survival of animals as expected. While, we found that injection of stabilized DPPC 30/HSPC 60 liposomes plus two-step HT resulted in the best survival of animals compared to all other treatment approaches. Administration of stabilized DPPC 30/HSPC 60 formulation with

two-step HT was possibly associated with the enhanced extravasation in the tumor area. Though DPPC 90-LTSL plus one-step HT provides bioavailable drug delivery, premature drug leakage and poor tissue penetration of cisplatin hamper treatment effects. Stabilized DPPC 30/HSPC 60 liposomes on the other hand plus two-step hyperthermia improved survival of animals. On the basis of the present data, we conclude that the balance between the *in vivo* stability, blood circulation time and release kinetic of TSLs determines the desired therapeutic efficacy. Further, the optimal tuning of TSL and moment of triggered release is dictated by the physicochemical properties of the encapsulated drug.

DISCUSSION

Stabilizing liposomal formulation is a prerequisite to ensure the delivery of sufficient drugs to the tumor site. We hypothesized by increasing HSPC fraction in LTSL matrix, the stability of TSL liposomes would improve. We observed that DPPC 30/HSPC 60 formulation improved drug retention in plasma under physiological conditions, while DPPC 90-LTSL displayed considerable leakage under the same circumstances. Therefore, our next question was whether a 2-step HT approach with stabilized liposomes outperforms the simultaneous HT together with DPPC 90-LTSL. *In vivo* data following tumor size evaluation and survival clearly indicates the positive impact of the choice of HT treatment on therapeutic outcome. When DPPC 90-LTSL was used plus two-step HT, no improvement in tumor growth delay was observed at ethical endpoint. In this case, the fast-release liposomes were apparently depleted of drug due to instability.

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MULTIFUNCTIONAL NANO-IN-NANO-COMPLEX DESIGNED FOR ENHANCED CELL UPTAKE, FAST ENDOSOMAL ESCAPE AND IMPROVED THERAPEUTIC EFFICIENCY

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Over the past few decades, exciting advances in nanotechnology have led to significant progresses in the biomedical field, with extensive focus being drawn to cancer diagnostics, targeted drug delivery and therapy.[1] More recently, breakthroughs in nanotechnology have paved the way for a new era of cancer theranostics, by creating the opportunity to design and engineer multifunctional nanoplatforms, which simultaneously integrate therapeutic and multimodal imaging modalities.[2]

Among the large variety of multifunctional nanosystems that have been revolutionizing the biomedical field,[3] porous silicon (PSi) nanoparticles have demonstrated tremendous potential for drug delivery applications, owing to its advantageous physicochemical and biological properties, with particular emphasis in cancer nanomedicine.[4] The sponge-like nanostructure of PSi nanoparticles enables the incorporation of diverse therapeutic agents within its pores, including hydrophobic drug molecules, with reproducible loading and release profiles.[5] In addition, PSi nanoparticles are characterized by superior biocompatibility,[6] biodegradability[7] and surface chemical versatility.[8] However, its limited cellular association and internalization, as well as the incapacity for breaking out from the intracellular endosomal compartments still stand as major deadlocks defying the implementation of these nanocarriers as effective anticancer drug delivery systems. Therefore, there is a continuing need for improving the performance of PSi nanoparticles at the cellular and intracellular levels, while preserving their unique properties for biomedical applications.
An interesting approach for improving the cellular internalization and intracellular trafficking of nanomedicines involves the design of multivalent cationic non-viral vectors, particularly relevant for transporting and delivering therapeutics into the cytosolic compartment of malignant cells.[9] For that purpose, cationic polymers, especially the widely investigated polyethyleneimines (PEIs), have been used to complex the negatively charged moieties, generating supramolecular nanostructures known as polyplexes.[10] However, major concerns arising from PEIs' acknowledged severe cytotoxicity and non-biodegradability, have hindered their further application in cancer nanomedicine.[11]

OBJECTIVE

Herein, we envisioned to fabricate a multifunctional nano-in-nanocomplex platform encapsulating both sorafenib (SFB)-loaded PSi and gold (Au) nanoparticles into a polymeric nanocomplex (CPP). This novel approach aims to enhance the interaction of the PSi nanocarriers with the cancer cells and induce their endosomal escape, ultimately improving the cytoplasmatic delivery and, consequently, the therapeutic efficacy of the loaded anticancer agents.

RESULTS

The PSi nanoparticles were fabricated by an electrochemical etching method, after which the surface was stabilized and rendered hydrophobic by thermal hydrocarbonization, and subsequently functionalized with carboxylic acid moieties (UnTHCPSi).[12] The CPP nanocomplexes composed of three different polyelectrolytes, namely L-cysteine, namely L-cysteine, PEI and poly(methyl vinyl ether-alt-maleic acid) (PMVE-MA) were prepared by an ionotropic gelation technique. The UnTHCPSi@CPP (UnCPP) and UnTHCPSi/Au@CPP (UnAuCPP) multifunctional nanocomposites were obtained by nanoencapsulating UnTHCPSi nanoparticles and both UnTHCPSi and Au nanoparticles into the CPP nanocomplexes, respectively, as depicted in Figure 1A.
The produced 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 (Figure 1B). The success of the nanoencapsulation of UnTHCPSi and Au nanoparticles into the CPP nanocomplexes was confirmed by investigating the presence of chemical elements characteristic of these nanoparticles, using energy dispersive X-ray (EDX) microanalysis (Figure 1C)

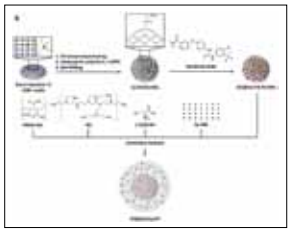
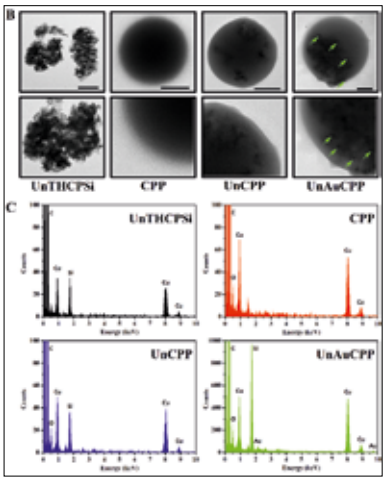


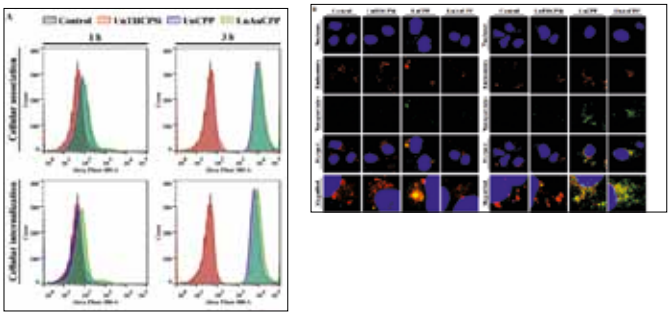
Figure 1. (A) Schematic illustration of the production of SFB@UnAuCPP nanocomplexes, including the fabrication of UnTHCPSi nanoparticles, loading of SFB into UnTHCPSi nanoparticles and nanoencapsulation of the SFB@UnTHCPSi and Au nanoparticles into CPP nanocomplexes by ionotropic gelation technique. (B) TEM images of UnTHCPSi, CPP,



UnCPP and UnAuCPP nanoparticles. Examples of the localization of encapsulated Au nanoparticles are highlighted by the green arrows. Scale bars are 100 nm. (C) EDX spectra of UnTHCPSi, CPP, UnCPP and UnAuCPP nanoparticles, confirming the presence of silicon (Si) and gold (Au) elements in the composition of the UnCPP and UnAuCPP nanocomplexes.

In addition, the multifunctional nanocomplexes were evaluated *in vitro* for their cytocompatibility and hemotoxicity, and the cellular association and internalization by MDA-MB-231 breast cancer cells was investigated by TEM and flow cytometry analysis (Figure 2A). Furthermore, the endosomolytic effect of nanocomplexes, after being internalized by the MDA-MB-231 breast cancer cells, was investigated by inverted confocal fluorescence microscopy (Figure 2B).

Figure 2. (A) Flow cytometric analysis of the cellular association and internalization of UnTHCPSi, UnCPP and UnAuCPP nanoparticles in MDA-MB-231 breast cancer cells. The measurements were performed before and after extracellular fluorescence quenching, using a trypan blue solution, for evaluating both cellular association and internalization of the nanoparticles with the cells, respectively. (B) Confocal fluorescence microscopy analysis of the MDA-MB-231 breast cancer cells after incubation with UnTHCPSi, UnCPP and UnAuCPP nanoparticles for 1 h and 3h.



The chemotherapeutic agent SFB was loaded into the pores of the UnTHCPSi nanoparticles by an immersion method, prior to their nanoencapsulation
into the CPP nanocomplexes, the *in vitro* drug release profiles of the SFB-loaded nanoparticles were studied in physiologically relevant media.
Finally, The *in vitro* chemotherapeutic efficacy of the different nanoparticles was evaluated by measuring their anti-proliferative effect on the breast cancer cell line studied.

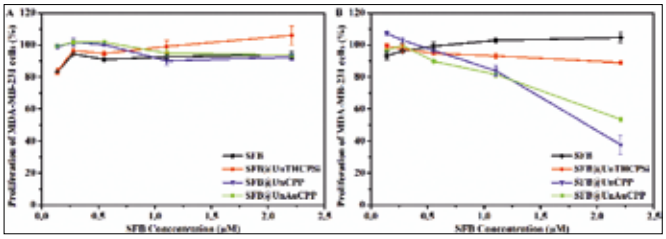


Figure 3. In vitro anti-proliferative effect of pure SFB, SFB@UnTHCPSi, SFB@UnCPP and SFB@UnAuCPP on MDA-MB-231 breast cancer cells after 6 h (A) and 24 h (B).

CONCLUSIONS

We have successfully produced a novel nanoplaform consisting of a polymeric nanocomplex simultaneously encapsulating PSi and Au nanoparticles, and further investigated its potential for cancer therapy applications. The resulting nanocomposites preserved some of the key physicochemical and biological characteristics of PSi nanoparticles and improved their *in vitro* performance at the cellular level, by significantly enhancing the cellular association and internalization. The developed UnCPP and UnAuCPP nanocomposites exhibited an increased endosomolytic effect, possibly by means of a PEI-mediated "proton-sponge" mechanism, enabling an efficient cytoplasmic delivery of the encapsulated SFB-loaded PSi nanoparticles. Finally, the SFB-loaded multifunctional nanoplatforms demonstrated a potent chemotherapeutic efficacy, preventing the *in vitro* proliferation of MDA-MB-231 breast cancer cells at very low SFB inhibitory concentrations. In addition to providing a robust evidence of the great promise of these multifunctional nanocomposites for the intracellular delivery of chemotherapeutics, the data reported here is a valuable proof-of-concept for the further development of these nanoplatforms for cancer theranostic applications.

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UPTAKE OF NANO-SIZED CARRIERS BY POLARIZED CELLS

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The use of nano-sized drug carriers in medical applications requires molecular-level understanding of how these objects interact with cells in physiological environment. So far, numerous studies have been conducted on standard cell cultures of different origin, typically constituted of individual non-polarized cells. However, it is well known that several processes – including for instance endocytosis - are strongly affected by cell polarization. Within this context, the aim of this project is to study the effect of cell polarization on the uptake of nano-sized materials. We have used primary human umbilical vein endothelial (HUVEC) cells as a polarized cell model. Endothelial cells were selected since they represent an important barrier that nanomedicines encounter before they eventually reach their target cells.
In order to ensure optimal polarization and barrier formation prior to performing nanoparticle uptake studies, extensive optimization of HUVEC cell growth was performed. Several techniques were used to characterize cell polarization and barrier formation, i.e. transendothelial electrical resistance (TEER) measurements, paracellular permeability (PP) assay, and confocal microscopy to monitor the expression and organization of tight junction proteins, together with gene expression of key proteins involved in endocytosis. Thus, the uptake of nanoparticles was assessed on the optimized HUVEC barriers using flow cytometry (Fig 1). Furthermore, we also investigated the effect of cell polarization on the uptake mechanisms by using a combination of endocytosis inhibitors.
Overall our results suggest that internalization of nanoparticles even within the same endothelial cells varies as they develop polarity and form a barrier.

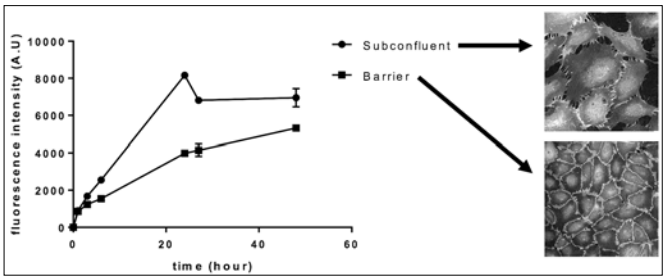

Figure 1. Nanoparticle uptake in HUVEC subconfluent cells or optimized barriers

HOW THE BIOLOGICAL PERFORMANCE OF TAT-MODIFIED LIPOSOME IS AFFECTED BY ITS ASSOCIATED PROTEIN CORONA AND LIPOSOME AVIDITY TO TUMOR CELLS

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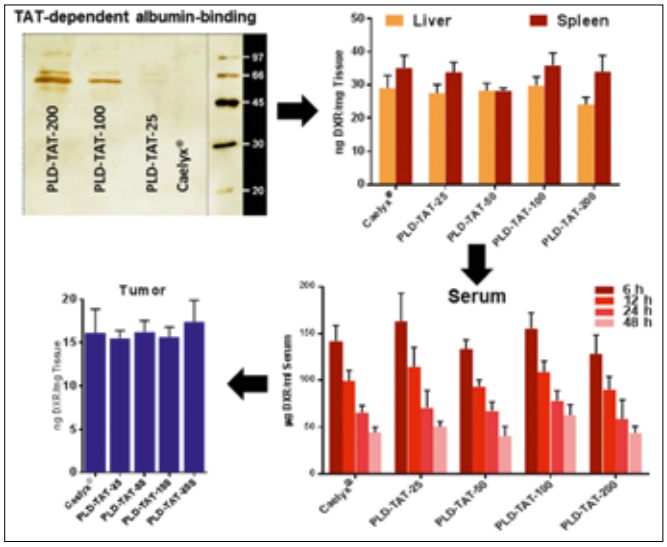
PEGylated liposomes are widely used and studied as carriers for chemotherapeutics. While pharmacokinetics of the encapsulated drug is drastically altered resulting in favorable circulation time, improved tumor accumulation and better manageable or reduced side-effects, therapeutic efficacy has been disappointing. Major drawbacks are a failure to reach the tumor cell, limited penetration depth and retarded uptake by tumor cells.
Here we study the implication of HIV-1 TAT-derived peptide inserted on PEGylated liposomal doxorubicin (PLD) and followed its fate *in vitro* and *in vivo*. PLDs were installed with 25 to 400 TAT-peptides per liposome, which can be done in an efficient and reliable way, without an effect on PLD stability. While TAT-peptides facilitate active endocytosis of the carriers, we observed that these peptides did not promote endosomal escape nor enhanced intracellular availability of doxorubicin. Interestingly, incorporation of TAT-peptides did not change pharmacokinetics or biodistribution of the liposomes, which we found to result from dysopsonization of the TAT-modified liposomes by serum proteins. An albumin-rich protein corona (PC) formed on TAT-peptide-modified PLDs shielding the active moieties from the reticulo endothelial system (RES) arms during circulation, which resulted in identical clearance rate and identical tumor accumulation for all targeted and non-targeted preparations (Figure 1).

Figure 1: SDS-PAGE analysis of serum treated liposomes revealed a TAT-dependent albumin binding on TAT-modified liposomes. Biodistribution of liposomes after single vein injection of 15 mg/kg liposomal doxorubicin revealed no specific uptake of TAT-modified liposomes by the spleen and liver which resulted in identical circulation life and finally similar tumor accumulation.



However, intratumoral fate was influenced by the number of TAT-peptides present. The best antitumor activity was observed with a TAT-peptide density of 100, while lower amounts showed results comparable to unmodified PLDs. At 200 TAT-peptides, the preparations appeared to be least effective, which we observed to result from augmented interaction with tumor cells directly upon extravasation (Figure 2). We conclude that optimized TAT-peptide PLDs balances pharmacokinetics with avidity and tumor penetration in the presence of a PC.

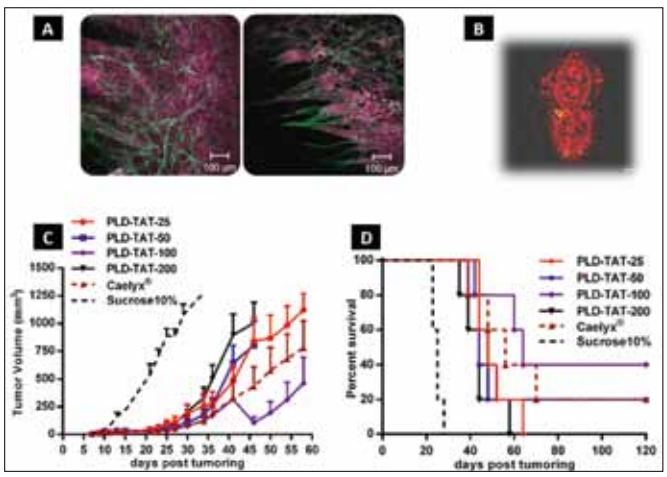


Figure 2: Intravital confocal imaging of intratumoral behaviors of TAT-modified liposomes revealed high avidity of TAT-liposomes against tumor cells which restricted their penetration depth just close to tumor vasculature (A). intracellular fate of TAT-modified liposomal doxorubicin revealed a delayed nucleus delivery of doxorubicin (B). therapeutic efficacy studies revealed that number of TAT installed on liposomes should be optimized to make avidity and penetration depth balanced. PLD-TAT-100 was the optimized preparation that resulted in better antitumor responses compared to other preparations after a single iv injection of 15 mg/kg liposomal doxorubicin (C and D).

NOVEL CELL PENETRATION ENHANCER DECORATED LIPOSOMES FOR OLIGONUCLEOTIDES DELIVERY

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INTRODUCTION

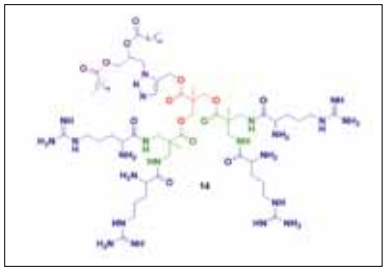
The use of oligonucleotides (OGNs) as therapeutic agents has emerged recently as a promising approach to treat genetic disorders such as cystic fibrosis or muscular dystrophy but also cardiovascular disorders or cancer, the latter being the major cause of mortality in the western population. In this scenario, the development of innovative and efficient methods to selectively deliver the therapeutic OGNs to the target cells has emerged as a major goal in the biopharmaceutical research arena [1].

Non-viral vectors are currently being studying since they are less immunogenic respect to viral vectors and particularly interesting for the delivery of short OGNs sequences, such as the siRNA and miRNA. Among non-viral vectors, cationic liposomes have been widely explored in virtue of their capability to condense the OGNs and protect them from enzymatic degradation[2, 3]. In this contest, we aim here to develop an innovative liposomal platform that can readily condense OGNs and possess high cell penetration capacity to deliver the therapeutic macromolecules. To do that, we exploited a newly synthesized polycationic cell penetration enhancer (CPE) lipid that was integrated in the liposome components. This non-peptidic CPE was designed to promote the OGNs loading efficiency in the liposomes and to favor access of the lipidic vesicles to the cytosol and the payload delivery. Notably, this synthetic CPE mimics the naturally occurring CPEs, such as the TAT peptide, but does not share the peptidic nature with them thus avoiding their drawbacks such as poor chemical and enzymatic stability, risk of toxicity and immunogenicity and the intrinsic biological activity [4].

The mechanism of activity of CPEs is hypothesized to rely on the high density of positive charges that ensures for the cell penetration activity according to a charge-to-charge interaction with the negatively charged proteoglycans on the cell membrane [5]. Notably, the CPEs do not possess cell type specificity which can yield unspecific tissue distribution of a carrier decorated with CPEs. Thus, the interfacial features of nanocarriers must be carefully considered when developing this class of therapeutic vehicles. For this reason, the liposomes surface decoration with mPE_{G2kDa}-DSPE or $mPEG_{5kDa}$-DSPE was included in the nanocarrier design in order to tune the lipoplexes stability and cell penetration behavior while conferring "stealth" properties to the nanosystem.

MATERIAL AND METHODS

Synthesis of the cell penetration enhancer (CPE). The CPE module was ex novo synthetized according to a multi-step procedure by conjugating 4 arginines to a second generation polyester dendron based on 2,2-bis(methylol)propionic acid, that was end terminated with 1,2-distearoyl-3-azidopropane by click chemistry as liposome bilayer anchor. The chemical structure of the derivative is reported in scheme 1. Each intermediate and the final CPE were characterised by NMR and mass spectrometry.



Scheme 1. Chemical structure of newly synthetized dendronic CPE.

Lipoplexes formulation and characterization.

Liposomes were prepared by the "thin layer hydration technique" using 2:1 HSPC/cholesterol molar ratio with 4% mol of the CPE with respect to the lipids. The lipidic film was rehydrated with a 19 bases dsDNA solution in HEPES buffer in order to obtain lipoplexes with an N/P ratio from 1 to 10. The dsDNA non-associated to liposomes was removed by dialysis. Then, the lipoplexes were surface decorated with 1.25-5% mol of mPEG2kDa-DSPE or mPEG5kDa-DSPE with respect to the lipids. Lipoplexes loading efficiency and capacity were assessed by spectrophotometric analysis. Lipoplexes with NP ratio of 5 and 10 were selected for further investigations. Size, zeta potential (ZP) and stability of lipoplexes were assessed by DLS analysis in HEPES buffer and cell culture medium (DMEM) supplemented with 10% of FBS at 37°C. Cell studies were performed using lipoplexes generated with cyanine3-labelled dsDNA (dsDNA-Cy3). Cell studies. MTT cell viability assay was performed on human breast adenocarcinoma MDA MB 231 by 24h incubation with dsDNA-loaded lipoplexes obtained with a 5 and 10 N/P ratio and coated with 2.5 and 5 mol% of mPEG-DSPE 2 or 5 kDa at a dsDNA concentration of 50, 125 and 250 nM. Lipoplexes obtained with a 10 N/P ratio with or without either of the two PEG coatings were used at a 125 nM dsDNA concentration to perform flow cytometric analysis. Cells were incubated for 2h with 300 µL of each lipoplex sample and then were harvest in flow cytometer tubes, washed several times with PBS and analysed with a FACS CANTO Instrument (BD Bioscience Instrument, USA).

RESULTS AND DISCUSSION

An innovative CPE to be combined with liposomes was efficiently synthetized. The novel dendronic CPE was design to possess high cationic charge density provided by 4 arginines. Furthermore, we hypothesized that this CPE can promote OGNs loading into liposomes. Indeed, the CPE was end terminated with a dialkyl moiety for the anchoring to the liposome bilayer thus generating a vesicular carrier for OGNs. Investigations were carried out to identify the formulation conditions and compositions that allow for the most efficient dsDNA loading of lipoplexes while ensuring adequate colloidal stability. Lipoplexes obtained with CPE decoration (NO mPEG-DSPE) and formulated with a N/P ratios above 5 showed high stability and dsDNA loading capacity. These formulations displayed a size of about 250 nm and a positive ZP of +16 mV. The lipoplexes loading efficiency was calculated to be 52% and 70% for the formulations obtained with 5 and 10 N/P ratios, respectively, corresponding to loading capacities of 16.0 and 10.8 µg dsDNA/mg lipid, respectively. The control lipoplexes prepared without CPE yielded 6.3- and 8.1-fold lower encapsulation efficiencies than that obtained with the CPE decorated lipoplexes prepared at 5 and 10 N/P ratios, respectively.

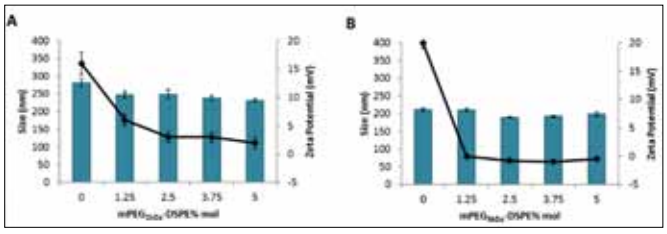


Figure 1. Size (histograms) and zeta potential (solid line) profiles of lipoplexes obtained with 10 N/P molar ratios and coated with increasing ratio of mPEG2kDa-DSPE (A) and mPEG2kDa-DSPE (B).

The lipoplex formulations obtained with CPE at 5 and 10 N/P molar ratios were selected since they demonstrated to be very stable

over seven days in HEPES buffer. However, since these formulations underwent rapid opsonisation in DMEM added of FBS, mPEG-DSPE coating was added (Figure 1). The stability studies in DMEM+FBS revealed that the $mPEG_{5kDa}$-DSPE coating provides for a better shielding of lipoplexes compared to the $mPEG_{2kDa}$-DSPE, with size and ZP values that remain constant up to 48h. The ZP analysis showed that the PEG coating shield, at least in part, the lipoplexes charge which may be responsible for the opsonisation and aggregation of these formulations in the presence of FBS proteins.Figure 2 shows the results of the *in vitro* studies on MDA MB 231 cells incubated with lipoplexes at 10 N/P molar ratio and coated with increasing ratio of the 2 and 5 kDa mPEG-DSPE. Cell viability analysis by MTT assay (Figure 2A) revealed that CPE-decorated lipoplexes without PEG coating possess a quite low toxicity while all formulations coated with PEG did not cause any significant alteration in term of cell viability compared to naked (CPE-free) lipoplexes.
Figure 2B shows the cytofluorimetric results of the lipoplexes coated or non-coated with PEG at 10 N/P molar ratio. Notably, the cell uptake of the CPE-decorated lipoplexes (NO mPEG-DSPE) is significantly higher with respect to naked lipoplexes obtained without CPE at the same N/P molar ratio. Moreover, despite the overall neutral charge of lipoplexes coated with $mPEG_{5kDa}$-DSPE, the cell association remains remarkable, suggesting that the CPE on the lipoplexes surface, while being hidden within the PEG chains, can be still revealed when the vesicles approach cell membrane and favour the interaction.

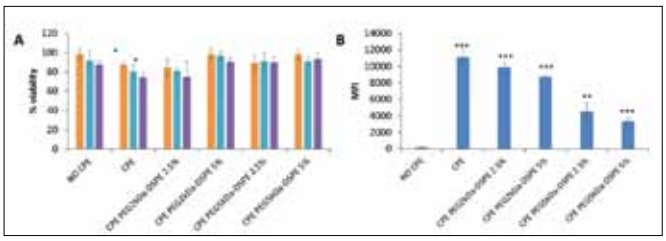


*Figure 2. A) Cytotoxicity profile of lipoplexes (10 N/P molar ratio) with different 2 kDa and 5 kDa mPEG-DSPE molar ratio incubated 24h with MDA MB 231 cells at 50 nM (•), 125 nM (•) and 250 nM (•) dsDNA concentration. (B) Cytofluorimetric cell association profile of the same lipoplexes formulation at 125 nM of dsDNA concentration incubated 2h with MDA MB 231 cells. Statistical analysis: * p<0.05; ** p<0.005; *** p<0.0001 respect to cells treated with lipoplexes without CPE.*

CONCLUSIONS

The formulation of lipoplexes using an innovative non-peptidic oligo-arginyl cell penetration enhancer provides for highly OGNs loading thsu providing a platform for the delivery of therapeutic siRNA. Systematic formulation studies showed that the surface decoration of these lipidic vesicles with mPEG-DSPE endows the lipoplexes with increased stability either in buffer or in cell culture media supplemented with 10% of serum. Preliminary *in vitro* studies on MDA MB 231 demonstrated that these carriers are devoid of toxicity on cells and that they promptly interact with cells. High association of these lipoplexes were obtained also when they were coated with mPEG5kDa-DSPE which decreased the zeta potential attributed to the CPE surface exposure, highlighting that CPE still plays a key role in lipoplex/cell interaction.

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EXOSOMES: DETECTION AND CHARACTERIZATION BY BIOPHOTONIC TECHNIQUES

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Exosomes have been proposed as potential biomarkers of multiple diseases. The quantity of exosomes released from cells and their composition changes according to the physio-pathological condition of the tissue of origin, mirroring their direct involvement in specific pathogenic mechanisms, including neurodegenerative processes like Alzheimer's disease. Nowadays, an effective method for the detection and characterization of exosomes is still needed. In order to better understand exosomes' biological function for a future exosomes-based clinical personalized application, we propose herein two biophotonic approaches: Surface Plasmon Resonance imaging (SPRi) that allows the study of exosomes interactions with biomolecules, and Raman spectroscopy (RS), suitable for exosomes characterization.
For SPRi experimental set up, first of all we tested commercial serum exosomes and then we extracted exosomes from plasma of healthy donors by size-exclusion chromatography. All samples were injected at low flow on a SPRi chip conveniently treated. The chip was coated with a self assembled monolayer, used to perform the conjugation of antibodies and to reduce unspecific binding. SPRi signals were collected to evaluate the interaction of antigens on exosomes membranes with the antibodies spotted on the chip.
RS was used for the chemical characterization of exosomes extracted by ultracentrifugation from different human mesenchymal stem cells of diverse tissue origin (bone marrow and adipose tissue).
We used RS with 532 nm laser source in the spectral ranges 500-1800 cm-1 and 2600-3200 cm-1 on air-dried drops of exosome suspensions, subsequently spectra were analyzed by means of multivariate statistical analysis (PCA-LDA) and Classical Least Squares (CLS) fitting with reference lipid molecules.
Taking advantage of the multiplexing capability of SPRi technology, both generic (i.e. CD9) and tissue specific (i.e. CD171/L1 as neuronal marker) biomarkers have been simultaneously analyzed on exosomes from plasma. The SPRi approach allowed to perform the concomitant characterization of multiple exosomes subpopulations(Figure 1).

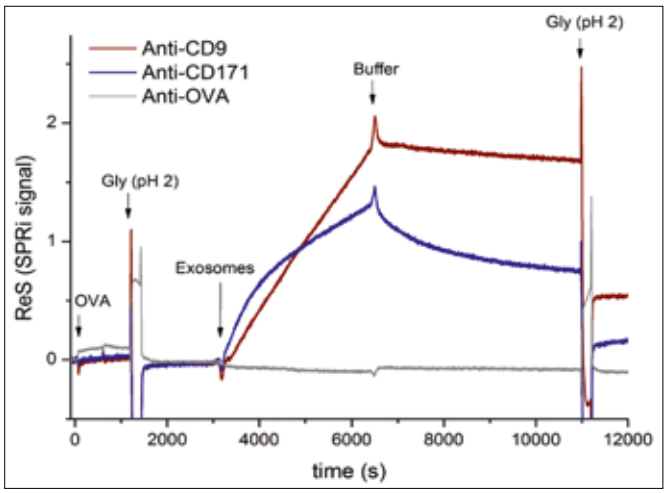

Figure 1: Multiplexing characterization of exosomes.The SPRi signals reveal the presence of specific antigens on the membrane of exosomes, showing the effective isolation of exosomes that express CD9 and CD171.

The collected Raman spectra showed the ability of the method to provide an overview of the chemical composition of exosomes,

with Raman peaks related to cholesterol, phospholipids, proteins and nucleic acids visible within the fingerprint (Figure 2).

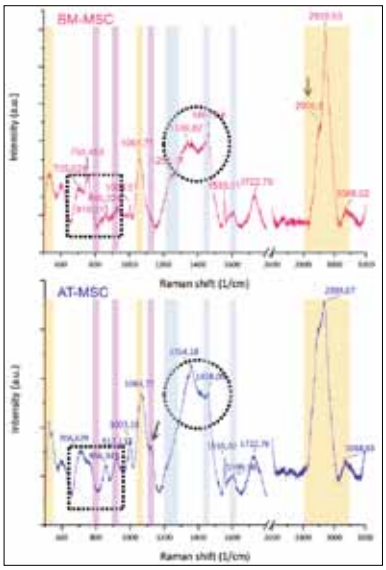

Figure 2: Raman fingerprint of exosomes from bone marrow (BM-MSC) and adipose tissue cells (AT-MSC). Average Raman spectra obtained with 532 nm excitation wavelength. Raman bands corresponding to lipids, proteins and nucleic acids are highlighted in yellow, blue, and red, respectively.

The analysis of the main Raman peaks, demonstrated a remarkable contribution of lipids to the recorded spectra that were further investigated by CLS fitting. RS allowed the calculation of the relative contribution of specific reference lipids to the recorded spectra, demonstrating the importance of ceramides and gangliosides in determining the fingerprint of MSC derived exosomes. PCA-LDA analysis demonstrates that, by RS. we can clearly distinguish vesicles released by different cell-types with good accuracy thanks to biochemical features typical of the cell/tissue of origin. The proposed approaches couple nanomedicine and biophotonic techniques to a biomedical issue that is currently limited in its clinical application by the lack of robust and innovative characterization methods. Our preliminary data suggest that SPRi technology has the potential to detect simultaneously different exosomes subpopulations and to provide information about the interaction with biomolecules, paving the way for a better understanding of their biochemical role. Regarding Raman analysis we can conclude that it can become an effective routine quality check method for exosomes before *in vitro* or *in vivo* use, being also more informative compared to other complementary techniques.In conclusion, SPRi coupled with RS could represent a valid and fast approach for exosomes characterization for their lipidic and proteic components

NANOPILOT PROJECT: HOW TO BRIDGE THE GAP BETWEEN BASIC RESEARCH AND FIRST GMP MANUFACTURING

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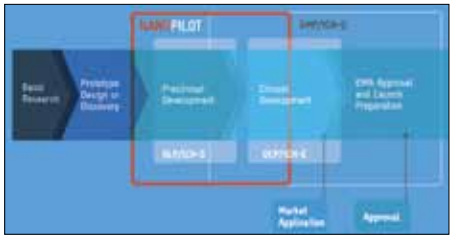
The European Technology Platform for Nanomedicine recorded more than 700 SMEs/Industries working in the field of Nanomedicine few years ago. This number doubles when including research institutions. There is a high potential of innovation in the field, however in most cases, clinical validation is still required. The production of innovative nanopharmaceuticals in sufficient quantity and quality (under Good Manufacturing Practices-GMP) to enter clinical trials remains a challenge. In this context, it is urgently needed to provide those SMEs with the tools that can validate their technologies to bring their products closer to the market.
NanoPilot project is funded under European Union Framework Programme for Research and Innovation Horizon 2020 to meet that need. The aim of NanoPilot is to set-up a flexible and adaptable pilot plant operating under GMP for the production of small batches of polymer-based nanopharmaceuticals, which exhibit significant potential in the field of drug-delivery particularly for the design of second-generation nanopharmaceuticals. The pilot plant resulting from this H2020 project will provide service to those SME, research groups and industries working in the field of Nanomedicine. The production plant will be specialized in polymer-based nanopharmaceuticals and provide the small quantities of products that technology developers will require for full preclinical and first clinical validation.



NanoPilot consists of nine complementary partners composed by: one industry and two academia developers of the nanosystems to scale-up; a research institute expert in nanoparticle characterization (already operating in compliance with Good Laboratory Practices, GLP); an SME and larger company that will develop ad-hoc continuous flow reactors for the optimization of two of the three processes; a consultancy SME expert in Quality System implementation and laboratory information management systems; a second consultancy SME in charge of the business plan, that will also help the coordinator in dissemination and exploitation activities; and finally, a research center with a recorded track in nanomedicine, already operating under ISO 9001 that will operate the pilot plant and technology transfer activities required during the project.The main objective of GMP is to ensure that the quality of the manufacture and control of a pharmaceutical is such that the quality, safety and efficacy of the final product are guaranteed. Small companies usually do not have the resources (facilities, quality system, trained personnel…) to up-scale and implement GMP standards to their potential nanopharmaceuticals. This can limit the capacity of these organizations to develop further their technology, and as a consequence, slow-down the technology translation of innovative nanopharmaceutical into product to treat patients in needs. In some cases, there is also a lack of knowledge about the challenges, from a manufacturing point of view, of transferring a nanomedicine candidate to Phase I clinical trial. In that sense, Nanopilot pilot plant will provide not only a GMP manufacturing of polymeric nanopharmaceuticals, but also a scale up service under GMP conditions to facilitate the technology transfer.

During the project:

- Three different processes for three selected nanopharmaceuticals at sufficient TRL with positive commercial evaluation are under development:
  a. A topical treatment of ocular pain associated with dry eye syndrome containing short interfering RNA and lactic acid.
  b. A resuspendable HIV nanovaccine for intranasal vaccination.
  c. Hyaluronan based particles intended for intravesical instillation, for the treatment of interstitial cystitis/painful bladder syndrome.
- Working flow for technology transfer is being implemented in order to achieve GMP scaling-up of all three nanopharmaceuticals.
- State-of-the-art production processes including micro-reactors and highly advanced characterization techniques will ensure the quality of the nanodrugs.
- Existing laboratories in compliance with GMP and owned by the coordinator, are being adapted to get the certifications required to enable the operability of the pilot plant.
- Quality System is being implementing according to GMP guidelines.

USING LABORATORY µCT FOR THE VISUALIZATION OF THE MOUSE BRAIN

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Micro computed tomography using synchrotron radiation has been proven a particularly powerful method for the, complementary to histology, non-destructive visualization of biological tissue, down to true micrometer resolution, even revealing subcellular detail [1]. On the other hand, access to synchrotron radiation facilities is limited and costly. In this context, we have proposed the use of a laboratory-based microCT system with an operation voltage of 40 to 60 kV for the visualization of paraffin-embedded human brain samples [2]. In the present communication, we show that by using a desktop X-ray system that can operate reliably at a lower accelerating voltage of 20 kV, a time-efficient visualization of the entire mouse brain and soft tissues of other model animals of similar size is feasible with considerably increased contrast.
An extracted mouse brain was fixed in 4 % paraformaldehyde and embedded in paraffin, following a standard histology protocol. A cylindrical sample with a diameter of 8 mm was then carved out of the paraffin block, using a robotic drill. For the µCT measurements, the laboratory system Bruker Skyscan 1275 was used, with the acceleration voltage set to 20 kV and the beam current set to 175 µA. Effective pixel size was 5.5 µm. A time-efficient scan of two hours allowed for the visualization of the entire mouse brain hemisphere, revealing several anatomical structures of interest, including the cerebral cortex, the caudate putamen, the corpus callosum, the thalamus, hypothalamus and hippocampus. Thanks to the use of photons with energies below 20 keV, the contrast of the acquired tomograms was sufficient for the semi-automatic segmentation of brain structures of interest, such as the ventricles, vessels and hippocampus. Image quality was comparable to our previously reported use of synchrotron-radiation double-grating interferometry for the investigation of the mouse brain [3], not only because of the photon energies below 20 keV, but also due to the paraffin embedding itself. Thanks to the ease of use, desktop size and reasonable acquisition and running costs, such systems are a prime candidate for complementing histology in several research applications involving a wide range of laboratory animals.

Keywords: micro computed tomography, laboratory X-ray systems, three-dimensional, micrometer resolution, nervous tissue, mouse brain

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FLUORESCENCE-BASED APPROACHES TO STUDY RELEASE OF ACTIVE MOLECULES FROM LIPID NANOCARRIERS IN BIOLOGICAL MEDIA

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Lipid nanocarriers (LNCs) are an important nanocarrier platform, as they can be prepared from FDA approved agents and their hydrophobic core can encapsulate drugs and contrast agents. One of the key criteria for their optimal application in biomedical field, is to maintain their integrity until it reaches the target, for example a tumor. For this purpose and in order to rationalise the designed nanocarriers drug delivery systems, it is necessary to fully characterize their drug encapsulation, retention and release properties by using a simple and effective in situ assay. However, a remaining challenge in this area is the difficulty of obtaining rapid, quantitative characterization of the cargo release directly in biological media. Here, using specially designed dyes as models of drugs, we propose several fluorescence-based approaches to address this problem: Fluorescence correlation spectroscopy (FCS) [1] and Forster resonance energy transfer (FRET) [2] as well as bleaching of a fluorescent dye by a chemical agent. In case of FCS, we found an approach to quantify the release of active molecules based on analysis of nanocarrier fluorescence fluctuations within the laser focal volume (Fig. 1). In case of FRET, the release can be detected due to separation of donor and acceptor molecules. Finally, using sodium dithionite as a bleaching agent [3], it was possible to directly evaluate the fraction of poorly encapsulated Nile Red-based dyes inside nanocarriers. Importantly, these methods enable direct monitoring and quantification of cargo release from nanocarriers in serum containing media as well as *in vivo*.

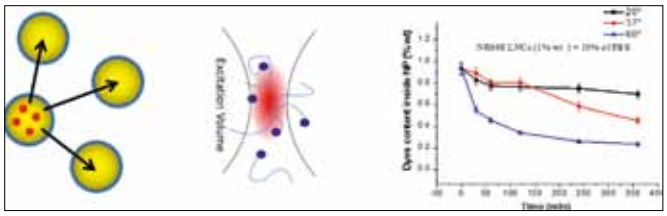

Figure 1. Concept of using FCS (Fluorescence correlation spectroscopy) in order to quantify the release of NR668 dyes from lipid nanocarriers in biological media.

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A NOVEL ORGANOTYPIC 3D MICROTISSUE MODEL TO ASSESS NANOPARTICLE UPTAKE AND EFFECTS AT THE HUMAN PLACENTAL BARRIER

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The use of medicines in pregnancy is widespread despite the fact that comprehensive drug safety and efficacy data for pregnant women are often lacking [1]. Nanoparticle (NP)-based drug carriers hold great promise for the development of targeted therapies to specifically treat the mother, the placenta or the fetus with reduced side effects as compared to conventional therapies [2]. However, knowledge on NP-placenta interactions is limited and concepts how to steer placental translocation are mostly lacking.

Since the placenta is the most species-specific mammalian organ with unique anatomical and physiological properties, findings from *in vivo* animal studies cannot be simply translated to humans. Currently available human placenta models are technically challenging (ex vivo placenta perfusion model) or highly artificial (2D cell cultures, static Transwell models). A promising approach to bridge the gap between oversimplified 2D cell cultures and ex vivo placenta tissues is the development of organoid-like placenta models.

To study the interaction of NPs with the trophoblast barrier in an organotypic environment, we developed 3D placental MTs consisting of a core of human villous mesenchymal fibroblasts (HVMFs) surrounded by a continuous layer of human trophoblastic choriocarcinoma cells (BeWo cells) using the scaffold-free hanging drop technology [3] (Figure 1). Morphological analysis revealed the highly reproducible formation of well-organized core-shell MTs resembling the *in vivo* placental barrier structure. Trophoblasts exhibited a polarized morphology with extensive apical microvilli and formed tight junctional complexes. BeWo cells secreted more of the placental hormone human chorionic gonadotropin (hCG) if cultivated in co-culture MTs as compared to 2D monocultures indicating that differentiation of BeWo cells on 3D MTs is enhanced.

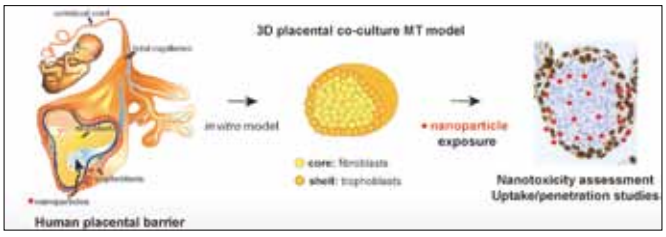

Figure 1: We developed a novel 3D in vitro microtissue (MT) model representing the human placental barrier structure and investigated the impact of different NPs on MT viability and functionality as well as Au-NP uptake and penetration in dependence of particle size and surface modifications.

These 3D placental MTs were successfully used to assess the impact of NPs (non-toxic TiO2 versus toxic CuO and COOH-CdTe NPs) on cell viability and hormone production (hCG). Interestingly, CuO and COOH-CdTe NPs were more toxic to 2D BeWo cultures than 3D MTs. Thus toxicity studies in 3D co-culture MTs provide more tissue-like dose-response curves as they represent a morphology and exposure scenario closer to the *in vivo* situation than 2D monocultures. In addition, CdTe and CuO NPs significantly reduced hCG levels at low concentrations.

The potential of the 3D MT model for mechanistic studies on NP uptake, localization and penetration was explored using gold nanoparticles (AuNPs) with different sizes (3 versus 4 nm) and surface modifications (sodium carboxylate versus PEGylation). Label-free high-resolution elemental bioimaging and quantification (laser-ablation ICP-MS and conventional ICP-MS) revealed higher uptake and deeper penetration was for smaller (3-4 nm) or carboxylate-modified AuNPs than for larger (13-14 nm) or PEGylate AuNPs, which barely passed the trophoblast barrier layer (Figure). Quantitative uptake of Au-14-COONa NPs in co-culture MTs was similar after the incubation at 4 or 37°C, which is indicative for the involvement of a passive uptake pathway.

Conclusively, we demonstrated that the human placental co-culture MT model can deliver novel mechanistic insights on NP uptake, penetration and localization of NPs in dependence of different physicochemical properties or surface modifications. Our results provide first insights how NP-placenta interactions can be steered via their size or surface modification, which is a prerequisite for the development of targeted therapies and diagnostics in pregnancy. Finally, we anticipate that the inclusion of additional cell types such as immune cells as well as the replacement of BeWo cells with primary trophoblasts will further improve the predictive value of this promising new 3D placental microtissue model.

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SHEAR RESPONSE OF NANO-CONTAINERS FOR TARGETED VASODILATION

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To overcome the high risk of mortality caused by cardiovascular diseases, shear-responsive liposomes [1] loaded with a vasodilator are proposed. To know the morphology of such promising phospholipid nano-containers under mechanical stimuli, small-angle X-ray scattering technique has been combined with microfluidics [2]. Tomographic reconstructed datasets of human coronary arteries [3] are the ground for flow simulations providing the average wall shear-stress range of healthy and constricted vessels.

Keywords: Nano-containers, drug carrier, microchannel, small-angle X-ray scattering, micro computed tomography.

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NANO-FORMULATION OF A DRUG FOR SKIN CANCER PENETRATES THE STRATUM CORNEUM AND INDUCES HIGH RATE APOPTOSIS IN TUMORAL CELL LINE AND ALTERATIONS IN IN VIVO ZEBRAFISH MODEL

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Ultradeformable liposomes (UL) are a drug delivery nanosystem with an elastic modulus lower than conventional liposomes. This feature makes UL capable to penetrate the stratum corneum (SC) of the skin and release their content into the viable epidermis, where neoplastic events occur in skin cancer. 5-Fluorouracil (5FU) is a classic antineoplasic drug, administered parenterally, with severe side effects. Therefore, the incorporation of 5FU in UL could improve specific-site delivery and aims to reduce side effects.

In this work, a UL 5FU-loaded formulation (UL5FU) of soy phosphatidylcholine and sodium cholate, as border activator, was obtained as a future topical treatment for skin cancer. The nanosystem was biophysically characterized in size, encapsulation efficiency, stability in time, lamellarity, drug release, ultradeformability, drug to lipid ratio and drug-membrane interaction.

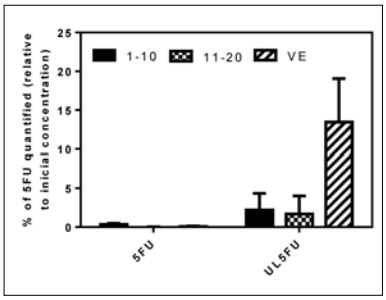

Figure 1. Human skin penetration profile after tape stripping of free 5FU and encapsulated into LU.

Penetration properties were studied on a Saarbrücken Penetration Model device with human skin explants. The skin penetration was determined by tape stripping technique (Figure 1), removing each layer of SC and analyzing the presence of the drug in the upper SC (layers 1-10), bottom SC (layers 11-20) and viable epidermis plus dermis (VE). Also, skin was incubated with UL5FU performed with two fluorescent labels, Rhodamine-DPPE for the tracking of membrane lipids and FITC for the tracking of aqueous content. Intact skin and transversal sections of 20 µm in thickness (Figure 2) were studied by confocal laser scanning microscopy. Penetration profile of 5FU was quantified in each layer of the SC and in the VE after tape stripping by fluorescence.

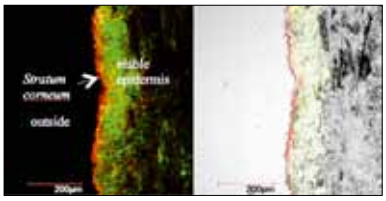

Figure 2. Skin transversal section of an explant incubated with UL5FU with two fluorescent labels by confocal laser scanning microscopy (20x).

In vitro studies were carried out in two human cell lines: HaCaT (non tumoral) and SK-Mel-28 (tumoral). Cytotoxicity was studied by MTT, Crystal Violet and Neutral Red at 4 and 24 hs. Uptake of UL5FU with Rhodamine-DPPE and FITC was analyzed at 4 and 37 ºC in both lines. Induction of apoptosis after 6 hs of incubation was assessed by flow cytometry, it was detected with annexin V conjugated with FITC (Figure 3).

In vivo studies in zebrafish (Danio rerio) larvae (4-7 days post-fecundation) were performed to determine toxicologic and teratogenic effects of 5FU and UL5FU. Zebrafish is an increasingly accepted animal model for nanotoxicological studies because it high correlation effects and other advantages if compare to other animals. Effects were assessed by determination of swimming activity, with an automated system with infrared microbeam arrangement that detects interruption by the larva body in real time; alterations in the heart rate; morphological changes (changes in eye area, rostrocaudal length and spinal cord length, absence of swim bladder, arched body, tissue ulceration and pericardial edema); and histological analysis of brain -particularly the raphe populations-, spinal cord and liver in parasagittal serial sections of 10 µm thickness staining with hematoxylin-eosin (Figure 4).

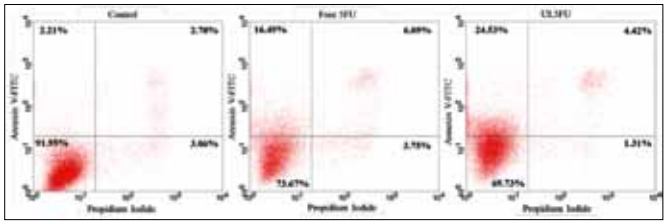

Figure 3. Flow cytometric detection of apoptosis with annexin V – FITC of free 5FU and UL5FU in SK-Mel-28 human cell line of skin malignant melanoma.

The UL5FU formulation was stable over time and incorporation of 5FU did not alter significantly its deformability properties, although it interacts with the liposomal membrane. It was capable to penetrate the SC and deliver 5FU in the VE of intact skin, at similar conditions of temperature and water gradient of *in vivo* context.

Even though, UL5FU affected both cell lines, the formulation increased strongly the cytotoxic effect of 5FU in the tumoral line after 24 hs of treatment. Furthermore, SK-Mel-28 shown a higher uptake than HaCaT and apoptosis studies shown a differential effect between both lines. After 6 hs of incubation there was significant induction of apoptosis only in the tumor line by UL5FU and higher than the free drug.

From *in vivo* studies, valuable toxicological and teratogenic information was obtained. UL5FU and the free drug produced alterations in the swimming activity -but a very different range of doses-, which could be related to neurological damage, and induced morphological changes in larvae. Cardiological effects of 5FU observed correspond to a secondary effect of the drug, because the heart is a target organ of it.

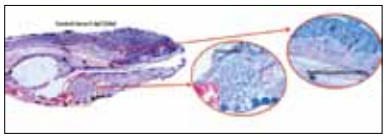

Figure 4. Hematoxylin-eosin staining of control larvae at 5 dpf (10x). Liver and raphe populations are pointed in the control larvae and amplified (40x).

The research continues to develop a novel formulation of UL as delivery system of Vismodegib for topical treatment of basal cell carcinoma. Vismodegib is a recently approved by FDA drug for oral administration.

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DISCOIDAL POLYMERIC NANOCONSTRUCTS: THERANOSTIC AGENTS IN CANCER AND CARDIO-VASCULAR DISEASE

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Over the last decade, a plethora of nanoparticles have been developed for the smart delivery of therapeutic and imaging agents, exhibiting a variety of physico-chemical properties. Here, we demonstrate multifunctional Discoidal Polymeric Nanoconstruct (DPN) for the treatment and early detection of malignant tissues, atherosclerotic plaques and vascular occlusions. DPNs are derived from a sacrificial-template fabrication strategy allowing for the precise control of the nanoconstruct size, shape, surface properties and, most importantly, mechanical stiffness – the 4S design parameters. Inspired by the behavior of blood cells, our nanoconstructs are designed to efficiently navigate the circulatory system, minimize recognition and sequestration by professional phagocytic cells and maximize accumulation at diseased sites.[(1)] In the sequel, DPN interaction with cells of the immune system, biodistribution in healthy and tumor bearing mice, deposition in malignant tissues and thrombolytic efficacy are presented and discussed. [(2)]

MATERIALS AND METHODS:

DPNs were fabricated via a top-down approach combining lithographic techniques, wet etching and polymer chemistry.[(2)] By changing initial polymer concentration and PEG/PLGA ratio, DPNs with different mechanical stiffness (soft and rigid) were synthesized. Physico-chemical properties were extensively characterized via multisizer and DLS analyses, and electron, optical and atomic force microscopy imaging.

To perform cell-uptake experiments, fixed amounts of fluorescent (lipid-RhB) soft and rigid DPNs were incubated for 24 h with macrophages, and after washing, cells were analyzed via flow cytometry. *In vivo* biodistribution studies in healthy mice were performed upon iv injection of soft and rigid particles synthetized with Gd(DSPE) entrapped in DPN polymeric matrix. 48 h post DPN injection, main organs were collected, homogenized and digested with a mixture of nitric acid and hydrogen peroxide (1:3). The amount of Gd accumulated in the organs was quantified using ICP-MS.

Tumor biodistribution studies were performed in primary breast cancer models established following 106 MDA-231-luc cells injection in nude mice mammary glands. Tumor growth was monitored via bioluminescence imaging up to six weeks. Then, 1 µm RhB-labeled soft-DPNs (109/mice) were tail vein injected and their progressive tumor accumulation was followed via whole animal fluorescence imaging at different time points.

The relaxometric characterization were performed using DPNs loaded with Gd(DSPE) via a STELAR Fast Field Cycling (0.01 - 20 MHz). At higher fields, the 1H relaxivity measurements were performed on a STELAR Variable Field Electromagnet (20 - 70 MHz) at the frequencies of 20, 30, 40, 60 and 70 MHz. [(2)]

For the treatment of thrombosis, an antithrombotic molecule (XC) was encapsulated into DPNs. *In vitro* matured blood clots were treated with saline solution (control), free XC and XC-DPNs to compare their thrombolytic efficacy for over 300 min.

RESULTS:

DPNs are constituted by chains of poly(lactic-co-glycolic acid) (PLGA) and polypropylene glycol (PEG) entangled together to form a spongy matrix. By changing the polymer relative concentrations, soft and rigid DPNs (s- and r-DPNs) are synthesized presenting the same geometry and surface charge (-14 mV) but different Young's moduli: 0.1 and 1 MPa, respectively. Flow cytometry analyses demonstrate that s-DPNs are less susceptible (~ 4-time) to macrophage (RAW-267 and primary BMDM from rat) internalization as compared to r-DPNs (Figure 1A).

This stiffness dependent macrophage uptake directly correlates with the biodistribution performance of DPNs. Specifically, soft DPNs efficiently escape macrophage phagocytosis and less abundantly accumulate with the major filtering organs (liver, spleen and lungs) as compared to rigid DPNs (Figure 1B). As a consequence, soft DPNs circulate longer and accumulate more in the tortuous tumor vasculature.

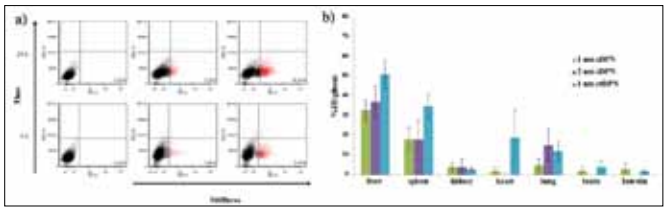

Figure.1: A. FACS analysis for primary macrophages (BMDM) incubated with s,r-DPNs at different time points; B. Biodistribution in healthy animals for s,r-DPNs. Data are expressed as %ID/g.

To analyze DPN accumulation in malignant tissues, breast cancer cells (MDA-MB-231 Luc+) are orthotopically implanted in the mammary fat pad of nu/nu mice (Figure 2A). DPNs are iv injected and whole animal fluorescent imaging was used for assessing the nanoconstruct accumulation over time. Figure 2A demonstrate a partial overlap between the bioluminiescene signal associated with the tumor mass (upper row) and the fluorescent signal associated with the DPNs (lower row). Ex vivo imaging further confirms the significant tumor accumulation of DPNs at 48h post injection (Figure 2B). As expected for any nanoparticle, a portion of DPNs also deposited in the liver.

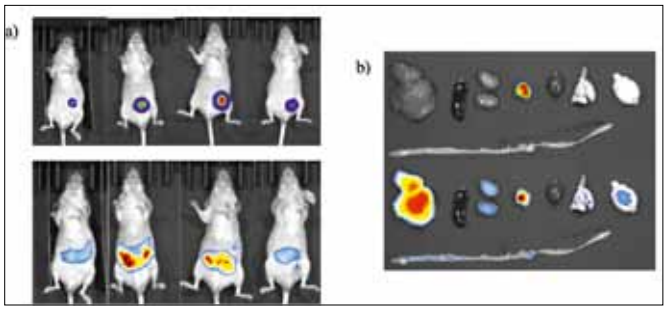

Figure 2: IVIS imaging of primary breast cancer and RhoB-DPNs accumulation in vivo (A) and ex vivo (B)

For relaxometric studies, DPNs are loaded with Gd(DSPE) providing the Magnetic Resonance Imaging capability. The confinement of Gd(DSPE) chains within the spongy DPN matrix boosts the longitudinal relaxivity r1 of 1 µm Gd-DPNs up to 55 mM-1s-1, which is about 15-times larger than for the clinically available Gd(DOTA) (Dotarem®) (Figure 3A-B). This demonstrates that the confinement of Gd-complexes within porous matrices amplifies the longitudinal relaxivity and returns highly efficient MRI contrast agents.

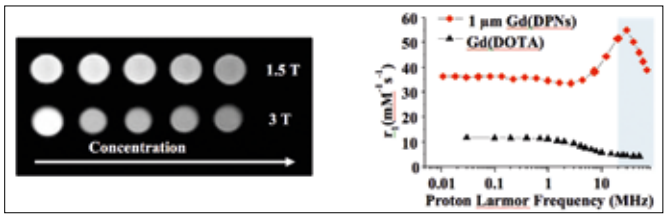

Figure 3: MRI phantoms for DPNs acquired using the gr-MRI Spin Echo Inversion Recovery sequence at 1.5 and 3 T; NMRD profiles of 1 µm DPNs loaded with Gd(DSPE), compared with the clinical agent Gd(DOTA).

Finally, for the treatment of vascular occlusions, XC-DPNs are synthesized and tested *in vitro* on blood clots. The thrombolytic efficacy of XC and XC-DPNs was assessed for different XC concentra-

tions and incubation times. *In vitro*, the analysis shows a similar clot dissolution speed when clots are treated with XC-DPNs and free XC (Figure 4). Since XC-DPNs are expected to have a much longer circulation half-life and higher affinity for vascular occlusions than free XC, a significant advantage for the XC-DPNs over free XC is anticipated *in vivo*. In particular, XC-DPNs are expected to increase the thrombolytic efficacy of XC and reduce the injected doses.

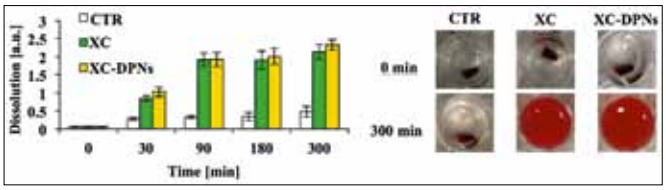

Figure.4: Blood clot dissolution over time for different experimental groups: saline solution, XC and XC-DPNs.

CONCLUSIONS:

These results suggest that DPNs offer unique opportunities in the treatment of cancer and cardiovascular diseases. The high blood longevity, low liver sequestration and enhanced tumor deposition of soft-DPNs make this multifunctional platform suitable for the early diagnosis and treatment of different diseases.

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IRON SUCROSE VS IRON SUCROSE SIMILARS: DIFFERENT CLINICAL OUTCOMES RELATED TO DIFFERENT PHYSICOCHEMICAL CHARACTERISTICS?

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INTRODUCTION

Iron sucrose (IS) is a complex colloidal drug used to reestablish iron levels in patients suffering from severe anemia. During the last decade, several pharmaceutical companies introduced "intended copies" of IS in various markets. Nevertheless, retrospective and perspective studies proved that patients treated either with IS or iron sucrose similars (ISSs) experienced divergent clinical outcomes [1, 2]. It is not clear as to what physicochemical characteristics might be attributed to these clinical differences.

Recently, the European Medicines Agency (EMA) published a reflection paper addressing the need for new assays to prove quality, efficacy and safety of both IS and ISSs [3]. Moreover, the European Directorate for the Quality of Medicines and Healthcare (EDQM) is currently working on the elaboration of a monograph on IS to clearly identify the key characteristics of this compound. Evaluation of equivalency of complex drug products, such as iron colloids and liposomes, continues to remain as one of the US Food and Drug Administration (FDA) regulatory science priorities in 2017 [4].

In this brief project, IS and several other commercial ISSs are studied for their physicochemical attributes. All the assays were designed to mimic the clinical injection of the drugs in the blood stream.

MATERIALS AND METHODS

Size and shape of the colloidal particles were investigated with Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM) and Low Voltage Electron Microscopy (LVEM). Stability of the drugs was evaluated through zeta potential measurements and the amount of the potential toxic labile iron was estimated using a colorimetric procedure. *In vitro* dissolution kinetics of IS and ISSs were elucidated in relevant media over time.

RESULTS

Significant differences were identified between IS and other commercial ISSs for all the assays performed.

Dynamic light scattering results revealed that IS presented a monomodal distribution by Intensity with an average size of 12 nm, whereas all the ISSs showed a bimodal distribution with the presence of aggregates of micron size. TEM and LVEM images showed significantly divergent arrangements of particles in the different products, as reported in Figure 1:

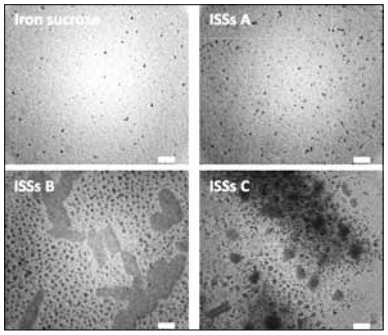

Figure 1: Low voltage electron microscopy (LVEM) images for Iron Sucrose and three different ISSs (named A-C). Scale bar = 100 nm.

Zeta potential measurements proved that both IS and ISSs are stable colloidal suspensions with average values of around -30 mV. The amounts of labile iron identified in both IS and ISSs were significantly different, with values comprise between 3 and 6%. Finally, the *in vitro* dissolution kinetic studies showed different degradation pathways for IS and ISSs.

CONCLUSIONS

Results from this ongoing study suggest that there might be differences between IS and ISSs that can be discerned through physicochemical characterization and advance our understand between originator and a generic drug. Moreover, the protocols developed might be used to create a list of most appropriate assays to lay the basis for testing equivalency between IS and ISSs.

Disclaimer: The views expressed in this poster do not necessarily represent those of the U.S. Food and Drug Administration.

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SYNTHESIS AND BIOLOGICAL CHARACTERIZATION OF MICROSCOPIC POLYMERIC IMPLANTS FOR THE LOCAL TREATMENT OF PATHOLOGICAL CONDITIONS

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INTRODUCTION:

Drug delivery systems provide significant advantages over conventional molecular therapy, such as reduced side effects and payload degradation, increased drug bioavailability, sustained release over time. Moreover, new fabrication techniques allow us to realize particles with a priori defined size and shape combinations.[1-3] Taken together, these features allow the realization of platforms with suitable characteristics for the specific purpose, not only for systemic administration, but also for the local and prolonged treatment of pathological conditions. Here, polymeric micro-plates (μPLs), in which, more importantly, stiffness can be modulated among the common properties (size, shape, surface properties), are proposed as an anti-inflammatory reservoir system.

MATERIALS AND METHODS:

μPLs are fabricated via a top-down, replica-molding approach. First, a silicon master template is realized by Direct Laser Writing. The resulting template is replicated in a sacrificial PVA template and finally loaded with a poly(lactic-co-glicolic acid) (PLGA) paste. After dissolving in aqueous solution the PVA template, microscopic particles (μPLs) made out of PLGA are released in their pre-designed size and shape combination. As an anti-inflammatory drug, dexamethasone (DEX) was used and mixed with PLGA during the synthesis step for direct loading into the μPLs. The physico-chemical properties of μPLs were characterized by confocal and electron microscopy (SEM), atomic force microscopy (AFM). HPLC analyses were performed for estimating the pharmacological properties of μPLs. The biological efficacy of DEX loaded μPLs was tested on Bone Marrow-Derived macrophages (BMDM) by analyzing gene expressions via qPCR.

RESULTS AND DISCUSSION:

μPLs are square particles with an edge length of 20 μm and a height of about 5 μm (Figure.1) Because of the top-down fabrication approach, particles are very homogeneous in size and shape (Figure.1A and B). Moreover, confocal analyses with a fluorescent cargo, show a quite uniform distribution of the loaded molecules (Figure.1B).

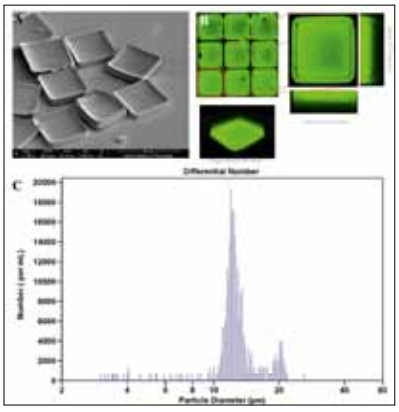

Fig. 1. μPLs images. A) Scanning Electron Microscope of μPLs. B) Z-stack confocal images , showing the well patterns and 3D structure, and distribution of curcumin, as a reference molecule.

By changing the PLGA amounts, the Young's modulus of μPL can be readily modulated (Figure.2). This feature can be efficiently used to reproduce the mechanical stiffness of the hosting micro-environment upon implantation, thus favoring integration and modulating immune responses. Figure.2A gives a morphological image of a μPL, produced via atomic force microscopy under wet conditions. Figure.2B presents the mechanical properties of μPLs corresponding to different amounts of PLGA, documenting a Young's modulus ranging from about 1 to 5 MPa.

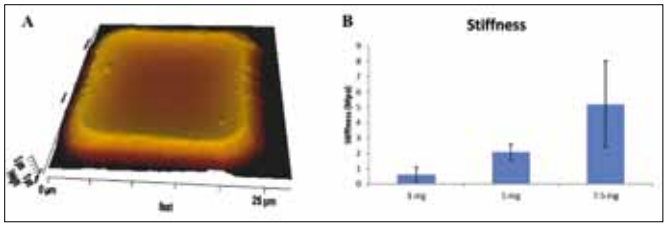

Fig. 2. Atomic Force Microscopy characterization. A) Morphological structure of a representative μPLs. B) Results for the stiffness analysis of μPLs made out with different PLGA feeding amounts, namely 1, 5 and 7.5 mg.

As per the pharmacological properties, μPLs display an encapsulation and a loading efficiencies as from Figure.3. Notice that, DEX amounts per μPLs are elevated (Figure.3A and B) thus limiting the number of particles required for therapeutic intervention. The DEX release profile (Figure.3C) demonstrates a diffusion-driven kinetic with a sustained release for over a week. Indeed, only about 70% of DEX is released within the first week, as per the linear and slow release profile visible at longer time points.

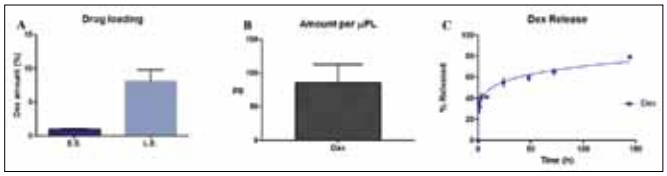

Fig. 3. DEX- μPLs pharmacological characterization. A) Drug loading and encapsulation efficiencies. Loading efficiency is defined as the weight percentage of DEX over the μPL weight; encapsulation efficiency is defined as the weight percentage of DEX in μPLs over the initial feeding amount. B) DEX amount in a single μPLs. C) DEX release profile from μPLs in physiological conditions (pH = 7.4, temperature = 37 °C).

Finally, the ability to act as an anti-inflammatory system is tested by incubating DEX-loaded μPLs with BMDM, exposed to a pro-inflammatory stimulus (LPS) (Figure.4). At two DEX concentrations (1 and 10 μM), μPLs drastically reduce the expression of pro-inflammatory cytokines.

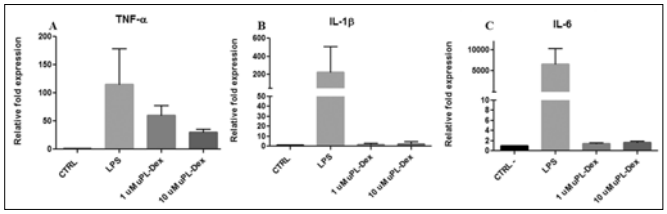

Fig. 4. DEX- μPLs biological efficacy in reducing pro-inflammatory cytokines expression. A) TNF-α relative gene expression. B) IL-1β relative gene expression. C) IL-6 relative gene expression

CONCLUSIONS:

Polymeric μPLs are fabricated as 20 x 20 x 5 μm square particles, with a tunable mechanical stiffness. These μPLs are efficiently loaded with the anti-inflammatory molecule dexamethasone and used to alleviate inflammation in macrophages stimulated with LPS. μPLs represent a promising anti-inflammatory platform for a variety of biomedical applications.

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IN VIVO BIOCOMPATIBILITY OF SITE-DIRECTED APOFERRITIN-BASED NANOCARRIER FOR CARDIOTOXIC ANTITUMOUR DRUG DOXORUBICIN

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Doxorubicin (DOX), a potent anthracycline antibiotic [1], is used in conventional cancer therapy for the treatment of various solid and haematological malignancies, such as breast [2], lung [3], bladder [4] or Hodgkin lymphoma [5]. However, up to 26% of patients treated with DOX develop arrhythmia or cardiomyopathy [6]. Co-administration with cardioprotective drugs can lead to other unwanted side effects, such as the development of secondary malignancies in case of dexrazoxane [7]. This effectively limits DOX dose that can be administered to the patients [6].
Targeted delivery to cancer cells using nanocarriers seems like an effective way to eliminate these unwanted side effects [8]. Only two nanopharmaceuticals containing DOX are currently on the market – Myocet (bare liposomes) and Doxil (polyethylenglycolated (PEGylated) Stealth® liposomes) and these are only used in case of stage IV cancer and in patients who are prone to cardiotoxicity, such as the elderly [9]. Moreover, bare liposomes are often recognized by the immune system's cytotoxic cells of the patient and removed from the body before they can reach the tumour [10]. Due to this, PEGylated liposomes that are able to evade the immune system are used more often. However, PEGylation hampers the cellular uptake of the liposomes by cancer cells, as well as causes side effects of its own, such as the palmar-plantar erythrodysesthesia [11]. Also, these liposomes are not actively targeted to the cancer cells but rather depend on the enhanced permeability and retention (EPR) effect, a passive targeting mechanism caused by higher accumulation of suitably sized nanoparticles in the vicinity of tumour, which is not very efficient in humans [12].
Experimental use of other nanocarriers, often modified by actively targeting moieties, has shown that upon administration of exogenous particles to body fluids, their surface is quickly covered by protein corona, hampering their entry into cancer cells [13]. For these reasons, our group focuses on protein apoferritin (APO) that is naturally found in body of most living organisms, with high interspecies sequence homology, thus not causing immune response [14]. Due to its structural responsiveness to surrounding pH, it provides a very universal and easy system for encapsulation of various drugs [15].
We have previously employed APO modified with prostate cancer-targeted antibodies for *in vitro* site-directed delivery of DOX to cancer cells [16]. In this work, we focused on *in vivo* applications of this targeted system and its potential drawbacks. DOX was encapsulated in APO (creating APODOX) and this nanocarrier was subsequently modified with antibodies against prostate specific membrane antigen according to our pilot study [16] (creating APODOX-anti-PSMA). The formation of protein corona around these non-targeted and prostate cancer-targeted APODOX upon their introduction to plasma was tested (Fig. 1A). Micrographs obtained using the transmission electron microscopy (TEM) revealed formation of large protein coronas around APODOX with negligible coronas around APODOX-anti-PSMA showing higher compatibility of the targeted nanocarrier with plasma.
To evaluate the *in vivo* biocompatibility of these nanocarriers, twelve five-week-old male nude athymic BALB/c nu/nu mice were used for xenograft studies. The use of the animals followed the European Community Guidelines as accepted principles for the use of experimental animals. The experiments were performed with the approval of the Ethics Commission at the Faculty of Medicine, Masaryk University, Brno, Czech Republic. The mice were housed in individually ventilated cages at 12/12 h light/dark cycle and provided ad libitum with standard diet and water. Mice were subcutaneously injected with LNCaP cells and after 38 days, their treatment was carried out intravenously (through tail vein) once a week for 21 days (total of 4 applications) using 5 μg·g-1 of body weight of DOX, either free or in form of APODOX or APODOX-anti-PSMA.

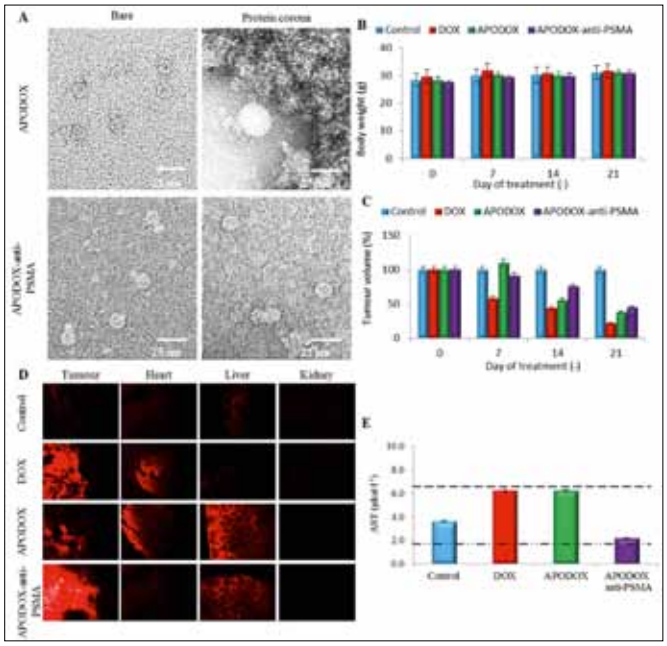

Fig. 1: In vitro and in vivo biocompatibility of APODOX and APODOX modified with anti-PSMA antibodies. A) TEM images of bare APODOX and APODOX-anti-PSMA and these nanocarriers after formation of protein corona in plasma environment. B) Body weight of mice with prostate cancer xenograft tumours untreated (control) and treated with free DOX, APODOX and APODOX-anti-PSMA. C) Percentage changes of tumour volumes in mice with prostate cancer xenograft tumour untreated (control) and treated with free DOX, APODOX and APODOX-anti-PSMA. D) DOX fluorescence observed in histological sections of tumour, heart, liver and kidney collected from mice with prostate cancer xenograft tumours untreated (control) and treated with free DOX, APODOX and APODOX-anti-PSMA. E) AST levels in plasma collected from mice with prostate cancer xenograft tumours untreated (control) and treated with free DOX, APODOX and APODOX-anti-PSMA. Dashed line shows higher limit of normal reference range. Dash-dot line shows lower limit of normal reference range.

The body weight and volume of the tumours were continuously monitored throughout the course of the treatment (Fig. 1B and 1C, respectively). The weight of all tested mice did not significantly lower during the treatment. On the other hand, the tumour volumes were significantly decreased, the most in case of treatment with free DOX, where the tumour volume decreased to 22% of its original volume after 21 days of treatment. In case of treatment with APODOX, the tumour volume decreased to 38% of its original volume and in case of treatment with APODOX-anti-PSMA, the tumour volume decreased to 45% of its original volume.
After 21 days of treatment, the mice were euthanized by intraperitoneal injection of 1% Narkamon + 2% Rometar, 0.5 mL/100 g of

weight, followed by intracardiac blood collection in EDTA-treated tubes. DOX was previously shown to most prominently damage heart, liver and kidneys [17, 18]. These organs (as well as the tumours) were collected after the euthanasia and subjected to microscopic analysis to asses DOX distribution (Fig. 1D). The tumours showed highest DOX fluorescence in case of treatment with APODOX-anti-PSMA. The heart showed unwanted DOX fluorescence in case of treatment with DOX and non-targeted APODOX, while there was no observed fluorescence after treatment with APODOX-anti-PSMA. Kidneys showed no observed DOX fluorescence after any of the treatments. The liver of mice treated with both non-targeted APODOX and APODOX-anti-PSMA showed DOX fluorescence in the connective tissue. To assess whether this caused damage to the liver tissue, the levels of liver enzymes in plasma were determined. As can be seen in case of aspartate aminotransferase (AST, Fig. 1E), the level of enzymes in mice treated with free DOX and APODOX reached the higher limits of normal reference range, showing possible damage to the liver. However, these enzymes were well in the normal reference range for mice treated with APODOX-anti-PSMA.

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A PROMISING APPROACH TOWARDS SAFER DESIGN OF NANOMEDICINES

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With large amounts of nanotoxicology studies delivering contradicting results and an unstable, moving regulatory framework, potential risks surrounding nanotechnology appear complex and confusing. The European chemical regulation, REACH, is trying to adapt its annexes to fit nanomaterials [1], however this is not a process achieved swiftly. In order to develop safe and legally compliant products, we focussed on the development of an implementable safety concept [2], which may help to plot a sensible path through the nano-risk landscape, without stifling innovation (see Fig. 1).

Figure 1. Overview of the different phases of the suggested nanosafety approach

The outlined methodology follows the general REACH (CSA) approach [3] applied to chemicals and has dual focus: One being the creation of a risk profile for a given nanomaterial (e.g., classify which materials and/process operation pose greater risk, where these risks occur in the lifecycle, and the impact of these risks on society) using state-of-the-art safety assessment approaches/tools (ECETOC TRA, Stoffenmanager Nano and ISO 12901-2) [4-6].
The other being the development concrete, practical guidance to industry and regulatory authorities (such as European agencies, scientific committees, national competent authorities) on how to deal with environmental health and safety (EHS) issues of manufactured nanomaterials (NMs) and nano-enabled products, including, as appropriate, legislation/sector specific issues. Risk mitigation actions focus on hazard/risk avoidance rather than address them as an exposure (e.g., "design out" physico-chemical parameters that are identified as being drivers of toxicity of NMs whilst retaining the functional aspects, "re-design" process and operational conditions, develop solutions for exposure reduction and personal protective equipment).
The concept is currently applied in the European project Smart-4-Fabry in order to compile a risk profile along the manufacturing and encapsulation processes of GLA-nanoliposomes. Moreover, the model may be universally applicable for various nano-related innovations and thus bringing them closer to the market.

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IN VITRO CYTOTOXICITY AND CELLULAR UPTAKE OF POTENTIAL IRON OXIDE NANOPARTICLES FOR BREAST CANCER TREATMENT

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Superparamagnetic iron oxide nanoparticles (SPIONs) are promising tools for the treatment of different diseases. Their magnetic properties enable therapies involving Magnetic Drug Targeting (MDT), hyperthermia or imaging. Depending on the intended treatment, specific characteristics of SPIONs are required. While particles used for imaging should circulate for extended periods of time in the vascular system, SPIONs intended for MDT or hyperthermia should be accumulated in the target area to come into close proximity of, or to be incorporated into, specific tumor cells.
In this study, we determined the impact of several accurately characterized SPION on various human breast cancer cell lines to identify the most suitable particle for the future breast cancer therapy. We analyzed cellular SPION uptake, magnetic properties, cell proliferation and toxicity using atomic emission spectroscopy, magnetic susceptometry, flow cytometry and microscopy.

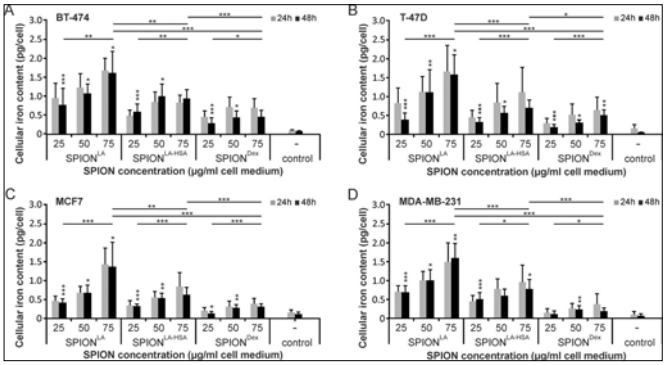

*Figure 1: Quantification of the cellular Nanoparticle Load via Microwave Plasma-atomic Emission Spectroscopy (MP-AES). Cells were incubated for 24 h and 48 h with 0-75 µgFe/ml SPION^{LA}, SPION$^{LA\text{-}HSA}$ and SPION^{Dex} and cell lysates were investigated by MP-AES. The cellular iron content (pg/cell) is shown for (A) BT-474, (B) T-47D, (C) MCF7 and (D) MDA-MB-231. Negative controls are samples with corresponding amount of H2O instead of water-based ferrofluid. .Statistical significance of 48 h datasets are indicated with *, ** and ***. The respective confidential intervals are p < 0.05, p < 0.001 and p < 0.0001. Asterisks shown directly on bars indicate dose-dependent significance to the next lower SPION concentration. Asterisks over the lines indicate dose-dependent significance between lowest and highest SPION concentration and between the highest SPION concentrations of different SPIONs.*

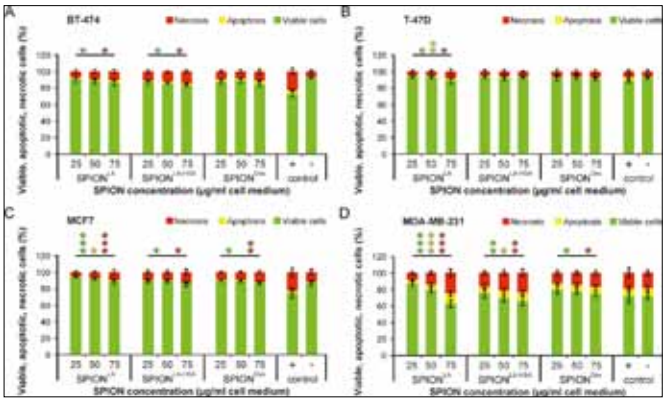

*Figure 2: Viability after SPION Treatment. Cells were incubated for 48 h with increasing amounts of SPION^{LA}, SPION$^{LA\text{-}HSA}$ and SPION^{Dex}. Cell viability was determined by Annexin A5-Fitc/propidium iodide staining and analyzed by flow cytometry. The amount of viable (Ax-PI-), apoptotic (Ax+PI-) and necrotic (PI+) cells are shown for (A) BT-474, (B) T-47D, (C) MCF7 and (D) MDA-MB-231 cells. Positive controls contain 2% DMSO, negative controls represent the corresponding amount of H2O instead of water-based ferrofluid. Statistical significance are indicated with *, ** and ***. The respective confidential intervals are p < 0.05, p < 0.001 and p < 0.0001. Colored asterisks indicate dose-dependent significance between lowest and highest SPION concentration on necrosis (red), apoptosis (yellow) and viability (green).*

SUMMARY

We found that the particle internalization by cells is strongly related to the SPION-surface coating. Moreover, our studies demonstrated a cell type-dependent SPION uptake and toxicity that determine the possible areas of application. SPIONLA are relatively non-toxic to breast cancer cells and could easily be functionalized to target these cells, but they are harmful to HUVECs and possibly also to other healthy cells. Due to this fact, there is a risk of enhanced toxicity if these particles escape the tumor targeting, which excludes their clinical use. SPIONDex, showing a high stability, extremely low toxicity and barely detectable cell uptake, can be considered a suitable candidate for MRI. Compared with SPIONDex, SPIONLA-HSA are more suitable for hyperthermia and, upon drug-loading, for MDT, based on their good magnetic properties, adequate stability and low toxicity. Additionally, SPIONLA-HSA could easily be utilized for MRI, thus enabling theranostic applications. Future *in vivo* experiments are necessary to determine the theranostic capabilities of SPIONLA-HSA.

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COMPLEMENT ACTIVATION-RELATED PSEUDOALLERGY TO CORTICOSTEROID-CONTAINING PEGYLATED LIPOSOMES IN PIGS: ROLES OF NATURAL ANTI-PEG ANTIBODIES AND INFUSION RATE

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INTRODUCTION

Non-IgE-mediated (pseudoallergic) hypersensitivity reactions (HSRs) are frequently reported acute adverse effects of intravenous administrations of liposomal drugs and many other nanomedicines and biologicals. Earlier studies provided evidence that activation of the complement (C) system plays a causal role in this phenomenon. Hence its name, C activation-related pseudoallergy (CARPA) [(1)]. Earlier studies also established the *in vivo* "porcine CARPA model" for sensitive evaluation of the CARPA-genic potential of liposomal drug candidates [(2)], and the model was used to study the CARPA caused by PEGylated liposomal doxorubicin (Doxil) [(3, 4)].

It is known that in addition to Doxil, also other PEGylated nanopharmaceuticals can cause CARPA in patients, including PEGylated liposomal prednisolone disodium phosphate (Nanocort). This formulation has been in clinical trials for patients with rheumatoid arthritis, inflammatory bowel disease and atherosclerotic cardiovascular disease [5], and was shown *in vitro* to have a mild C activating capability in normal human serum [6]. Nanocort, however, has never tested in pigs for CARPA, thus, the first goal of the present study was to explore the presence and characteristics of CARPA in pigs. Since it is a widely used practice in pharmacotherapy to administer reactogenic drugs via slow infusion in order to reduce the risk of HSRs, the second main goal of our study was to explore if slowing of the infusion could also help prevent Nanocort-induced CARPA in pigs. Based on previous studies suggesting that pre-existing (naturally occurring) anti-PEG IgM may play causal role in PEGylated liposome-induced CARPA [7], an additional goal of our study was to establish if the natural anti-PEG IgM titers of Nanocort recipient pigs could serve as a predictor of CARPA.

MATERIALS AND METHODS

Nanocort® was provided preformulated in 20 mL sterile glass vials. It was characterized earlier in detail [5, 8]. Fourteen domestic male and female Yorkshire-Hungarian Landrace mixed breed pigs were treated with the human equivalent dose (HED) of Nanocort via infusion according to 2 protocols referred to „slow" and „fast" infusion protocol. The infusion in the former group was performed in 3 steps of 0.04 - 0.4 - 4 mL/kg/h for 40, 20 and 100 min, respectively, while the 40 min slow initial infusion step was skipped in the fast infusion protocol starting with the 10-times faster speed of 0.4 followed by 4.0 mL/kg/h for 20 and 100 min respectively. Before and during infusion pigs were monitored for changes of pulmonary arterial pressure (PAP), systemic arterial pressure (SAP), heart rate, ECG, respiratory rate, core body temperature, tissue oxygen saturation and end-tidal CO2. Blood samples were collected pre-administration and at various time points post-administration for the measurement of blood cell counts, and blood thromboxane B2 levels were measured by ELISA. Here we only report the area under the PAP curve during the first 15 min because it appeared the most sensitive and reproducible quantitative measure of CARPA. The titer of anti-PEG IgM was measured by ELISA, as described elsewhere[7].

RESULTS

CARPA could be induced by Nanocort in pigs with symptoms similar to those described for many nanoparticles, including some liposomes, dendrimers, polymers, polymeric micelles, carbon nanotubes and many other nanoparticles [2]. However, the slow infusion protocol showed no reaction (4 pigs), while the fast infusion protocol induced a mild but clear reaction in 4 out of 10 pigs, thus duplicating the empirical observations in man (data not shown). As for the role of anti-PEG IgM, Fig. 1 shows a remarkable and unprecedented observation: in case of fast infusion CARPA showed significant (linear) correlation with the titer of natural anti-PEG IgM in the blood of pigs, suggesting that these antibodies played a causal role in CARPA, just as it was shown for Doxil-incuded HSRs [7].

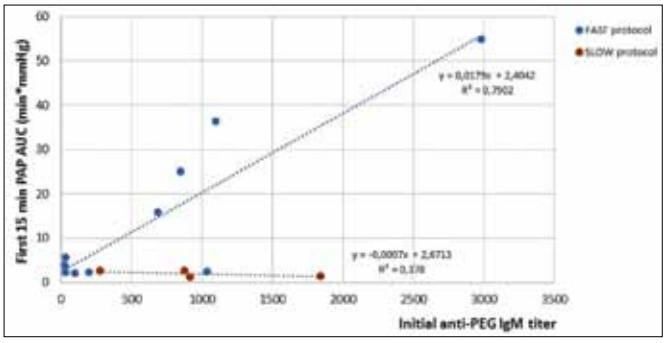

Figure 1: Effect of pre-existing anti-PEG IgM on Nanocort-induced CARPA in pigs. CARPA is quantitated by the area under the PAP curve (AUC) during the first 15 minutes of the reaction. Pigs obtained Nanocort either rapidly in 2 steps ("fast" protocol, blue) or slowly in 3 steps ("slow" protocol, red).

DISCUSSION

Our results suggest that 3-step infusion protocol starting at a slow infusion speed can help prevent HSRs and it provides validation of the porcine CARPA model as an important nonclinical safety assessment for nanopharmaceutical drug products. The observations on the correlation between CARPA and the plasma concentration of natural anti-PEG IgM, on the other hand, suggest that IgM is causally involved in the reactions. This means that measuring IgM may help predict HSRs, and that elimination of these antibodies, for example by depletion in a non-reactogenic procedure, may prevent the reaction. An example for the latter proposal was served earlier by the use of Doxebo for the prevention of Doxil-induced CARPA in pigs [3]. Perhaps most importantly for clinicians facing the possibility of CARPA with new PEGylated nanopharmaceutical drug products, our data suggest that prescreening of patients for natural anti-PEG IgM before treatment and special attention to individuals with high antibody titer by using of slow infusion protocols can be effective measures to avoid the CARPA problem.

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DESING AND IN SILICO EVALUATION OF NEW MOLECULES THAT MANTAIN SSA1-42 IN ∂-HELIX CONFORMATION: AS POSIBLE THERAPEUTIC IN ALZHEIMER DISEASE

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Alzheimer's disease (AD) is a public health problem, as it is the leading cause of senile dementia worldwide affecting about 10% of people over 65 and 50% of people aged 85 years. Actually, there are numerous theories to explain the pathophysiology and development of AD one of the most correctly theory based on evidences is the amyloidogenic pathway, since a histological data in the majority of these patients is consisting of the presence of neurofibrils made-up of β-amyloid peptide of 40-42 (βA1-42) capable of producing cytotoxicity in anatomical sites of the brain related to the control of cognitive abilities. This βA1-42 neurofibrils are the end product of the amyloidogenic pathway which is a pathophysiological phenomenon that starts from the processing of amyloid precursor protein (PPA), a transmembrane protein that upon being sequentially hydrolyzed by β-secretase and γ-secretase culminates in the release of a β-amyloid peptide of 40-42 (βA1-42) amino acid residues in α-helix conformation. However only peptides with 42 or 43 residues will be fibrinogenic due to the presence of hydrophobic residues at their C terminal favoring the formation of hydrophobic interactions. The βA1-42 in α-helix conformation will not be prone to aggregation in the form of oligomers and fibrils until there is a conformational change towards β-sheet dependent on an electrostatic interaction between residues.
Using in silico studies have been showed that molecules that have an tertiary amine able to be protonate at physiological pH plus an aromatic ring at 4 carbon atoms of distance from the tertiary amine as well as an aliphatic chain residues capable of making a interaction with Phe19 and Phe20 are able to stabilize βA1-42 in its conformation α-helix. These chemical characteristics have been obtained previously by our work group through the design of new molecules. The best molecule was named F3S4 based on its free energy and binding mode obtained by in silico studies. However, the addition of new pharmacophores with the previously mentioned characteristics to F3S4 could increase its free energy and improve its binding mode. Therefore, in this work is proposed to add new molecules to F3S4 structure and know if these favored the interaction with βA1-42. Then, in the amine group of F3S4 was binding several types of carboxylic acids, among them, some have aromatic rings in their structure such as butylbenzoic, phthalic, isophthalic and terphatidic acid; As well as aliphatic chain moieties such as adipic acid, fumaric acid, gutaric acid, maleic acid, succinic acid and valproic acid. This chemical structural change could increase their affinity and consequently their anti-aggregation abilities of βA1-42 being these molecules a better alternative of the therapeutics than many other that are used, since nowadays the first line for the treatment of AD are the inhibitors of cholinesterase, however only generate a beneficial effect in the symptomatology of the disease and only can be used in early step of the disease.

METHODS

The F3S4 molecule was chosen as the best molecule based on the affinity obtain by in silico studies, then were designed ten compounds binding a carboxylic group from diferents carboxylic acids to the amine group of F3S4 to form an amide bond. The amine tertiary of F3S4 was free to be protonated an acquiring the positive charge. The carboxylic acids that were employed are adipic, Butyl benzoic, Phtalic, Fumaric, Glutaric, Isophtalic, Maleic, Succinic, Terphtalic, and Valproic. In addition, Resveratrol and Cucurmin were used as positive control molecules, due these avoid the βA1-42 aggregation. All the molecules were drawn with ChemBioDraw Ultra 12.0 and optimized using Hyperchem6.0. After, docking analysis were performed with βA1-42 in α-helix and β-folded conformation employing the structures obtained from the Protein Data Bank (PDB) code 1Z0Q and 2BEG. In addition a βA1-42 structure obtained from molecular dynamics studies were employed as random coil structure. The results obtained for each ligand was subjected to analysis by its free energy using AutoDock 3.4 and their binding geometry using PyMol Viewer.

RESULTS

The free energy obtained by the molecules were as follows; F3S4-Valproic acid (-9.74),F3S4 (-7.75) F3S4-Adipic (-7.17) F3S4-Terphatilic (-6.99), F3S4-butylbenzoic acid (-6.96), F3S4-), F3S4-Phthalic (-6.18), F3S4-succinic acid (-5.88), F3S4-Fumaric acid (-5.71), F3S4-Maleic acid (-5.44), Resveratrol (-5.39) and Cucurmin (-5.02) in α-helix conformation . All presented higher affinity for βA1-42 in its conformation α-helix, followed by its β-folded conformation and finally the structure in random coil.

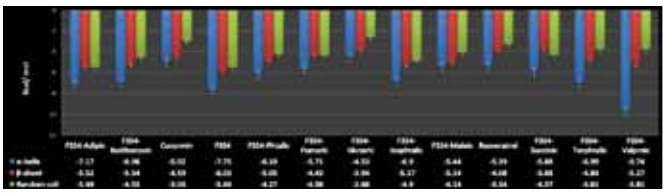

Figure 1 Free energy of the compounds in interaction with the βA1-42 in its three conformations.

In the binding mode, all molecules were capable to interact with Asp23 and Glu22 by the presence of a tertiary amine able to make electrostatic interactions with carboxyl groups. All compounds are capable of interacting with Phe19 and Phe20 by the formation of π-stacking interactions, however there is a notable difference between those compounds which has a carboxilic acid in the aromatic ring and those that have aliphatic chains due to these have the highest affinity (F3S4-valproic and F3S4-adipic). The binding mode between F3S4-valpoic and βA1-42 in α-helix shows interactions between the lateral chain of GLN15 and the amide bond of F3S4-valproic, in addition hydrophobic interactions between the aliphatic chains of valproic acid and the aliphatic chains of VAL18 and GLN15. In the binding mode of F3S4-adipic to βA1-42 in α-helix interaction between the amide bond and the lateral chain of GLN15 by hydrogen bonds with the carbonyl groups of the adipic acid are observed. This results shows that the interaction with GLN 15 is important which was not observed in the compounds with less affinity.

CONCLUSIONS

The results shows that the presence of aliphatic chains in the molecule could confer additional hydrophobic interactions improving the affinity without avoid the interaction of the ligand with GLU22 and ASP23. However, the presence of aromatic ring produce hindrance effects that avoid the approach of the ligand to GLN15. For future studies it is proposed the evaluation of the same molecules on polymeric or fibrilar βA1-42.

UNDERSTANDING NANOPARTICLE UPTAKE AND TRAFFICKING IN CELLS TO IMPROVE DRUG DELIVERY APPLICATIONS

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Nanomedicine has the potential to design novel devices capable of targeted, site-specific drug delivery. In particular, nano-sized drug

carriers allow functionalization with specific biomoleculesto promote active targeting and can help to overcome several of the barriers that drugs need to pass in order to arrive to their site of action. During the last decade, researchers have focused on phenotypic screenings of lots of different candidate drug carrier to improve active targeting and internalization by the cells. Nevertheless, there is a growing realization that it is necessary to first understand the biological mechanism by which nano-sized materials interact with cells to ultimately be able to fully exploit their potential and translate nanomedicines to the clinic. Within this context, our work is focused on understanding how nano-sized materials are internalized and trafficked inside cells. We use a combination of flow cytometry and cell fluorescence microscopy in order to follow and quantify nanoparticle uptake and define their intracellular location. By adding the nanoparticles to cells under energy depletion conditions, such as 4ºC, we determine nanoparticle adhesionto the plasma membrane. It is in fact established that nano-sized materials enter cells by energy-dependentpathways.1As a result, the nanoparticlesadsorband accumulateon the cell membrane.Thus, the adsorbed nanoparticles are allowed to undergo endocytosis for different periods of time.Finally, immunostainingofproteins of different intracellular organelles is used to follow the nanoparticles as they are trafficked between the different compartments of the cell, typically to lysosomes.

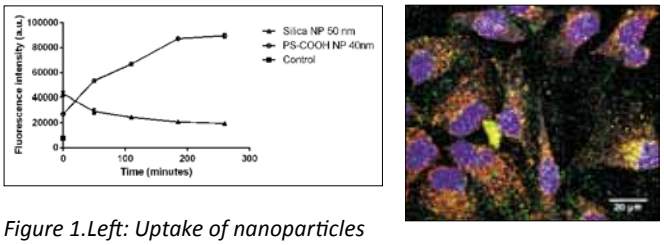

Figure 1.Left: Uptake of nanoparticles adsorbed to Hela cell membrane. Cells were exposed to silica and carboxylated-polystyrene nanoparticles at 4ºC for 1h, thus the extracellular nanoparticles were washed and cells further grown at 37ºC in nanoparticle-free medium for increasing times. Right: Accumulation of nanoparticles in the lysosomes. Confocal microscopy image of Hela cells incubated 24h with carboxylated-polystyrene nanoparticles (green) showing nanoparticles trafficked to the lysosomes. Blue: DAPI stained nucle;, red: immunostaining of lysosomal protein LAMP1.

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RAMAN SPECTROSCOPY, A SENSITIVE METHOD FOR BONE QUALITY EVALUATION

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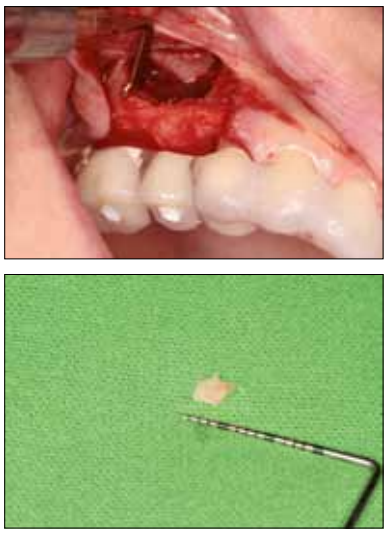
RESUME:

Our days it has been involved a large number of surgical techniques involving the implantation of various types of bone graft and /or bone substitutes in order to achieve periodontal regeneration. Despite positive observations in animal models and successful outcomes reported for many of the available regenerative techniques and materials in patients, including histologic evidence, robust information on the degree to which reported clinical improvements reflect true periodontal regeneration remains just limited. [1, 2]
The bone quality has primary influence on treatment planning, implant design, surgical approach, healing time and initial progressive bone loading during prosthetic reconstruction. [2] Bone quality encompasses factors other than bone density such as skeletal size, the architecture and 3-dimensional orientation of the trabecula, and matrix properties. Bone quality is a matter of mineral content (mature / immature bone ratio) and of structure as well.
The calcium phosphates compounds crystals toward their way to the phase of HA (hydroxyapatite) in bone, are organized as plate-like habit and are nano sized, with a length of ~20–50 nm and a width of 12–20 nm; ratio of bone mature / immature is depending on age or disease problems (periodontal most). [3]
Outcomes include mineral crystallinity, elemental composition, and collagen crosslink composition. Advantages include the detailed material characterization; disadvantages include the need for a biopsy (histomorphometry) for better results / evaluation. Bone samples were harvested by drilling during the piezo surgery protocol, before and after sinus bone augmentation healing period (about 8 month's interval) [Fig.1].



(a)

(b)

Fig. 1 Bone harvesting: (a) harvesting area, preparing for sinus augmentation; (b) cortical bone sample.

Regarding bone quality before and after healing period (sinus lift bone augmentation), investigation was performed by RAMAN technique [Fig. 2]. [4, 5, 6] There were evaluated following peaks:

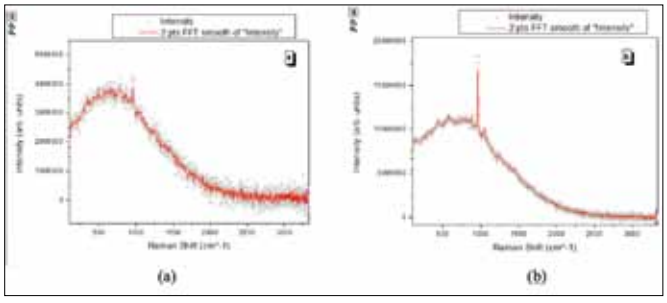


Fig. 2 Raman spectra for a patient: harvested bone samples before (a) and after healing time period (b)

The normalized peak intensity values, are related to the compounds concentration. A proper behavior quantification can be achieved (clinical as well). In bone, cementum and dentin, apatite crystals develop with their long c-axes parallel to the collagen fibril axis. Octacalcium phosphate (OCP, $Ca_8(HPO_4)_2(PO4)_4{\cdot}5H_2O$,) is considered very important because it is regarded as an *in vivo* precursor of HA [4, 5, 7] and has become an important candidate for use as a biomaterial for bone augmentation. The collagen and associated proteins play an important role in determining nucleation,

growth, and proliferation of these crystals – a nanoscale process. Trying to find traces of transformation of OCP to HA, the presence of HA nano rods and plate-like HA particles can be utilized as signs of bone augmentation process and a good quality future bone. [7]

The goal of our future study is that a correlation must be established between RAMAN spectra and bone main organic / inorganic fractions value in order to obtain a one-step complete investigation. Method easily can be adapted for *"in vivo"* bone quality evaluation, being much less invasive method then the well known CT (computer tomography) or CBCT (con beam computer tomography) already used and more accurate. [7, 8]

Acknowledgment: Part of financial support was obtained from B&W Tek Company USA, by equipment parts acquisition.

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AUTOMATIC CELL IDENTIFICATION IN HARD X-RAY TOMOGRAMS OF HUMAN BRAIN TISSUE

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2 Institute of Pathology, Department of Neuropathology, Basel University Hospital, Basel, Switzerland;

Tissue quantification including cell counting is a typical task in biological and medical research. Since the automatic cell identification is demanding within inhomogenous tissue, the inspection is often done manually. Most automatic approaches provided in quantification software tools are limited to the two-dimensional analysis of images with one cell type. In this study we present a cell identification approach to extract Purkinje cells within a human brain specimen represented by a three-dimensional hard X-ray tomogram. The human cerebellum sample was measured in local phase-contrast mode using synchrotron radiation at ESRF, Grenoble, France. The segmentation approach is derived from Frangi-filtering for vesselness and tailored to identify the characteristic geometry of the elliptical Purkinje cells. After filtering a level set approach is used to identify misdetections of spherical accumulation of hyaline masses featuring the target volume as well as vessels that locally resemble the shape of the target cells. Finally, the algorithm rejects outliers of the Purkinje cell layer. The algorithm determines an average cell volume of 4,850 μm^3 within the tomogram volume of 43 mm^3 and a surface density of 164 cells/mm^2 at the layer. The identification of the Purkinje cells is validated using a comparison to a histological analysis within a volume of interest and features an error of 5%.

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CONTACT ANGLE MEASUREMENTS ON NANO-STRUCTURED PDMS FILMS

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Silicone elastomers such as polydimethylsiloxanes (PDMS) are applied in medical implants, soft robotics, and microfluidic devices. For improved interface between PDMS films and biological systems, nanostructuring of the surface was performed, as previously reported for other polymers [(1)]. PDMS films form nanostructures resembling wrinkles on their surface when exposed to oxygen-plasma treatment. The amplitude and periodicity of these wrinkles could be tuned by varying the film thickness, treatment time, and partial oxygen pressure [(2)]. The surface chemistry of the nanostructured PDMS films was studied by dynamic water contact angle measurements. The wetting behaviour was improved for films with smaller amplitude and wrinkle periodicity.

Keywords: polydimethylsiloxanes (PDMS), dynamic contact angle measurements, oxygen-plasma surface nanostructuring.

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THE IMPACT OF LIPOSOMAL FORMULATIONS ON THE IN VIVO RELEASE AND BRAIN DELIVERY OF METHOTREXATE: A MICRODIALYSIS STUDY

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BACKGROUND:

The blood-brain barrier (BBB) is the primary reason for ineffective delivery of drugs from blood to the brain. One promising strategy to enhance brain drug delivery via the systemic circulation is to utilize nanocarriers (e.g. liposomes) encapsulating the active drugs. The composition of liposomes could be an important factor that may influence their *in vivo* "fate". However, quantitative evaluations regarding how different liposomal formulations may affect the *in vivo* release and brain uptake of the cargo have not yet been reported. Therefore, the purpose of this study was to quantitatively assess the impact of liposomal formulations on the *in vivo* release and brain delivery of methotrexate (MTX) in rats.

METHODS:

Two PEGylated liposomal MTX formulations based on hydrogenated soy phosphatidylcholine (HSPC) or egg-yolk phosphatidylcholine (EYPC) were prepared using an ethanol injection method with pre-insertion of PEG-lipid, followed by stepwise size extrusion through filters, before removing non-encapsulated drug via ultrafiltration. The drug release and uptake into the brain after intravenous administration of both formulations were compared with unformulated MTX by determining the released, unbound MTX in brain and plasma using microdialysis, together with total MTX in plasma using regular blood sampling.

RESULTS:

The preparation of both formulations yielded comparable liposomal batches, with average size of 110 nm and 105 nm for HSPC and EYPC liposomes, respectively. The loading efficacy was 4.2% for HSPC and 10.4% for EYPC liposomes. The extent of MTX release from EYPC liposomes in plasma was 10 times higher than that from HSPC liposomes ($p < 0.05$). MTX itself possessed limited brain uptake with steady-state unbound brain-to-plasma concentration ratio (Kp,uu,brain) of 0.10 ± 0.06. Encapsulation in HSPC liposomes had no impact on MTX brain uptake at all (Kp,uu,brain 0.11 ± 0.05). In contrast, EYPC liposomes significantly improved MTX brain delivery with approximately 3-fold increases of Kp,uu,brain, at both high ($p < 0.05$) and low dose ($p < 0.01$) used (Figure 1).

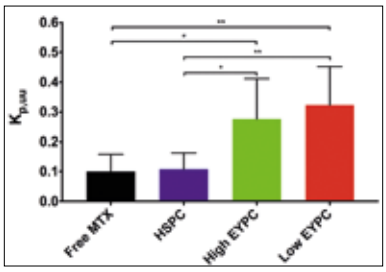

*Figure 1. The ratio of unbound MTX concentration in brain compared to that in plasma (Kp,uu,brain) after an intravenous short infusion (30 min) of PEGylated liposomal MTX or a constant infusion of free MTX. *p < 0.05, **p < 0.01, indicate significantly higher brain uptake of MTX with the administration of high and low dose EYPC liposomes compared with HSPC liposomes or free MTX, using a Kruskal-Wallis test followed by a Dunn's post hoc test, n = 5-6 per group.*

CONCLUSION:

This study showed that liposomal formulations based on different phospholipids can result in very different release properties and brain delivery of MTX. Due to the ability to separate different *in vivo* processes such as the drug release in blood and drug uptake into the brain, microdialysis can be a very valuable tool in investigating nanocarrier-mediated brain delivery, providing unique, detailed and quantitative information.

COMPARISON OF DEXTRAN NANOPARTICLE-COATED FLUORESCENT NANOCRYSTALS SYNTHESIS METHODS USING HYDRODYNAMIC FOCUSING

MAGDALENA JANCZEWSKA, Piotr Kowalczyk, Iga Wasiak, Tomasz Ciach

Hydrodynamic focusing is a highly valued repeatable process for formation of nanocrystals and copolymeric nanoparticles. Simplicity and scalability of this process makes it convenient for such applications where batch to batch consistency plays crucial role thus also for medical applications of nanotechnology.

Dextran nanoparticles (NPs) can serve as a platform for chemotherapeutics and diagnostic agents. By using glucose polymer we intend to exploit the Warburg effect of increased glucose need due to impaired metabolism.[1] Due to triggering metabolic abnormalities – present in a large variety of tumors - nanoparticles could reach cancer tissues that lack specific receptors on their cell membrane. This attempt is used e.g. by fluoro-deoxyglucose (FDG), a popular radiopharmaceutical used for PET diagnostics.

The aim of presented work was to compare two methods of nanoprecipitation and coating fluorescent, organic nanocrystals with dextran nanoparticles. For the NPs synthesis polyaldehydodextran (PAD) and dodecylamine (DDAC) were used as reagents, and 9-aminoacridine stearate (9-AA) was used for formation of nanocrystals. Briefly, nanoparticles were synthetized by a covalent attachment of dodecylamine to oxidated dextran chain. The Schiff base was obtained and product was purified by dialysis for 1 h. The organic fluorescent salt was obtained in an one-step synthesis of 9-aminoacridine hydrochloride and sodium stearate. The product was purified by double recrystallization.

In the first method – Parallel Precipitation and Reaction (PPR), the mixture of reagents for NPs synthesis was freshly mixed in the syringe and the nanoprecipitation was carried out. Afterwards the NPs synthesis was finished by the controllable Schiff base formation. Second method – Nanoparticle Supported Precipitation (NSP) was performed by first: synthesis of NPs and then nanoprecipitation in microfluidic system. Nanocrystals were expected to be preferably formed inside the hydrophobic areas of micellar structure of nanoparticle. Nanoprecipitation was carried in microfluidic system for four flow ratio values R (9-AA flow / reagents/NPs flow): 0,033; 0,02; 0,01; 0,0066. All samples were lyophilized and then analyzed using NTA (Nanoparticle Tracking Analysis) and SEM microscopy. As a control crystals were also obtained using bulk method and nanoparticles without nanocrystals were characterized.

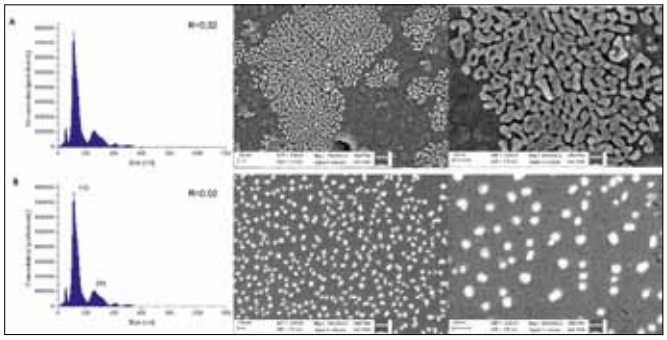

Figure 1. Comparison of PPR (A) and NSP (B) method of fluorescent nanoparticle formation.

The obtained results showed the preference of nanocrystals to precipitate in local hydrophobic areas – such as micelle interior (due to self assemble of dextran molecule modified with hydrophobic chains). SEM microphotographs showed that fluorescent nanoparticles obtained with NSP method are more homogeneous compared to the same R parameter for PPR method. Figure 1 shows comparison of two methods for R parameter equal 0,02.

Furthermore, the higher flow ratio (R parameter) resulted in smaller and less aggregating nanocrystals. With decreasing R values more heterogenous nanocrystals were observed. What is more method with using solely mixture of reagents in aqueous phase (PPR) resulted in similar crystals to bulk method – growing on earlier formed crystals. With decreasing value of R parameter higher polydispersity of size distribution was observed which is consistent with SEM microphotographs.

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MODIFICATION OF THE TUMOR MICRO-ENVIRONMENT BY TARGETED INDUCTION OF IMMUNOGENIC CELL DEATH

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Innovative strategies fighting cancer by inducing anti-tumor responses from the immune system are urgently needed. Previously, chemotherapeutics from the class of the anthracyclins have been shown to induce immunogenic cancer cell death and triggering anti-tumor immune responses [1]. Problematically, however, the patient`s immune system often becomes severely impaired by the unspecific action of these cytotoxic drugs in systemic chemotherapy precluding any effective immune reactions. To reduce systemic side effects and to accumulate the drug exclusively in the tumor region, we developed an iron oxide nanoparticle-based system (SPIONs) for the magnetically-targeted delivery of mitoxantrone (MTO) to the tumor [2,3], which has proven its long-term therapeutic effectivity in tumor bearing rabbits previously [4]. This study aims to analyze the cell death phenotype induced by SPIONs loaded with mitoxantrone (SPIONMTO) in comparison with the free drug.
Flow cytometry was performed to determine cell cycle and kinetics of apoptosis and necrosis in Jurkat and HT-29 cells treated with SPIONMTO. AnnexinA5-Fitc/propidium iodide (PI) staining and PI-Triton staining revealed that both SPIONMTO and the free drug induced a cell cycle arrest with concomitant DNA degradation, exposition of phosphatidylserine and plasma membrane rupture (Fig.1). Quinacrine staining, luciferase chemiluminescence assay and Westernblot showed that SPIONMTO efficiently induced release of the danger signals ATP and HMGB1.
In sum, we showed that SPIONMTO induced a cell death phenotype which was slightly delayed but comparable to that of free MTO, which is a well-established immunogenic cell death inducer. We conclude that the targeted induction of immunogenic cell death exclusively in the tumor region (e.g. by magnetic drug targeting of SPIONMTO) might be a promising possibility to selectively modulate the tumor microenvironment and to stimulate immune responses against the tumor in the presence of an intact immune system in future clinical applications.

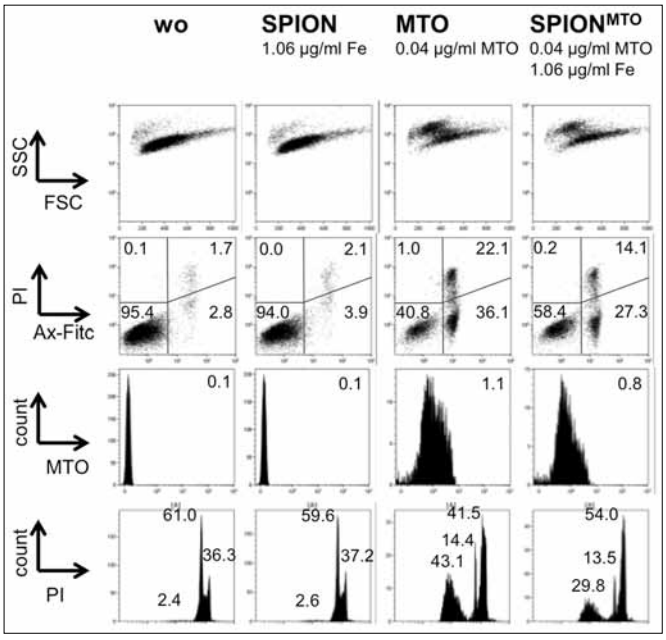

Figure 1: Flow cytometry of Jurkat cells after 24 h treatment with free MTO, SPIONMTO and unloaded SPIONs. AnnexinA5-Fitc/ propidium iodide staining discriminates between viable (Ax-PI-), apoptotic (Ax+PI-) and necrotic (PI+) cells. PI-Triton staining provides information about cell cycle (G1 and S/G2 phase) and DNA degradation (subG1). Due to its inherent fluorescence cellular MTO loading can be additionally assessed.

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ENTRAPPING IRON OXIDE NANOPARTICLES FOR LOCAL MAGNETIC HYPERTHERMIA

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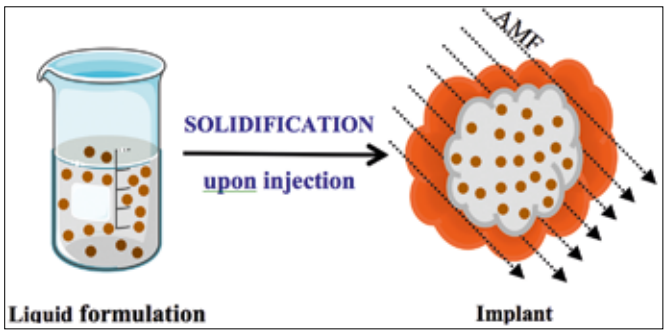
Local moderate hyperthermia based on superparamagnetic iron oxide nanoparticles (SPIONs) offers a promising therapeutic approach in cancer treatment, especially in combination with radio- or chemotherapy [1]. One of the major challenges for clinical translation remains the deposition of a sufficient amount of SPIONs at the site of interest to reach the target temperature of 42-46 °C under well-tolerated alternative magnetic field (AMF) strengths and frequencies.
In order to circumvent these obstacles, we aim to develop polymer formulations that are able to solidify as implants upon injection, entrapping the incorporated SPIONs.



The water-insoluble polymer consists of a non-toxic, modified poly(vinylalcohol) (mono-/tri-iodo benzylether polyvinylalcohol, MTIB-PVA, Easyx®) [2]. Its covalently attached iodine moieties allow X-ray imaging of the implant due to its radiopacity. Such formulations are intended for minimally invasive tumor treatment through interventional oncology techniques, embolizing tumor-feeding blood vessels,
We present in this work two promising nanoparticle formulations: PMMA-coated SPIONs and SPIONs embedded in mesoporous silica (silica-SPION beads). In a first step, the heat release quantified as specific power loss (SLP) of the SPIONs was determined. To do so SPIONs were embedded in an agar gel to suppress the released

heat due to Brownian relaxation, mimicking the *in vivo* implant.
Formulations composed of different concentrations of MTIB-PVA and SPIONs were then evaluated for their rheological behavior and implant formation to ensure proper injectability while forming a homogeneous implant. Together with the determined SLP of the implants, the 2 formulations containing 18% of MTIB-PVA and 25.0% of PMMA-SPIONs or 29.8% (w/w) of silica-SPION beads were further tested for their syringeability by measuring the injection force when injecting the liquid suspension in tissue-mimicking material.
To mimic the physiological tumor cooling, *in vitro* temperature rise was measured with the SPIONs-containing implant cell surrounded by circulating thermostated water at 37 °C, under AMF exposure.
A temperature plateau of 50°C (± 3°C) was reached at 121 kHz and 9 mT.
Since the in-situ formation of the implant can be used to locally deliver an active drug, the incorporation of polidocanol as a sclerosing agent was investigated. Drug release over 14 days was monitored by UPLC-MS. Incorporation of the maximal recommended dose of polidocanol showed an initial burst within 1 day whereas for a 10 times lower concentration a sustained release until day 7 was observed.
To conclude, we were able to develop an injectable, in-situ forming implant adapted for magnetic moderate hyperthermia due to its high SPION payload, appropriate injectability and implant formation. We showed that co-delivery of polidocanol as a sclerosing agent is feasible at its maximal recommended dose to support the embolic effect of the implant.
The authors thank Dr. Martin Rudolph for providing the PMMA-SPIONs. The presented work is part of the MagnetoTheranostics project, funded by the Swiss National Program Nano-Tera.

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MUCOADHESIVE NANO LIPID GEL AS PROLONGED CARRIER SYSTEM FOR OROPHARYNGEAL ANTI-CANDIDAL DRUG DELIVERY

FRANKLIN KENECHUKWU, Attama A.A., Ibezim E.C.

EXECUTIVE SUMMARY

The objective of this study was to develop phospholipid-modified beeswax-based nano lipid gel for enhanced oromucosal delivery of miconazole nitrate (MN) in the treatment of oropharyngeal candidiasis (OPC). Lipid matrix containing beeswax and Phospholipon® 90H was used to formulate SLNs which were thereafter used to prepare mucoadhesive nano lipid gels of MN. Drug release from the nano lipid gels in simulated salivary fluid (SSF, pH 6.8) and anti-candidal activity against oral thrush swab (OTS) of Candida albicans were carried out. The SLNs were monodisperse, with nanometer-sized particles and had good physicochemical properties. Phospholipid-modified beeswax-based MN-loaded nano lipid gels exhibited better prolonged drug release and anti-candidal properties than marketed formulation (Daktarin® oral gel) ($p < 0.05$). The nano lipid gels were stable and possessed adequate mucoadhesive strengths. The developed phospholipid-modified beeswax-based mucoadhesive nano lipid gels could be employed to prolong localized oromucosal delivery of MN for effective treatment of OPC.

INTRODUCTION

The oromucosal route of administration is highly appropriate for treating local conditions such as oropharyngeal candidiasis (OPC), but current drug regimen has not been adequate. Several approaches have been explored to enhance the delivery of MN in the treatment of OPC [1, 2]. Nano lipid gel made up of lipid nanoparticles in a gel base [3] could be exploited as potential oromucosal formulation to target imidazole antifungals to the oromucosal layers. In this study, phospholipid-modified beeswax-based mucoadhesive nano lipid gels were formulated and evaluated for prolonged localized oromucosal delivery of MN for effective treatment of OPC.

EXPERIMENTAL MATERIALS

The following materials were used in the study: Miconazole nitrate USP (Gutic Biosciences Limited, India), Phospholipon® 90H (P90H) (Phospholipid GmbH, Köln, Germany), Daktarin® oral gel (McNeil Products Ltd., Maidenhead, Berkshire, SL6 3UG, UK), Sabouraud Dextrose Agar (SDA) (United Technology Trade Corp, USA), Polycarbophil (Noveon®) (Lubrizol Corporation, Ohio, USA), beeswax (white) (Ph. Eur. Carl Roth GmbH + Co. KG Karlsruhe, Germany) and distilled water (Lion water, University of Nigeria, Nsukka, Nigeria). Clinical isolate of C. albicans was used. All other reagents were analytical grade.

PREPARATION AND CHARACTERIZATION OF LIPID MATRICES

The lipid matrix (LM) was formulated by fusion [4] in paraffin oil bath using Phospholipon® 90H and beeswax and thereafter characterized by wide angle x-ray diffraction (WAXD).
Preparation and characterization of solid lipid nanoparticles (SLNs)
The SLNs were prepared using MN (0, 0.25, 0.5, 1.0 %w/w), LMs (5.0 %w/w), Polysorbate® 80 (Tween® 80) (2.0 %w/w), sorbitol (4.0 %w/w) and distilled water (q.s. to 100.0 %w/w) by the high shear hot homogenization method [5]. The formulated SLNs were characterized using encapsulation efficiency (EE%), drug loading capacity (LC), average particle size (z-average), polydispersity indices (PDI), zeta potential (ZP) and morphology.
Preparation and characterization of nanolipidgels
The nano lipid gels were prepared using the mucoadhesive agent (Polycarbophil®, PCP) (1.0 %w/w) SLNs from each batch [(20 ml) (to yield 0, 0.05, 0.10, 0.20 %w/w MN)] and glycerol (3.0 %w/w), sorbic acid (0.02 %w/w) and distilled water [6]. The pH of the developed nano lipid gels was adjusted to 6.8 using 0.5 M NaOH. The formulations were evaluated for drug content, mucoadhesion on everted cow buccal mucosal tissue and drug dissolution in dialysing membrane in simulated salivary fluid (SSF, pH 6.8), anti-candidal activity against OTS of C. albicans and stability.The results were analysed statistically using analysis of variance and inter- and intra-group variability were considered significant at $p < 0.05$.
Results and discussion
X-ray diffractograms (Figures 1 and 2) confirmed the amorphous nature of MN in the LM. Monodisperse SLNs were obtained (Figure 3) with PDI of 0.238 – 0.274 and z-average, ZP, EE% and LC values in the range of 204.0 to 363.0 nm, -32.9 to – 40.1 mV, 36.23 to 58.87 % and 9.82 to 18.24 %, respectively. Beeswax-modified MN-loaded nano lipid gels exhibited better prolonged release and anti-candidal properties than marketed formulation (Daktarin® oral gel) (Figures 4 and 5a) owing to the higher inhibition zone diameters obtained [1, 5]. The formulations were stable and possessed adequate mucoadhesive strengths (Figure 5b) for prolonged delivery of MN for effective treatment of OPC. The nano lipid gels were also spreadable.

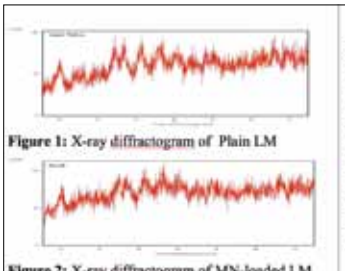
Figure 1: X-ray diffractogram of Plain LM

Figure 2: X-ray diffractogram of MN-loaded LM

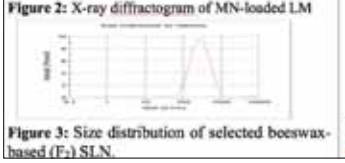
Figure 3: Size distribution of selected beeswax-based (F_1) SLN.

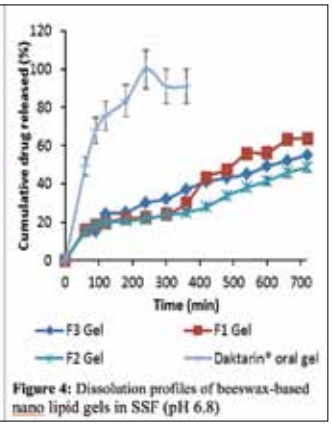

Figure 4: Dissolution profiles of beeswax-based nano lipid gels in SSF (pH 6.8)

Key: Z-Av means average particle size; PDI means polydispersity indices; ZP means zeta potential; EE means encapsulation efficiency; DL means drug loading; F0- F3 are beeswax-based SLNs; F1, F2 and

F3 contain increasing concentrations (0.25, 0.5 and 1.0 %w/w, respectively) of MN; F0 is plain or unloaded SLNs, F1 Gel, F2 Gel and F3 Gel contain increasing concentrations (0.25, 0.5 and 1.0 %w/w, respectively) of MN while Daktarin® oral gel is a commercially available gel containing 2.0 %w/w MN.

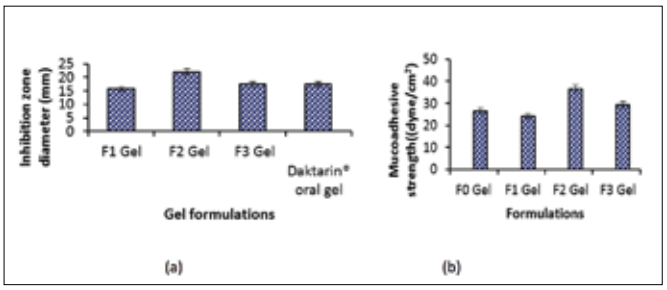

Figure 5: Anticandidal efficacy (a) and mucoadhesive property (b) of beeswax-based nano lipid gels

Key: F_1 Gel, F_2 Gel and F_3 Gel contain increasing concentrations (0.25, 0.5 and 1.0 %w/w, respectively) of miconazole nitrate; F0 Gel is plain or unloaded nano lipid gel, while Daktarin® oral gel is a commercially available gel containing 2.0 %w/w miconazole nitrate.

Conclusion

This study has shown that phospholipid-modified beeswax-based mucoadhesive nano lipid gels could be employed to prolong localized oromucosal delivery of MN for effective treatment of OPC.

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TB^{3+}-DOPED NANOPARTICLES FOR CORRELATIVE CATHODOLUMINESCENCE ELECTRON MICROSCOPY BIOIMAGING

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Correlative imaging has enabled some of the biggest scientific discoveries in the past. It has become a powerful analytical tool for unravelling structure-function relationships. However, correlating optical and electron microscopy is often challenging due to the different sample preparation requirements and the resolution mismatch between these methods. Interestingly, accelerated electrons can also generate luminescence signal from some materials; creating an effect called cathodoluminescence.

Here, we present the use of Tb^{3+}-doped nanocrystals as probes for high-quality correlative cathodoluminescence electron microscopy (CCLEM) bioimaging. We demonstrate correlative high resolution cathodoluminescence and electron back-scattering (BSE) imaging of focused ion beam (FIB) sectioned osmium-contrasted biological samples. Simultaneously obtained back-scattering and cathodoluminescence images reveal the cellular ultrastructure, including mitochondria and vesicles filled with nanocrystals, as well as identify the light-emitting nanoparticles inside the cells. This first experimental demonstration of correlative cathodoluminescence back-scattering electron microscopy on FIB-sectioned biological samples illustrates the potential of this approach for the acquisition of high-quality luminescence and electron microscopy images with nanometric resolution In addition, we show a comprehensive study on stability of crystal phases under accelerating electrons.

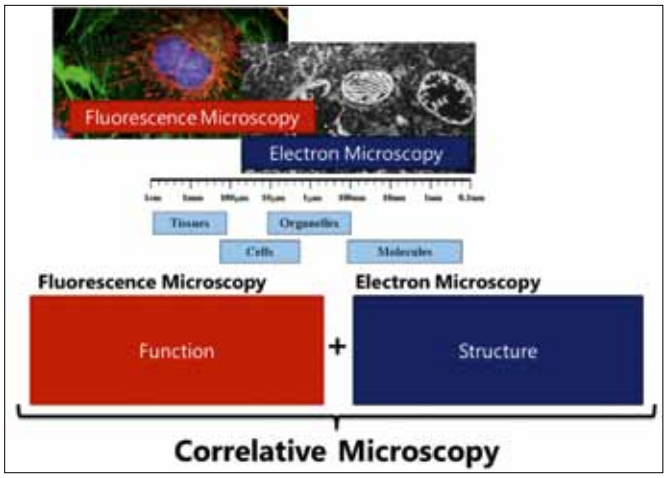

Figure 1: Correlative Microscopy combines information on the function and the structure and provides a more comprehensive picture.

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IMAGING HUMAN BRAIN TISSUE DOWN TO CELLULAR LEVEL USING HARD X-RAY TOMOGRAPHY WITH CONVENTIONAL AND SYNCHROTRON SOURCES

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Hard X-ray imaging is a powerful method for three-dimensional (3D) investigations, which can provide a sub-cellular resolution. As it was recently shown on the example of formalin-fixed paraffin-embedded (FFPE) human cerebellum, laboratory-based micro computed tomography in absorption-contrast mode, yields a density contrast, comparable to conventional histological sections [1], and synchrotron radiation-based in-line X-ray phase-contrast tomography enabled the visualization down to the sub-cellular level and automatic feature quantification [2]. Here, we discuss the study of brain tissue ultrastructure using synchrotron radiation-based hard X-ray magnified phase-contrast nano-holotomography at beamline ID16 (ESRF, Grenoble, France). As an example, we present images of human cerebellum and cortex blocks embedded in JB-4 (water-soluble, GMA based, plastic resin), epoxy resin and paraffin in which structures at nanometer scale such as the nucleoli of Purkinje, granule or pyramidal cells can be resolved. We expect that nano-holotomography can provide valuable complementary information for clinical applications, quantitative diagnostics and basic research emerging accessibility of data from relatively large intact samples.

Keywords: Phase-contrast, micro computed tomography, hard X-ray, 3D visualization, soft tissue, synchrotron radiation-based hard X-ray magnified phase-contrast nano-holotomography, cerebellum, cortex.

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PHOTOTHERMAL, PHOTODYNAMIC, REDOX AND OPTICAL PROPERTIES OF GREEN SYNTHETIZED CARBON NANOPARTICLES: TOWARD A NOVEL CLASS OF SWITCHABLE NANO PLATFORMS FOR THERAGNOSTIC APPLICATIONS

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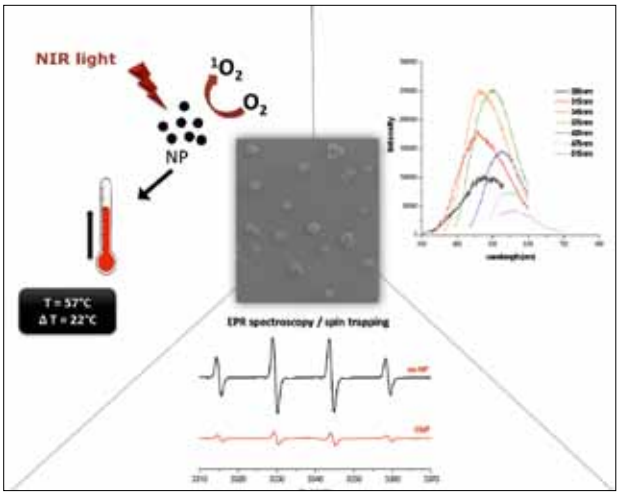
Photodynamic therapy (PDT) and photothermal therapy (PTT), are non-invasive and localized treatments that presents great potential in cancer.[1] Molecular photosensitizer like porphyrins are the most studied PDT agents. However, recently, carbon nanomaterials emerged as a possible alternative for PDT/PTT.[2][3] By respect molecular substances, nanoparticles have a higher potential as theragnostic tools. In fact, by functionalization of the surface, the design of multifunctional platforms is possible. Here, carbon nanoparticles (CNP) synthesized by an hydrothermal green-chemistry approach [4], have been prepared, characterized (SEM/TEM, TGA, DLS, ELS) and tested for their optical, antioxidant, photodynamic and photothermal properties by means of EPR spectroscopy in order to explore their possible development as novel multifunctional platforms for theragnostic applications .The optimized synthesis produces CNP exhibiting a spherical shape having a mean diameter of 100 nm and a narrow size distribution in a reproducible manner. The nanoparticles are negatively charged and form very stable reversible colloidal systems in aqueous media. When irradiated with Near Infrared (NIR) light, CNP are able to generate the highly cytotoxic singlet oxygen ($^1O_2$) an effect related to their partial graphenic bulk structure. Moreover, CNP efficiently convert the NIR radiation in heat, causing an increment of the temperature (T = 57°C) sufficient for cell death induction, this synergic effect make CNP good candidates for combined PDT/PTT therapy. On the other hand, in the absence of photo-activation, CNP act as hydroxyl radical scavengers suggesting a possible antioxidant activity. Finally, CNP display fluorescence which could be useful for imaging or diagnostic purposes. This study represent a first step towards the development of novel morphologically and structurally tailored switchable theragnostic platforms for the treatment of cancer and other diseases.



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POLYETHYLENE GLYCOL (PEG)-INDUCED ANTIBODY RESPONSE IN THE SPLEEN OF PIGS VIA T CELL INDEPENDENT IMMUNOGENICITY

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INTRODUCTION

Coating of drug carrying liposomes and therapeutic proteins (e.g. monoclonal antibodies) with polyethylene glycol (PEG) is common practice today to extend the circulation time of these drugs. However, these structures sometimes cause pseudo-allergic (also

called infusion-) reactions during intravenous application mediated mainly by the complement system[1,2]. We have shown previously in a porcine model that the interaction of PEGylated liposomes with pre-existing anti-PEG IgM antibodies could trigger complement activation. As it is shown on Figure 1, even one low dose (0.1 mg phospholipid per kg (PL/kg)) injection of a PEGylated liposome, DOXEBO (doxorubicin-free Doxil look-alike), was able to induce strong anti-PEG antibody response causing often (4 out of 5) immediate death in the case of a second injection (data not shown). Interestingly, neither PEG immunogenicity nor physiological changes could be detected after 2nd DOXEBO treatment, if doxorubicin containing Doxil was also (co-) administered during sensitization (pre-treatment). As it could be expected from the repetitive structure of PEG[3]; from the kinetics of antibody response (Figure 1); and from the literature[4,5,6], PEG immunogenicity might be T cell independent (TI-2).
The aim of our present study was to understand the role of spleen cells in the anti-PEG IgM response to PEGylated liposomes, and the suppression of this response by Doxil pretreatment.

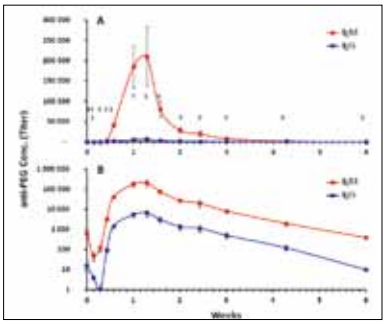

Figure 1. Immunogenicity of DOXEBO after pigs were injected (immunized) i.v. with 0.1 mg PL/kg DOXEBO on day 0. Titers were identified by ELISA. Anti-PEG IgG and IgM levels are presented both on linear (A) and logarithmic (B) scales in different time points over 6 weeks. The error bars are SEM from n pigs specified above the curves at each time-point in Panel A.

METHODS

The PEG binding capacity of spleen cells were investigated *in vitro* by flow cytometry in naïve pigs (control without immunization) and 1 week after i.v. treatment by DOXEBO or Doxil. Spleen cells were isolated by mechanical disruption and PEG binding cells were stained by naturally auto-fluorescent Doxil (because of doxorubicin), or by fluorescently labeled PEG micelle/DOXEBO followed by red blood cell lyses. The *in vitro* antibody production potential of spleen cells was investigated in the mononuclear fraction of spleen cells obtained by Ficoll density gradient cell separation. 10x106 cells from control, DOXEBO, or Doxil pre-treated pigs were plated in one mL complete medium (containing RPMI-1640, FBS and supplements) in 24 well plates, 1 week after *in vivo* treatment; and they were stimulated by DOXEBO in different concentration from 0.7 pM to 670 nM phospholipid per liter (PL/L) for 5 days. After the supernatants were collected, anti-PEG IgM levels were identified using an ELISA (SeroScience Ltd).

RESULTS AND DISCUSSION

As it is shown on Figure 2, DOXEBO immunization (0.1 mg PL/kg) markedly increased the frequency of Doxil binding IgM+ B cells or IgM- lymphocytes in the spleen one week after treatment, suggesting that these B cells are directly involved in IgM production against DOXEBO. Similar results was found (data not shown), when spleen cells were stained by fluorescently labeled PEG micelles, or DOXEBO, indicating that nano-structures were recognized by their PEG coat.

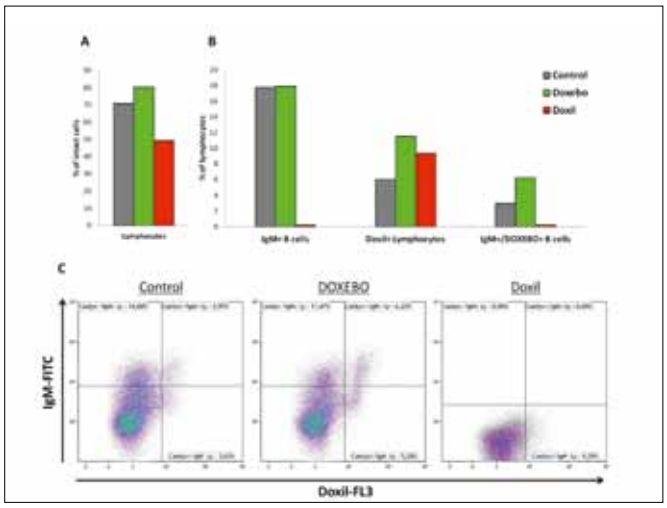

Figure 2. Representative flow cytometric analysis of spleen cells isolated from a control pig, or from 0.1 mg PL/kg DOXEBO, or from human equivalent dose (HED, 6.4 mg PL/kg) Doxil-treated pigs, 1 weak after immunization. A and B show the frequency of different cell types among intact cells and lymphocytes, respectively, identified by side and forward scattering. Cell gating of B is also shown on C.

Doxil pre-treatment during sensitization eliminated the IgM positive B cell population after one weak, explaining the lack of IgM production and the physiological changes during a 2nd antigen challenge. Cytotoxic effect of doxorubicin might be specific to the IgM+ cells, since the frequency of the whole lymphocyte population decreased less dramatically (Figure 2A), than the frequency of IgM+ cells (Figure 2B and C).
Spleen mononuclear cells of non pre-treated control animals produced a substantial amount of anti-PEG antibody when they were stimulated by 70 pM PL/L DOXEBO as it could be seen on Figure 3. However their antibody production was close to zero if DOXEBO was used *in vitro* in other than 70 pM PL/L concentration. Similar tendency of antibody production was measured from the supernatant of DOXEBO sensitized pig's spleen cells, but these cells produced 10 times less antibody than the spleen cells of control animal. As it was expected, *in vivo* Doxil pre-treatment one week before the *in vitro* stimulatory experiment, completely blocked the antibody producing potential of spleen cells. These results may confirm the direct role of spleen in PEG immunogenicity. The shortness of *in vitro* antibody production also suggests T cell independent mechanisms.

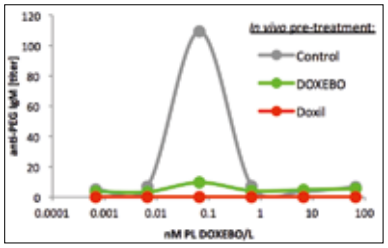

Figure 3. Anti-PEG IgM titer in spleen mononuclear cell supernatants 5 days after treatment by DOXEBO in different concentration as it is indicated on the X axes. Spleens were isolated from a placebo treated (control) pig (gray); a 0.1 mg PL/kg DOXEBO-treated pig (green), or from a pig treated with the human equivalent dose of the minimum therapeutic dose of Doxil (HED, 6.4 mg PL/kg), 1 weak after immunization. Antibody measurements were performed by ELISA (SeroScience Ltd).

CONCLUSIONS

Our results provide new evidences that immunogenicity against PEG is developed in spleen tissue by T cell independent mechanisms, and doxorubicin prevents this immunogenicity effect by eliminating the IgM positive B cells. Since these cells are not directly involved in classical, T cell dependent, IgG mediated immune response, selective IgM+ cell toxicity might be a mechanism of the special effect of doxorubicin, which does not affect immunity of humans against infections as it was observed in man using Doxil therapy, but eliminates the immunogenicity of PEG.

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NOVEL PEPTIDE-BASED TARGETED THERAPEUTICS FOR TREATMENT OF PANCREATIC CANCER

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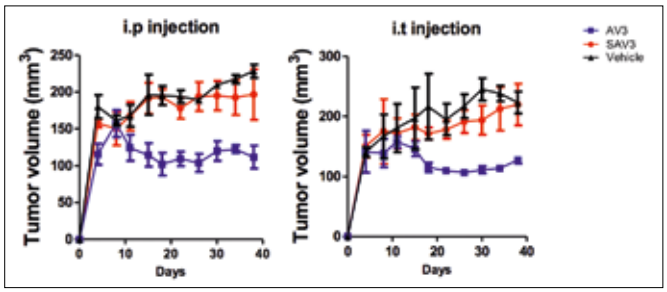
Cancer-associated fibroblasts (CAFs) are the key cell type in the pancreatic tumor microenvironment, which induces tumor growth and metastasis. Here, we have identified integrin alpha5 (ITGA5) as a key target overexpressed in pancreatic CAFs, designed a novel peptide for ITGA5 targeting, and studied its significance on pancreatic cancer.

In human patient tumor samples (n=137), 66% of the patients were positive for ITGA5 and well co-localized with α-SMA, as shown with double immunostaining. Overall, clinical data analysis reveals that the overexpression of ITGA5 (log-rank p=0.022) is linked to significant poor overall survival. *In vitro*, activation of human primary pancreatic stellate cells (PSCs) either with recombinant TGFβ-1 or with conditioned medium obtained from Panc-1 tumor cells significantly induced ITGA5 expression in PSCs.

A novel 7 amino acid peptide (AV3) designed from the region of fibronectin for targeting ITGA5. Importantly, ITGA5 targeting in hPSCs with a novel peptide led to a reduction of differentiation markers such as α-SMA and Collagen1 at gene and protein level. Furthermore, targeting ITGA5 in hPSCs showed inhibition of TGFβ-1 induced collagen contractility compared to control hPSCs. *In vivo*, co-injection of Panc-1 and PSCs in SCID mice showed a significant increase in tumor growth compared to Panc-1 tumors. Fig. 1 shows tumors from co-injection of Panc-1 and PSC treated with AV3 peptide {either intraperitoneal (i.p) or intratumoral (i.t)} had less tumor growth compared to scrambled peptide (SAV3) and vehicle group, indicating the significance of ITGA5 in controlling PSC-induced tumor growth *in vivo*.

Fig1. Tumors from co-injection of Panc-1 and PSCs treated with novel AV3 peptide (20mg/kg (i.p) or 4mg/kg (i.t)) inhibits tumor growth compared to scrambled peptide and vehicle group.



Altogether, this study reveals ITGA5 as a novel therapeutic stromal target in the pancreatic tumor, which is in potential for clinical application.

APPLICATION OF GOLD NANOPARTICLES FOR ANATOMICAL EX VIVO MICROVASCULAR X-RAY MICRO-CT IMAGING IN MOUSE KIDNEYS

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X-ray imaging of the vasculature in the kidney poses additional challenges compared to other organs. They perform their function as the body's excretion mechanism in a first step through hyperfiltration, pressing the incoming blood into the glomeruli, where compounds smaller than 6 nm in hydrodynamic diameter are filtered into the primary urine [1]. Commercially available iodine-based organic X-ray contrast agents are well below this size, as excretion through the kidneys allows fast clearance of the contrast agents in patients after the scan. Blood pool contrast agents on the other hand are designed for longer retention time, to allow for additional scan time with the same injection. Gold nanoparticles with hydrophilic coating are available in the necessary sizes in order to not get filtered.

X-ray microcomputed tomography scanners are able to scan objects the size of a mouse kidney with isotropic micrometer resolution with the potential to image the three-dimensional microvasculature. However, immersion fixation is insufficient in kidneys to retain full tissue integrity, as blood vessels and tubules collapse after blood pressure drop caused by euthanasia. The injection of nanoparticles into the blood stream would not lead to their even distribution throughout the body by the natural action of blood flow, because they would be flushed out during the perfusion step. Instead, they have to be perfused after the fixative by artificial action.

We have perfused Nanoprobes Aurovist 15 nm gold nanoparticles along with gelatin into mouse kidneys and scanned them with a GE Phoenix Nanotom m X-ray micro-CT scanner. Their performance was compared with resin-based hydrophobic vascular casting.

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BENEFITS AND EFFECTS OF NANOPARTICLE ASSISTED MICROWAVE IMAGING

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Microwave Imaging (MWI) is a novel and inexpensive technique that has recently been studied for cancer detection in the breast. Microwave sensing is an emerging technology that can be used in healthcare applications and particularly in the detection of malignancies. This method is attractive because it uses non-ionising electromagnetic radiation and breast compression is avoided, making it safer and suitable for patient needs. The prospective of using microwaves for breast imaging has been suspect due to published studies of wideband dielectric characterization of various breast tissues, which have shown that the contrast between malignant and healthy tissue can be as low as 10% in the case of dense fibroglandular tissue[1,2]. Utilization of nanoparticles (NPs) as effective contrast agents to assist in imaging may be applicable to microwave imaging. We aim to understand how nanoparticles would be able to provide a clear and visible contrast enhancement in water-based suspensions In this study we characterize the nanoparticles in water based suspensions, and observe how dispersion and concentration of these NP effect the electrical properties at specific microwave frequencies.

METHOD

Preparation of samples

The nanoparticles used in this study are silicon dioxide (SiO_2), zinc oxide (ZnO), and titanium oxide (TiO_2). Concentrations of 2 mg/mL, 1 mg/mL, 0.5 mg/mL, and 0.25 mg/mL for each nanomaterial were prepared in a final volume of 20 mL with 1 v/v% Pluronic surfactant. Samples were vigorously agitated for 2 minutes and sonicated for another 30 minutes (frequency = 37 kHz, Ultrasonic power = 160 W) at room temperature (20oC) to ensure a homogenous solution.

PEGylation of zinc oxide NPs

Further improvement of the dispersion and stability of ZnO NPs in water is required to record meaningful results. Polyethylene glycol (PEG) can be used as a coating on ZnO NP to prevent aggregation and sedimentation ZnO nanoparticles may require PEGylation to maintain colloidal stability. We investigated the effect of PEG on size and colloidal stability using Atomic Force Microscopy (AFM), Scanning Electron Microscopy (SEM) and UV-Vis spectrophotometry. 750 mg of PEG (8000 MW) was stirred into 100 mL of deionised water until fully dissolved (prepared in stock solution). Then, 606.9 mg (15 mg/mL) of zinc oxide powder was suspended into 40mlof deionised water and sonicated vigorously for 60 minutes, and agitated for 1 minute at 20 minute intervals. The pH of the solution was adjusted to 8.5 by adding NaOH (aqueous) followed by 30 minutes of stirring and the same sonication phase as before. 10 mL of PEG stock solution was added to the ZnO NPs suspension. The reaction mixture was stirred for 48 hours at room temperature. ZnO NPs were collected in a centrifuge and washed in water, followed by further washing procedure, to remove unabsorbed polymer. The pellets was then dried overnight[3,4].

Characterisation of Dielectric Properties

The dielectric properties were measured using a well-established open-ended coaxial probe technique. The dielectric probe was calibrated using three known dielectric materials; air, short block (conductive elastomer which replicates the electrical properties of metal), and de-ionised water (20oC). The minimum and maximum frequencies for the dielectric measurements was set to 1 GHz and 4 GHz. At the completion of the calibration, the measured dielectric constant of water is compared to factory stored dielectric data of water, in order to validate the accuracy of the measurement.

RESULTS

Size characterisation of NPs were confirmed by SEM and AFM (Fig 1). Average size obtained for ZnO, $SiO_2$and TiO_2 NPs by SEMwas 55.7nm, 24.4nm and 14.3nm respectively. Size for PEG-ZnO NPs was confirmed by AFM and the average size obtained was 67.2nm. The stability of PEGylated ZnO NPs and ZnO NPs was measured by Ultraviolet/Visible spectrophotometer (UV-Vis).The stability of ZnO suspension in distilled water showed an increase of transmission from 72.75% ± 4.14% to 99.08% ± 1.86% in 2-hour interval indicative of aggregation phenomena. The suspensions of ZnO-PEG showed transmission of 91.87% ± 1.84% at t0 and at t2h was 90.23% ± 3.80%.

Carbon nanotubes (CNTs) have been studied in the past as potential contrast agents for MWI[5] and were measured in this study asa potential control. The relative permittivity of CNT-OHat concentration of 2 mg/mLin water at 2GHz was 87.11 ± 2.14. The relative permittivity of SiO_2 and TiO_2 at a concentration of 2mg/mL at 2GHz was 79.76 ± 0.01 and 79.81 ± 0.01 respectively. However, the relative permittivity of ZnO at same conditions was 80.42 ± 0.07. Therelative permittivity obtained for ZnO-PEG was 84.04 ± 0.35. The maximum average change (ε) in unaltered zinc oxide NPs in 2, 1, 0.5, 0.25 mg/mL is 1.99 %, 1.58 %, 2.11 % and 1.70 %, respectively. The maximum average change of PEGylated zinc oxide dielectric constant in 2, 1, 0.5, 0.25 mg/mL is 6.76 %, 7.58 %, 4.85 % and 4.70 % accordingly, over the whole frequency range.

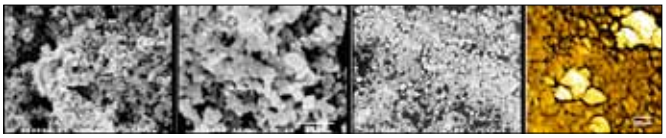

Figure 1: Scanning electronic microscopy (SEM) images starting from the left are of TiO_2, SiO_2, ZnO and Atomic force microscopy (AFM) image of ZnO-PEG.

CONCLUSION

Currently, our results suggest that ZnO-based NPs are promising contrast agents for microwave imaging and merit further investigation. PEGylation of ZnO NPs improves its stability and dispersion in water. However physical properties of ZnO nanoparticles need to be observed to optimize the possible contrast.

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A NOVEL LIPOSOMAL FORMULATION FOR INTRAVENOUS DELIVERY OF VORICONAZOLE

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INTRODUCTION

Voriconazole (VCZ), a second generation triazole with a large spectrum of action is one of the most recommended systemic antimicrobial agents as the first line therapy against several types of systemic mycoses. VCZ exhibits a nonlinear pharmacokinetic profile due to metabolic clearance saturation (YAMADA et al., 2015).

VCZ is extensively metabolized in the liver, into its main circulating metabolite, voriconazole-N-oxide (VNO), which has minimal antifungal efficacy. Voriconazole-N-oxide also interferes in the metabolic activity of CYP3A4 and CYP2C19, thus interfering in the metabolism of VCZ, a substrate of the aforementioned enzymes (MARTINEZ, 2006; THEURETZBACHER;et al, 2006).

The solubility of VCZ in water is very low. As a result, the commercially available formulation of VCZ (VFEND®) for injection contains sulfobutyl ether-beta-cyclodextrin (SBECD) to improve drug solubility in water. Similar to other cyclodextrins, SBECD could be associated with hepatic and renal toxicity due to potential accumulation. As an alternative, the use of liposomes as carriers of VCZ might encompass additional advantages besides eliminating toxicity caused by SBEC. These advantages include targeting to infectious sites with higher local bioavailability, which may contribute to overcome microorganism resistance mechanisms (ROFFEY et al., 2003; LUKE et al., 2010; KISER et al., 2015).

The aim of this study was to develop, characterize and compare the pharmacokinetics (PK) and tissue distribution of a VCZ liposomal formulation versus the marketed formulation, VFEND® in mice.

METHODS

A liposomal formulation for intravenous application containing voriconazole was prepared by lipid-film hydration followed by extrusion. Formulations were lyophilized using sucrose as cryoprotectant and ressuspended immediately before use.

Particle size and polydispersity index (PdI) were evaluated by dynamic light scattering (DLS) and the morphology by Transmission Electron Microscopy (TEM) following negative staining. Voriconazole was quantified in all formulations by High Performance Liquid Chromatography (HPLC) with UV detection. Liposome Entrapment Efficiency (EE%) and zeta potential were also was determined.

In vivo pharmacokinetics (PK) and biodistribution of liposomal VCZ were compared with VFEND® over a 24 hour period in Balb/c mice following 10 mg/kg intravenous administration. Parent drug and metabolite were simultaneous quantifed at predetermined times using HPLC coupled with a mass spectrometer (MS/MS). For the biodistribution assay, organs were collected 4h after IV administration. Animal experiments were approved by the Ethics Committee for Animal Use (CEUA) of the Universidade Federal de Goiás - UFG, protocol number 108/14.

RESULTS

Liposomes exhibited a narrow size distribution and diameter of ~115±0.9 nm, a polydispersity index of 0.08±0.01 and -0.32 ± 0.6 mV of zeta potential. TEM analysis confirmed liposomes spherical morphology (Figure 1).

Quantitative data from formulations (by HPLC-UV) and from biological tissues (by HPLC-MS-MS) are presented in Figure 2.

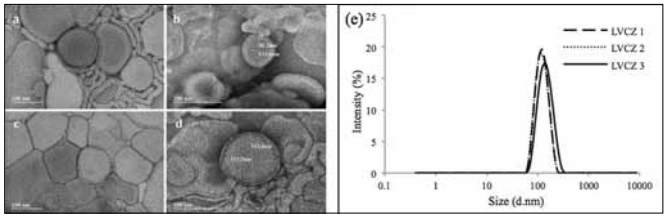

Fig. 1 - TEM micrograph of empty (blank) liposomes (a-b); VCZ liposomes (LVCZ) (c-d) and VCZ liposomes (LVCZ) size distribution profiles by intensity obtained by DLS from three independent batches (e). LVCZ: liposomes containing 2.0 mg/ml of VCZ in three different batches (1, 2 and 3).

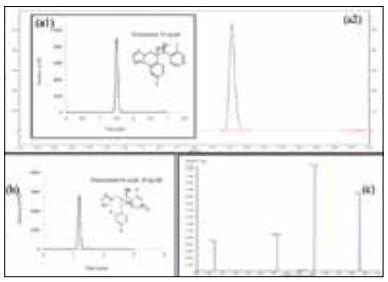

Fig. 2 - Representative chromatograms of VCZ in mice blood samples, using a mass spectrometer detector (a1); chromatogram from VCZ liposomal formulation at 2.0 mg/mL from a UV-VIS detector (a2); chromatograms of the metabolite (VNO) from mice blood samples (b) and ion scan spectrum of VCZ (c), both from a mass spectrometer detector.

Following the IV administration of 10 mg/kg of VCZ in Balb/c mice, either as liposomal voriconazole (LVCZ) or VFEND®, the main PK parameters were significantly different. Area under the curve (AUC) obtained for VCZ and VNO from both formulations are shown in Figure 3. The PK profile shows a maximum blood concentration (Cmax) of 1.23±0.28 and 0.61±0.15 µg/mL; area under curve (AUC0-24): 4.86±1.01 and 1.96±0.30 µg/mL.h and a clearance of 52.95±8.88 and 100.01±20.15 mL/h for LVCZ and VFEND®, respectively. Tissue distribution indicated a higher accumulation in the liver and kidneys.

Voriconazole-N-oxide (VNO) concentrations found in blood samples from mice receiving the liposomal formulation intravenously were 60% lower than in samples from mice receiving the same dose of VFEND® in the first 30 minutes. This indicates that liposomal entrapment protects the drug from metabolism in the initial stages of circulation and distribution. Decelerating the metabolism of VCZ thus slowing the formation of VNO, might be an additional mechanism of improving the existing therapy (PEER et al., 2007; LI et al., 2017).

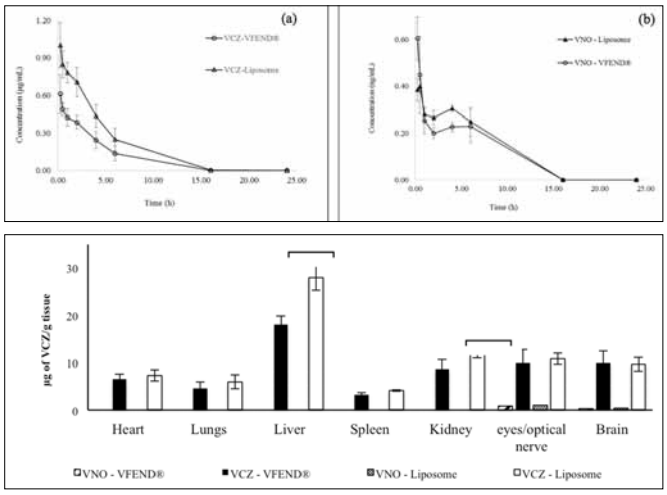

Fig. 3 - Mean blood concentration–time profiles of voriconazole (a); voriconazole-N-oxide (b), after IV administration of a single 10 mg/kg dose of voriconazole as VFEND® and voriconazole in liposomes (insert graph in (b) highlights the first hour of curve), (c) organ biodistribution of voriconazole and voriconazole-N-oxide (brain and eyes) 4h after IV administration. Bars represent the standard deviation (n = 6); concentration in µg/g of tissue. (indicates p<0.05)*

CONCLUSIONS

Encapsulation of voriconazole in liposomes can improve the pharmacokinetic profile of the drug, with AUC0-24 ~2.5 fold higher than VFEND® following IV administration in Balb/c mice. Higher amounts of voriconazole found in the liver and kidney after administration of the liposomal formulation indicate a potential therapeutic advantage since these organs are also important targets of microorganisms in systemic infections (KOBAYASHI, 1996; CALDERONE ;FONZI, 2001).

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STRESS AND IMMUNE RESPONSE OF PRIMARY HUMAN MYOBLAST TO TWO TYPES OF POLYMER COATED MAGNETIC NANOPARTICLES

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Nanoparticles (NPs), with their small size and related properties, have emerged as potent, multifunctional platforms that enabled new advances in biomedicine as well as development of different biotechnological applications. Among those, superparamagnetic NPs have been widely used in magnetic resonance imaging (MRI) and other imaging techniques as contrast agents. Numerous studies have thus focussed on the surface functionalization of NPs to improve their biocompatibility, *in vivo* distribution and targeting as well as their applicability for gene and drug delivery, imaging and tracking (Chomoucka et al., 2010; Jin et al., 2014; Ulbrich et al., 2016). However, despite thorough testing, some NP-based drugs are still being retracted from the trials and market due to toxicity problems (Fröhlich, 2012). NPs can induce cell stress through several different mechanisms, depending on their physicochemical properties such as size, shape, surface charge and surface chemistry, but their toxicity also depends on the properties of the cell type (Fröhlich et al., 2012).

Polyacrylic acid (PAA) is an anionic biocompatible polymer with carboxylic groups that allow further functionalization. PAA has already been used as a coating for magnetic NPs either alone or with additional modifications and has generally shown both good biocompatibility (Bregar et al., 2013; Couto et al., 2015; Lojk et al., 2015; Vasi et al., 2014). On the other hand, polyethylenimine (PEI) is a polycationic polymer most frequently used for transfection protocols. Similarly PEI coated NPs are used for magnetofection and drug delivery since PEI induces lysosomal leakage and thus PEI NPs are used for applications that require delivery into the cytosol, However, PEI toxicity still represents a major obstacle for its use since PEI NPs induce concentration dependent membrane damage, necrosis, lysosomal damage and release of lysosomal content, which can trigger apoptosis (Parhamifar et al., 2010).

The purpose of this study was to analyse the stress and immune response of PAA (63 nm diameter, -56 mV zeta potential in water) or PEI coated cobalt ferrite magnetic NPs (80 nm diameter in water, +50 mV zeta potential in water) (Bregar et al., 2013; Lojk et al., 2015a; Pavlin and Bregar, 2012). PAA coating of NPs enables high intracellular loading with relatively low toxicity, and can thus be applied for applications as cell labelling, separation and hyperthermia, while PEI coating is more appropriate for DNA and drug delivery to cytosol, since it can induce lysosomal leakage. We analysed the potential toxicity of these NP formulations through analysis of ROS induction, NF-κB activation and cell death. The experiments were performed primary human myoblasts (MYO) as an example of healthy, non-transformed cells.

To determine the toxicity of NPs, cells were incubated with increasing NP concentrations for 24 h and analysed with Propidium iodide (PI) viability assay. PAA NPs showed no negative effects after 24 h even at really high NP concentrations (Figure 1A), while PEI NPs induced a concentration dependent decrease in cell viability (IC50 at 5-6 µg/ml) and increase in PI positive (dead) cells (Figure 1B). Annexin V assay showed that PEI NPs do not induce apoptosis, but the observed decrease in viability resulted from necrotic cell death (Figure 1C), most probably due to PEI induced membrane damage (Parhamifar et al., 2010). Similarly, PAA NPs did not induce ROS formation, while PEI NPs, induced oxidative stress at 8 µg/ml NP concentration and higher. To confirm ROS were not induced at shorter time intervals following NP exposure, an incubation timeline was performed (from 0.5 to 24 h exposure), which confirmed the observed ROS levels were the highest after 24 h with no ROS induction at shorter time intervals.

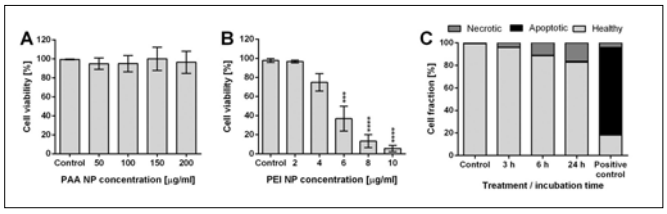

Figure 1: The effect of increasing nanoparticle concentration on cell viability for (A) polyacrylic acid (PAA) coated and (B) polyethylenimine (PEI) coated magnetic nanoparticles. Primary human myoblasts (MYO) were incubated with nanoparticles for 24 h and cell viability was determined using PI viability assay. Mean and standard error are shown for three independent experiments. (C) Percentage of apoptotic/necrotic cells was determined using Annexin/PI differential staining for different incubation times with 4 µg/ml PEI NPs in MYO cells. Staurosporine (1 µM, 6h) was used as a positive control.

The ability of NPs to trigger the activation of NF-κB, the main transcription factor of stress and immune responses, was assessed with Western blotting of phosphorylated NF-κB (pNF-κB). PAA NPs did not activate NF-κB at any tested time point in MYO cells (Figure 2A). NF-κB was activated (phosphorylated) only after incubation with 4 µg/ml PEI NPs. The increase in NF-κB phosphorylation was observed 15 min (2.4 fold increase) and 30 min (3.9 fold increase) after incubation, after which the activity dropped below the basal activity for up to 24 h (Figure 2B).

In most studies reporting NP-induced NF-κB activation, ROS has been implicated as the main mechanism of activation (Liu and Sun, 2010; Nishanth et al., 2011; Shi et al., 2014). However, we observed only a small time dependent increase in ROS (1.3 fold of control) at 4 µg/ml PEI NPs in MYO cells, which was the highest 24 h after incubation and no ROS increase 30 min after PEI NP incubation. So although NF-κB activation might represent a step in the activation of cellular antioxidant and anti-apoptotic mechanisms (Morgan and Liu, 2011), the time dynamics of both processes do not coincide.

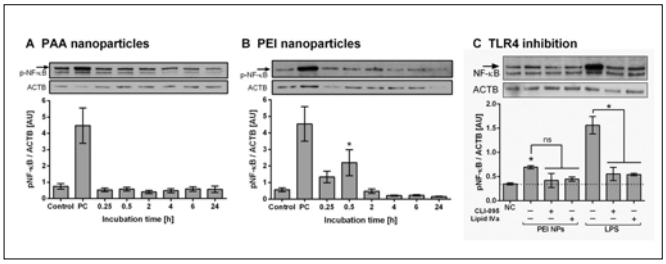

Figure 2: Phosphorylation of NF-κB (pNF-κB), normalized to actin (ACTB), in primary human myoblasts for different incubation times with 100 µg/ml polyacrylic acid (PAA) coated (A) and 4 µg/ml polyethylenimine (PEI) coated magnetic nanoparticles (B). 2 h incubation with 100 ng/ml LPS was used as a positive control (PC). Mean and standard error of relative arbitrary units as determined with densitometry are shown for four independent experiments. (C) Inhibition of NF-κB (pNF-κB) phosphorylation in MYO cells induced by 30 min incubation with 4 µg/ml polyethylenimine (PEI) coated NPs or 2 h incubation with 100 ng/ml LPS with CLI-095 and Lipid IVa inhibitors of Toll-like receptor 4 (TLR4) activation. Mean and standard error of relative arbitrary units as determined with densitometry are shown for two independent experiments.

The quick NF-κB response and the following drop in NF-κB activity shows the typical pattern of receptor mediated NF-κB activation. This suggests the activation is triggered either by membrane binding and damage or through receptor activation, and not by any mechanisms/damage occurring later during NP internalization. Such membrane receptors could be Toll-like receptors (TLR) or other receptors of the immune system that signal through NF-κB. For example, PEI coated NPs and PEI polymer per se have been shown to activate both TLR5 (Cubillos-Ruiz et al., 2009; Hu et al., 2013) and TLR4 receptors (Huang et al., 2013; Mulens-Arias et al., 2015). To confirm this, we inhibited TLR4 receptor signalling in MYO cells with CLI-095, an inhibitor of TLR4 intracellular domain (Ii et al., 2006), and with Lipid IVa, an LPS precursor, which binds to the TLR4/MD2 without inducing signalling transduction and in this way prevents binding of LPS (Saitoh et al., 2004). Both inhibitors reduced PEI NP-induced NF-κB activation (Figure 2C), which indicates that PEI might act through interaction with MD2 co-receptor, similarly to LPS, and not through the TLR4 receptor dimerization independent of the ligand presence. Taken together, in this study we compared the cell stress response to two types of polymer coated magnetic NPs in primary human myoblasts. Negatively charged PAA NPs induced no short-term cytotoxicity and cell stress, while positively charged PEI NPs were shown to induce concentration dependent necrotic cell death, ROS induction and activation of transcription factor NF-κB through activation of TLR4 receptor in primary human myoblast cells. Both PAA and PEI coatings induce specific interactions with the cells, so knowing potential stress and immune response related to each potential polymer can help design better and more biocompatible NPs based on the requirements of the application.

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THERANOSTICS IN CANCER WITH MULTIFUNCTIONAL NANOCLUSTERS

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Aim of the Section of Experimental Oncology and Nanomedicine (SEON) is to utilize SPIONs for the treatment of cancer and arteriosclerosis by MDT [1, 2]. Therefore, over a period of several years we developed SPIONs optimized for the purpose of magnetic drug delivery. These particles show a very good biocompatibility, are very stable in human and animal blood and can carry a sufficient drug load [3]. Due to their magnetic properties, these particles can be accumulated in a target area by magnetic fields and first results show they can be heated with alternating magnetic fields. These SPIONs were also suitable for MRI-imaging but from the theoretical point of view not optimal for Magnetic Particle Imaging (MPI), because they are clusters of a size between 60 nm and 70 nm with a single core diameter of approximately 7,6 nm. The aim of this study was to show, that multifunctional nanoclusters, which are suitable for magnetic drug targeting, hyperthermia, MR-imaging can also show a signal in MPI.

SEON^{LA-BSA} - IN VITRO EFFICIENCY

The nanoparticle system SEON^{LA-BSA} has been developed as a platform for delivery of drugs. At the beginning, the chemotherapeutic agent mitoxantrone (MTO) was chosen for cancer treatment.

In various experiments, SEONLA-BSA*MTO showed an effectiveness comparable to the free drug after 24 h and 48 h in killing cancer cell lines growing adherent in monolayers or in suspension [3].

In further *in vitro* tests the capability of these nanoparticles to infiltrate tumor tissue was investigated on three dimensional multicellular tumor spheroids [4].

Spheroids of the tumor cell line HT-29 were treated 72 h after the initial spheroid formation with free MTO and SEONLA-BSA*MTO in equivalent MTO doses. Fluorescence microcopy showed that both the free and the nanoparticle-loaded drug infiltrated efficiently into the tumor spheroids. Subsequently, the treated spheroids showed a highly reduced proliferation rate and higher rates of apoptosis and necrosis [4].

SEON^{LA-BSA} - BIOCOMPATIBILITY

Nanoparticles, which are intended to be used for the treatment of patients, have to be tested for their biocompatibility. Since, common toxicity assays, which are based on colorimetry, fluorescence or luminescence, show interference of the black iron oxide nanoparticles [5], we used different methods to assess possible toxic effects *in vitro*. For example, SEON^{LA-BSA} nanoparticles did not show induction of apoptosis or necrosis in Jurkat cells at doses of up to 100 µg/ml after 24 hours or 48 hours measured in flow cytometry [3].

Another important issue for nanoparticles that are dedicated for medical use is their behavior in blood.

Here, SEON^{LA-BSA} nanoparticles revealed a tremendously improved colloidal stability in blood compared to the precursor particle system SEONLA, which only coated with lauric acid for stabilization[3].

SEON^{LA-BSA} - Heating properties

One option for using SPIONs for the treatment of tumors is magnetic hyperthermia [6]. Therefore, we were interested, if combination of chemotherapeutic treatment with hyperthermia can enhance the outcome of the treatment. Hence, we investigated the heating properties of the SEON^{LA-BSA} system with alternating magnetic fields. It could be shown that these nanoparticles can be magnetically heated and that this is depended on the iron content, as could be expected [7].

SEON^{LA-BSA} - MRI-IMAGING OF IN VIVO

In an *in vivo* model with a VX2-tumor implanted subcutaneously at the hind limb of New Zealand White rabbits (NZW) we investigated the MRI-properties of SEONLA-BSA. It is known that in MRI imaging iron oxide nanoparticles are able to cause signal extinctions in the area, where they are accumulated.

The nanoparticles did not show signal extinction in T1 weighted sequences, while in a T2-weighted sequence, signal extinction was detected in several areas of the VX2-tumors (Figure 1). This shows that a theranostic approach is possible with MRI, giving the chance of estimating the particle load in the tumor region after the treatment. By this, it could also be possible to estimate the drug load, which was deposited in this area.

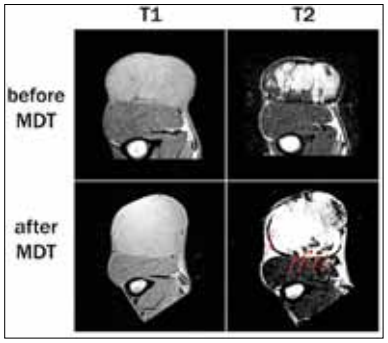

Figure 1: MRI-imaging of a VX2-tumor before and after MDT. The T1-sequence does not show any signal extinction after the administration of SEON^{LA-BSA}, while in T2 signal extinction is caused by nanoparticles as seen at the basis of the tumor (red arrows).

SEON^{LA-BSA} - MPI-IMAGING

Magnetic particle imaging (MPI) is a new imaging technique utilizing iron oxide nanoparticles imaging agents. The big advantage of MPI is that it not only generates images but it can also deliver quantitative data about the tracer content in a given volume. This could be tremendously helpful for MDT and the theranostic approach of an online monitoring of the nanoparticle content in the tumor region. Therefore, we were interested, if it is possible to image the SEON^{LA-BSA}-nanoparticles with a currently available preclinical MPI-device (Bruker/Philips) at Universitätsklinikum Hamburg-Eppendorf.

First, we measured a dilution series of these particles Magnetic Particle Spectroscopy (MPS). In comparison to the MPS-signal of Resovist® the signal of the SEON^{LA-BSA}-nanoparticles was weaker at higher frequencies but comparable at frequencies below 100 kHz (Figure 2A). Next, a sample of 20 µl (2 mm * 2 mm * 1 mm) was measured with MPI. Figure 2B to D show that it was possible to get an image of this point sample by MPI.

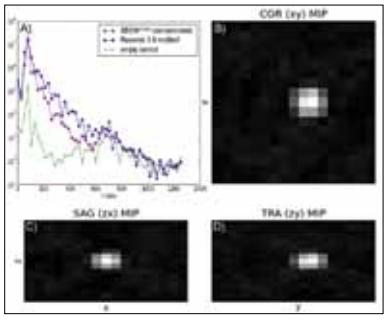

Figure 2: A) Comparison of the MPS signal of SEON^{LA-BSA}-nanoparticles and Resovist® at different frequencies. B) – D) MPI-signal of a point sample of SEON^{LA-BSA}-nanoparticles.

CONCLUSION

During the last years SEON developed a multifunctional nanoparticle platform SEON^{LA-BSA}, which is capable of carrying a variety of drugs. We showed that this nanosystem is very biocompatible *in vitro* and has excellent blood compatibility. *In vitro*, mitoxantrone-loaded SEON^{LA-BSA} are very effective in treating cancer cells in 2d-, suspension- and 3d-cell-culture, which was similar to the free, unbound drug. Furthermore, it is possible to use alternating magnetic fields for heating this particle system in a concentration depended manner up to ca. 65°C. Additionally and as could be expected, the SEON^{LA-BSA} particles are causing a signal extinction in MRI and is showing a signal in the new imaging modality MPI. Taken this together, the nanoparticle system SEON^{LA-BSA} could

be a promising candidate for an effective theranostic approach of cancer and arteriosclerotic diseases.
This preliminary study shows that in principal it is possible to image SEON$^{LA\text{-}BSA}$ using MPI. Further experiments will demonstrate how effective and quantitative this imaging is and if modifications on the particle platform or the technical equipment will be able to improve the imaging properties of this system. If this can be realized without impairing therapeutic efficiency, the combination of MDT and MPI could open new doors in the field of theranostics.

Acknowledgements: This study was supported by the German Research Foundation (DFG) (AL552/8-1), the EU project "NanoAthero", FP7-NMP-2012-LARGE-6-309820 the Cluster of Excellence Engineering of Advanced Materials (EAM), and the Manfred Roth Stiftung, Fürth, Germany.

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DUAL DRUG CONJUGATED NANOPARTICLE FOR SIMULTANEOUS TARGETING OF MITOCHONDRIA AND NUCLEUS IN CANCER CELLS

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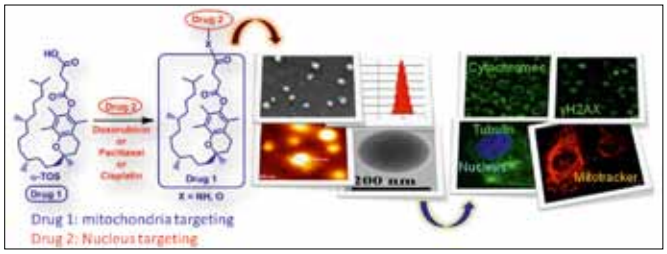
Effective targeting of mitochondria has emerged as an alternative strategy in cancer chemotherapy. However, considering mitochondria's crucial role in cellular energetics, metabolism and signaling, targeting mitochondria with small molecules would lead to severe side effects in cancer patients. Moreover, mitochondrial functions are highly dependent on other cellular organelles like nucleus. Hence, simultaneous targeting of mitochondria and nucleus could lead to more effective anticancer strategy. To achieve this goal, we have developed sub 200 nm particles from dual drug conjugates derived from direct tethering of mitochondria damaging drug (α-tocopheryl succinate) and nucleus damaging drugs(cisplatin, doxorubicin and paclitaxel). These dual drug conjugated nanoparticles were internalized into the acidic lysosomal compartments of the HeLa cervical cancer cells through endocytosis and induced apoptosis through cell cycle arrest. These nanoparticles damaged mitochondrial morphology and triggered the release of cytochrome c. Furthermore, these nanoparticles target nucleus to induce DNA damage, fragment the nuclear morphology and damage the cytoskeletal protein tubulin. Therefore, these dual drug conjugated nanoparticles can be successfully used as a platform technology for simultaneous targeting of multiple subcellular organelles in cancer cells to improve the therapeutic efficacy of the free drugs.



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A NOVEL MODEL OF HUMAN ARTERY TO INVESTIGATE MAGNETIC TARGETING IN CARDIOVASCULAR DISEASES

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BACKGROUND:

Magnetic targeting utilizes the properties of superparamagnetic iron oxide nanoparticles (SPIONs) to accumulate particle-bound drugs or particle-loaded cells in specified vasculature regions under an external magnetic field. Using this approach, SPIONs can be easily targeted to specified microvasculature regions, but little is known about the possibility of magnetic targeting in medium and large arteries. The behavior of SPIONs in arterial circulation may vary greatly depending on the nanoparticle characteristics, magnetic field strength and flow dynamics. In this respect, ex vivo models can provide critical information to predict their capture efficacy *in vivo*.

METHODS:

Our motivation was to develop an easy to handle flow-through model based on human umbilical artery. For this purpose, arteries were isolated from umbilical cords and 13 cm long fragments were embedded in agarose to mimic *in vivo*-like mechanical support (Fig. 1). To evaluate the model, the arteries were perfused with medium containing one of three different types of SPIONs with differing physicochemical characteristics. The experimental set-ups were modified to assess the influence of external magnetic field parameters, SPION circulation time and flow conditions. To evaluate the magnetic capture efficacy, arteries were subsequently cut into 11 segments and iron content was investigated by atomic emission spectroscopy and histology (Prussian blue staining).

RESULTS:

SPION-1 with lauric acid shell had the largest capacity to accumulate at the specific artery segment (Fig. 2). SPION-2 (lauric acid/albumin-coated) were also successfully targeted, although the observed

peak in the iron content under the tip of the magnet was smaller than for SPION-1. In contrast, we did not achieve magnetic accumulation of dextran-coated SPION-3. Effects of magnetic field parameters, as well as flow time and rate on capture efficacy were analyzed using SPION-1. Reduction of the magnetic field gradient from 40T/m to 30 T/m resulted in the iron peak decrease, in parallel with slightly increased accumulation in the other segments. Increasing the distance of the magnet tip to the artery led to reduction of the iron content by about 75%. Doubling of the circulation time had no major effect on the accumulation of SPION-1, but the reduction of the flow rate by half unexpectedly led to about 50% decrease in SPION-1 accumulation.

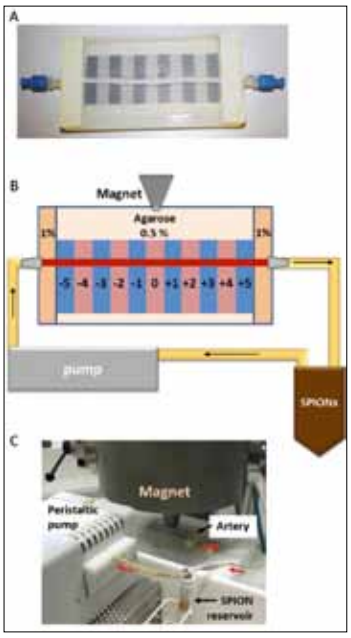

Figure 1. Umbilical artery model.
(A) Umbilical artery embedded in agarose gel; (B) Schematic presentation of the experimental set-up for magnetic targeting; (C) Example image showing the electromagnet, the artery, SPION reservoir, and peristaltic pump. Red arrows indicate the flow direction.

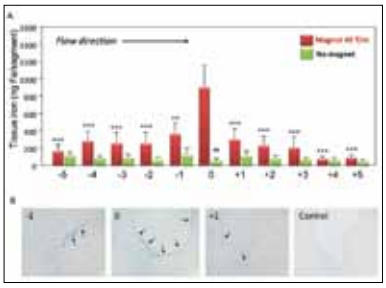

Figure 2. Magnetic targeting of circulating SPION-1.
*(A) SPION-1 suspension at 30 µg Fe/mL was circulated for 30 min under external magnetic field gradient positioned at segment "0" (red bars; the magnetic field gradient at the centre of artery: 40 T/m) or without magnetic force (green bars). Shown is iron concentration of respective segments (mean values ± SEM of n=5 experiments). ***P<0.001; **P<0.01 vs region "0" under the tip of the magnet; ##P<0.01 vs corresponding targeted region. (B) Representative images from n=3 experiments corresponding to the artery segments -1, 0 and +1. Iron accumulation, visualised with Prussian blue staining, is highlighted with arrows.*

CONCLUSIONS:

Taken together, the umbilical artery model constitutes a time- and cost-efficient, 3R-compliant tool to predict the efficacy of magnetic targeting under flow conditions *in vivo*. Our results further imply the possibility of an efficient *in vivo* targeting of certain types of SPIONs to superficial arteries. Diverse studies can be envisioned using this model, including the modifications of arterial wall geometry to mimic the presence of stenosis, or the implantation of stents to investigate the magnetic capture of SPION-loaded cells or SPION-based drug delivery systems.

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IMMUNOTOXICITY STUDIES OF LIPOSOMAL NANO-CONTAINERS FOR TARGETED DRUG DELIVERY

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Nanomedicines have been widely studied in preclinical and clinical trials as drug delivery systems. Local and controlled release of encapsulated medicament results in increase of the therapeutic effect of the drug, and decrease of its side effects. Recently discovered Pad-PC-Pad (1,3-palmitoylamido-1,3-deoxy-sn-glycero-2-phosphatidylcholine) [1] liposomes are promising candidates for targeted drug delivery against atherosclerosis. They possess a potential to deliver vasodilators preferentially to the constricted parts of atherosclerotic arteries. Thus, certain side effects from intravenous administration of vasodilators, such as the global fall in blood pressure and increased heart rate, can be well diminished. A common feature of nanomedicines is that they can stimulate the immune system, causing hypersensitivity reaction (HSR) and can occur in relatively significant number of patients. Such immunoreaction is caused by activation of the complement system, which leads to the formation of membrane attack complex (MAC) that disrupts the membrane of the pathogen. Therefore, to progress our nano-therapeutic system towards clinical trials, immunotoxicity studies of the artificial Pad-PC-Pad liposomes were conducted. We performed enzyme immunoassay versus SC5b-9 protein complex to detect the formation of MAC in the blood serum incubated with Pad-PC-Pad. Our previous results demonstrate that pure Pad-PC-Pad liposomes reveal absence of HSRs *in vitro* and *in vivo* [2]. Recently, we studied complement activation of Pad-PC-Pad liposomes, loaded with nitroglycerin, which acts as a vasodilator. Current results indicate that complement activation of Pad-PC-Pad liposomes within the human therapeutic dose is less than for FDA-approved drugs [3].

Keywords: nanomedicine; immunoassay; complement activation; Pad-PC-Pad; mechanosensitive liposomes; atherosclerosis; targeted drug delivery.

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ANAPHYLACTIC REACTIVITY AND IMMUNOGENICITY OF PEGYLATED LIPOSOMES IN PIGS PART II – INHIBITION VIA IMMUNE SUPRESSION BY DOXIL®

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Intravenous injection of pigs with PEGylated liposomes mimicking doxorubicin-free Doxil®/Caelyx (Doxebo) was previously shown to prevent complement activation-related pseudoallergy (CARPA) to Doxil® in a porcine model of human hypersensitivity reactions to nano-drugs. In the course of Doxebo's anaphylaxis-prophylactic (tachyphylactic) effect, levels of anti-PEG and anti-liposome (anti-LIP) antibodies showed major rises and anaphylactic shock was observed (see Part I – Inhibition via antibody scavenging). Astonishingly, both antibody production and the anaphylactic reactions were abolished by co-administration with Doxebo of the human equivalent dose of Doxil®, suggesting that an immune suppressive effect of Doxil® prevented the immunogenicity of liposomes.
Fig. 1 shows the changes of pulmonary and systemic arterial pressure (PAP, SAP) in pigs caused by sequential injections of 0.1 and 1 mg/kg PEGylated liposomes (LIP) and 0.1 mg/kg Zymosan 1 week after pretreatment of pigs with PBS ("nothing"), 0.1 mg/kg Doxebo ("Doxebo only") or Doxebo + the human equivalent dose (HED) of Doxil® ("Doxebo + 6.4 mg/kg Doxil"). The changes were expressed as area under the blood pressure curves normalized as % change relative to baseline. It is seen that the huge LIP-induced rises of PAP and SAP in Doxebo pretreated pigs was near entirely suppressed in Doxebo + Doxil® HED-treated animals, indicating that Doxil® provided significant suppression of Doxebo-induced immunogenicity. These data explain why Doxil® is not immunogenic in cancer patients, and suggest that Doxil® can be used for the prevention of CARPA.

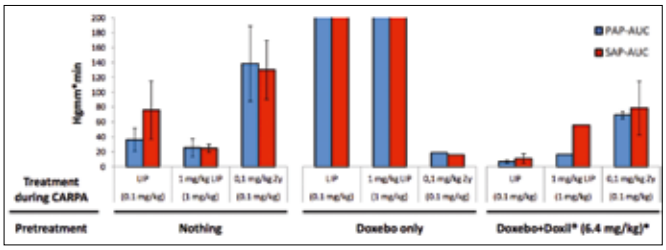

Figure 1. Blood pressure changes in pigs caused by PEGylated liposomes and zymosan 1 week after pretreatment of animals with 0.1 mg/kg Doxebo with or without 6.4 mg/kg Doxil pigs. „Nothing" means no pretreatment. AUC, area under the PAP or SAP curve.

COMPLEMENT ACTIVATION-RELATED HYPERSENSITIVITY REACTIONS TO AN AMPHOTERICIN B-CONTAINING LIPID COMPLEX (ABELCET) IN PEDIATRIC PATIENTS

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Amphotericin B is an antifungal medicine used in the treatment of Aspergillosis. This infection can pre-sent itself in various manifestations and invasive Aspergillosis can be observed in immunosuppressed patients. Abelcet is a lipid-complex containing Amphotericin B, and, along with AmBisome (the liposo-mal form of the same active agent), it is widely used in the treatment of severe invasive Aspergillosis, usually as a second tier treatment.
AmBisome is known to trigger complement activation-related pseudo-allergy (CARPA), a more or less severe hypersensitivity reaction, and our present data suggests that Abelcet does the same. We show that Abelcet causes massive complement activation in human serum *in vitro*, comparable to the action of the complement activator gold standard, zymosan. Furthermore, we analyzed the adverse effect reports of children undergoing infusion therapy with Abelcet at the Second Department of Pediatrics of Semmelweis University, Budapest, and found typical symptoms of hypersensitivity reactions corre-sponding to CARPA (tachycardia, rapid fever, shivering, fatigue, etc.) arising mostly at the beginning of the infusion. This type of pseudoallergy is usually attenuated by steroid and NSAID pre-treatment or by extending the infusion time (up to 6 hours), nevertheless CARPA remains to be a major safety issue with Abelcet. Interestingly, Abelcet causes significant hypertension in mice, along with blood cell changes typical of CARPA, yet without signs of complement activation (see poster by Örfi et al.). taken together, Abelcet-induced CARPA, or "pseudo-CARPA" represents a novel example for the adverse immune activi-ty of a nanomedicine. The significance of this recognition lies in the possibility to apply all theoretical and practical knowledge about CARPA to improve the safety of Abelcet therapy.

MICROFLUIDIC CHIPS FOR THE VASCULAR AND EXTRAVASCULAR TRANSPORT ANALYSIS OF MOLECULES, NANOPARTICLES AND CELLS

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Vascular and extravascular mass transport regulates the distribution of nutrients, the collection of waste products, the genesis and growth of cell aggregates and tissues, the migration of malignant cells to distant sites, and the cytotoxic potential and imaging performance of therapeutic and imaging agents. Soft polymers, such as poly-dimethyl-siloxane (PDMS), together with replica molding, imprinting and other micro/nano-scale fabrication techniques allow for the realization of complex microfluidic systems for the *in vitro* analysis of mass transport processes.[1] These are of crucial importance in the development of more precise nanomedicines, which are expected to deliver large payloads of therapeutic and imaging agents with unprecedented specificity to a variety of biological targets.[2]
In our group, microfluidic chips with different configurations have been developed for elucidating: i) the biophysical mechanisms that regulate the vascular transport of systemically injected nanopar-

ticles; ii) the distant migration of malignant cells; iii) the extravascular diffusion of nanoparticles and molecular agents. To this end, the following chips have been designed, fabricated and employed: i) a double-channel chip and authentically complex, naturally inspired microchannel systems for vascular and extravascular mass transport studies; ii) a single-channel chip for investigating the adhesion of circulating cells; iii) an intra-tissue diffusion chamber for extravascular transport analyses.

MATERIALS AND METHODS:

Single channel chips are produced via conventional SU-8 replica molding whereas double-channel chips are fabricated following a two-step lithography strategy (Figure 1).[3] Intra-tissue diffusion chambers are realized by curing the natural polymer of interest (collagen, hyaluronic acid, and combination thereof) around a needle and removing the latter upon complete gelification. The authentically complex vascular networks are obtained by replicating leaves with PDMS (Figure 2).

Single channel chips are used for studying the rolling and adhesive interaction of circulating cancer cells (CTCs) with endothelial cells, under physiological and pathological conditions. A confluent layer of endothelial cells (HUVECs) is realized by seeding cells at a density of 2×10^6 cells/mL and incubating the whole system for 48 h at 37°C. This same approach is also used for establishing a confluent HUVEC layer in the other microfluidic chip configurations. At the occurrence, the confluent HUVEC monolayer can be inflamed (pathological conditions) upon exposure to a 25 ng/mL solution of TNF-α, for 6 or 12 hours. For cell transport studies, colorectal cancer (HCT-15) and breast cancer (MBA-MD-231) cells are injected in the chamber, at a density of 10^6 cells/mL, via a syringe pump (100 nL/min).

Double-channel chips and intra-tissue diffusion chambers are used for characterizing the vascular transport, extravasation, and extravascular diffusion of molecules, nanoparticles and cells. For these experiments, Dextran molecules of different molecular weights (4, 40, and 250 kDa) and nanoparticles with a characteristic diameter of 200 nm are used. The extravascular diffusion coefficients are calculated via Fluorescence Recovery After Photobleaching (FRAP) and Mean Square Displacement (MSD) analysis, depending on the size of the agent.

Double-channel chips are also employed for investigating the efficiency of magnetic guidance in facilitating the extravasation of nanoparticles. In these experiments, spherical polymeric nanoparticles (~ 160 nm in diameter), loaded with iron oxide nanocubes (NCs), are employed. A square magnet (1 cm side × 0.4 cm height, grade N52) is placed in proximity of the channel and used to guide the polymeric nanoparticles towards an extravascular compartment filled with collagen.

RESULTS AND DISCUSSION

Channel microfabrication: Figure 1 presents on the photolithographic, etching, and replica molding steps needed for realizing single and double-channel chips. Note that in the specific configuration, two parallel channels are separated by a membrane of micropillars with high aspect ratio constituting the vascular membrane. This micro-pillars membrane separates the vascular and extravascular compartments. The authentically complex vascular network is realized by replicating a leaf as shown in Figure 2.

Cancer cell adhesion to the vascular endothelium: stimulation of HUVECs with TNF-α (pathological conditions) upregulates the expression of adhesion molecules thus increasing the vascular deposition of circulating tumor cells.[4] As in Figure 3, the average number of adhering tumor cells increases of about 80% and 200%, with respect to the control case, upon exposure of HUVECs to TNF-α for 6 and 12 h, respectively.

Intra-tissue permeability of molecules and nanoparticles: Figure 4 shows the tissue permeation of Dextran molecules in two different chip configurations, namely the intra-tissue diffusion chamber (top) and the double-channel chip (bottom). In both configurations, Dextran molecules are diffusing through a polymerized collagen type I slab, however the process is quasi-static for the intra-tissue diffusion chamber and dynamic for the double-chip configuration. The intra-tissue chip returns the actual diffusion coefficient in terms of size, so that $D_{dex4} = 7.42 \times 10\text{-}7 \pm 2.83 \times 10^{-8}$ cm^2 s-1, $D_{dex40} = 4 \times 10^{-7} \pm 3.36 \times 10^{-7}$ cm2 s^{-1}, $D_{dex250} = 2.92 \times 10^{-7} \pm 1.28 \times 10\text{-}7$ cm^2 s^{-1}. Differently, the double-channel chip provides the permeability of the molecule across the vascular membrane with and without HUVECs. As expected, the presence of endothelial cells on the walls of the vascular compartment dramatically hinders trans-vascular permeability.

Magnetically guided extravasation: Figure 5 shows the enhanced permeation of nanoparticles across the vascular barrier of a double-channel chip in the presence of an external magnetic field. 160 nm particles can penetrate up to 150 µm, which is about 3 times larger than the average collagen penetration without magnet.

CONCLUSIONS:

Microfluidic chips can be efficiently used for characterizing mass transport across multiple scales, from molecules to nanoparticles and cells, hence elucidating the mechanisms supporting the vascular extravasation of systemically injected nanoparticles and circulating cancer cells, as well as the deep tissue permeation of therapeutic molecules. Our preliminary results confirm that vascular inflammation favors the firm adhesion of circulating tumor cells and tissue permeation of nanoparticles is minimal and can be efficiently boosted via active, magnetic guidance.

Figure 1. Double-channel microfluidic chips. Fabrication steps and SEM images (left). Confocal images of a confluent HUVEC monolayer cultured in the vascular compartment, where nuclei are stained in blue and VE-cadherin receptors are stained in green (right).

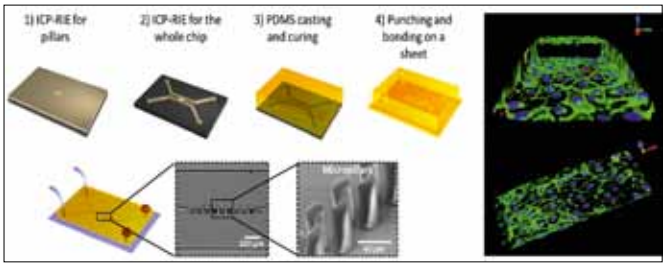


Figure 2. Authentically complex vascular networks. The leaf, embedding of the leaf in PDMS; replica; external tubing connection and perfusion of the complex vascular network (from the left to right).

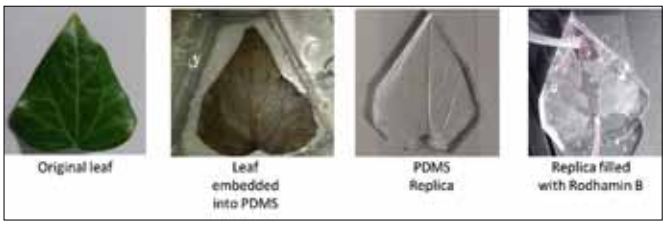


*Figure 3. Cancer cell adhesion to the vascular endothelium (metastatic process). Representative frames for the adhesion experiments in a single channel chip (left). Normalized number of adhering cancer cells (right). * = p<0.05, ** = p<0.01, *** = p<0.001*

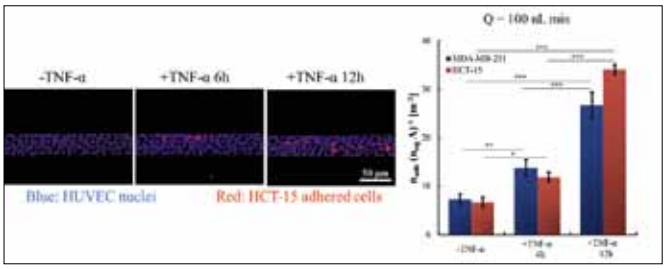


*Figure 4. Diffusion and permeation studies in collagen. Representative fluorescent images of free Dextran (4 kDa) diffusing in a intra-tissue chip (top-left) and estimation of the diffusion coefficient for different agents (top, right). Representative fluorescent images of Dextran (40 kDa) permeating across a vascular membrane (bottom, left) and estimation of the permeability coefficient (bottom, right). *** = p<0.001*

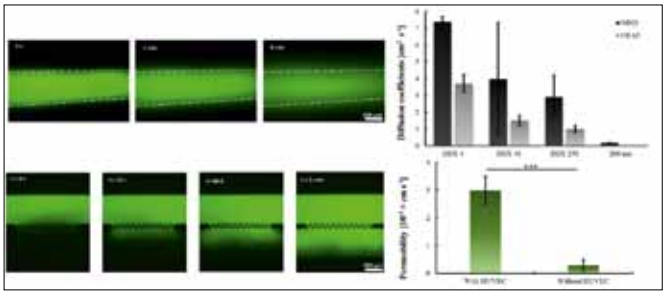
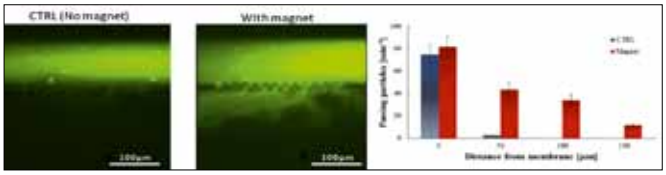


Figure 5. Magnetic permeation and accumulation of iron oxide based nano-particles. Representative frames of green-fluorescent spherical nanoparticles (~160 nm) progressively crossing the micropillars membrane; top channel is free, bottom one is filled with collagen type I (left). Quantification of the penetration depth (right).



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NANOSTRUCTURED ARCHAEOLIPID CARRIERS WITH ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITY FOR ORAL TREATMENT OF INFLAMMATORY BOWEL DISEASES

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INTRODUCTION

Inflammatory bowel diseases (IBD) such as Crohn's disease and ulcerative colitis are chronic relapsing disorders of the gastrointestinal tract, charac¬terized by chronic inflammation and epithelial injury induced by the uncontrolled activation of the mucosal immune system. Dendritic cells and macrophages are key cells in the inflamed mucosa, which produce large amounts of pro-inflammatory cytokines. The imbalance between pro-inflammatory and anti-inflammatory cytokines impedes the resolution of inflammation, leading to disease perpetuation and tissue destruction. On the other hand, oxidative stress is considered as one of the etiologic factors involved in several signals and symptoms of IBD that include diarrhea, toxic megacolon and abdominal pain. Once the uncontrolled activation of the immune system occurs, oxidative stress is a major contributing factor to tissue injury and fibrosis.

The treatment of IBD is symptomatic, and depending on the stage of the disease, ranges from oral aminosalicylates, anti-inflammatory and immunosuppressant drugs, to endovenous biological agents such as the anti-tumor necrosis factor (TNF)-α antibody infliximab. These treatments have limited benefits, because of their systemic adverse effects displayed during their long-term use.

More efficacious and safer therapies could rely on developing macrophages-targeted drug delivery systems capable of specifically delivering high doses of anti-inflammatory drugs and antioxidants with minimal exposure of healthy or distant tissues via oral administration.

Here we report the development of nanostructured archaeolipid carriers (NAC) for oral targeted delivery of natural antioxidants and the anti-inflammatory dexamethasone (Dex) to macrophages. NAC have a core of neutral and a shell of polar archaeolipids extracted from the halophilic archaebacteria Halorubrum tebenquichense. Polar archaeolipids are a mixture of saturated isoprenoid chains linked via ether bonds to the glycerol carbons at the sn 2,3 position. In contrast to conventional phospholipids, polar archaeolipids are hydrolytic, oxidative and enzymatic attack resistant. Besides, polar archaeolpids are ligands for the macrophages scavenger receptors class A. We have recently reported that ultra-small solid archaeolipid nanoparticles combine high resistance to gastrointestinal conditions with extensive uptake by macrophages (Higa et al., 2017). Neutral archaeolipids, on the other hand, are a mixture of carotenoids with C50 that have higher antioxidant activity than those extracted from algae, plants, yeast and cyanobacteria.

NAC made of a core of a mixture of solid (compritol) and liquid (neutral archaeal) lipids stabilized by a shell of polar archaeolipids and Tween 80 (2-2-1.2-3 % w/w) loaded with dexamethasone (NAC-Dex) were prepared by homogenization-ultrasonication.

NAC-Dex were characterized in terms of particle size, zeta potential, morphology, crystallinity and colloidal stability upon storage. The toxicity of NAC on macrophages (J774 cells) and human epithelial colorectal adenocarcinoma (Caco-2) cells was determined by MTT assay.

The *in vitro* scavenging capacity of NAC against 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azinobis (3-ethylbenzothiazoline-6 sulfonicacid) (ABTS) radicals was measured.

Stability of NAC incubated in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF), was determined in terms of size retention and lipolytic percentage, respectively.

Finally, the anti-inflammatory and antioxidant activity of NAC were measured in a model of inflamed mucosa using co-cultured Caco-2 and differentiated into macrophages human monocytes (THP-1 cells) stimulated with lipopolysaccharide (LPS). The anti-inflammatory activity was determined by measuring the release of pro-inflammatory cytokines. The capacity of NAC to reduce the generation of reactive oxygen species (ROS) by macrophages was measured using the carboxy-H_2DCFDA dye.

RESULTS

NAC resulted small, homogeneous, negatively charge and stable: NAC showed a mean particle size of 85.1 ± 23.3 nm with polydispersity index of 0.3 and zeta potential of -32.4 ± 5.8 mV. Dex incorporation into NAC resulted in a 7-fold increase in drug solubility with an encapsulation efficiency of 50 %. Transmission electron microscopy images showed spherical particles in the nanometer range while cryo-transmission electron microscopy images showed circular, ellipsoidal or elongated edged structures of high contrast. There was no significant change in mean particle size, PDI, zeta potential and drug content upon one-month storage at 4°C. The differential scanning calorimetry thermograms of NAC showed a decrease of melting point of compritol from 74.2°C to 56.7°C and lower crystallinity index compared to the bulk lipid, indicating the liquid lipid was dispersed within the compritol matrix.

NAC showed antioxidant activity *in vitro*: The DPPH radical scavenging test of NAC showed a Trolox equivalent antioxidant capacity of 35 ± 7 mM Trolox/g lipids, and the concentration of NAC that reduced 50 % of the radicals (IC50) was 0.70 mg/ml. Besides, at that concentration, NAC produced 60% of inhibition of the ABTS radical.

NAC were resistant to *in vitro* digestion: No modification in NAC mean size was revealed upon 4 h of incubation in simulated gastric fluid with respect to initial time. Lipolysis of NAC was relatively low, up to 60 min the accumulative lipolytic percentage was 45%.

NAC were not cytotoxic: Viability studies revealed no cytotoxicity of NAC in the range of 40- 200 µg/ml of compritol upon 24 hours of incubation with Caco-2 cells and J774 cells. NAC reduced the intracellular ROS levels: The subcellular ROS levels of J774A.1 cells significantly decreased upon co-incubation with LPS and NAC (figure 1).

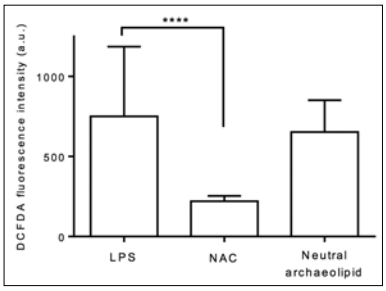

*Fig.1 Decreased generation of reactive oxygen species (ROS). Fluorescence intensity of carboxy-DCF-DA on J774A.1 cells after 24 h co-incubation with 1 µg/ml LPS and NAC. Statistical differences are represented by ***p < 0.001 compared to positive control.*

NAC reduced the production of pro-inflammatory cytokines on a model of inflamed mucosa: The stimulation of THP-1 cells in the co-culture with lipopolysaccharide was followed by a decrease in transepithelial electrical resistance, which is a marker of the integrity of the Caco-2 monolayer and an increase in TNF-α production by THP-1 cells and IL-8 by Caco-2 cells. NAC showed high anti-inflammatory activity as measured by reduced production of TNF-α and IL-8 in LPS stimulated co-cultures.

CONCLUSIONS

The highly stable under gastrointestinal conditions, nanostructured archaeolipid carriers that combines anti-inflammatory and antioxidant activity could be a new strategy to improve the oral treatment of inflamed mucosa.

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Ultra-small solid archaeolipid nanoparticles for active targeting to macrophages of the inflamed mucosa. Higa L, Jerez H, de Farias MA, PortugalRV, Romero EL, Morilla MJ.Nanomedicine. Future Medicine. In press 2017

GRAPHENE OXIDE NANOPARTICLES FOR DAMAGING SUB-CELLULAR DNA IN CANCER

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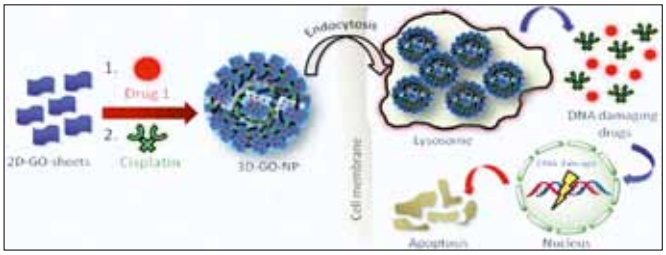
In the past few years, graphene oxide (GO) has attracted particular interest in drug delivery owing to its high aspect ratio and versatile chemical and physical properties. The multifaceted surface of graphene oxide (GO) arises from the oxygen functional groups on the planar carbon structure which allows easy functionalization by covalent or non-covalent linkage. Herein, we describe the hitherto unobserved cisplatin induced self-assembly of 2D-graphene oxide sheets into 3D-spherical nanoscale particles. These nanoparticles can encompass dual DNA damaging drugs simultaneously. A combination of confocal microscopy, gel electrophoresis and flow cytometry studies clearly demonstrated that these novel nanoparticles can internalize into cancer cells by endocytosis, localize into lysosomes, followed by DNA damage leading to apoptosis. Cell viability assays indicated that these nanoparticles were more cytotoxic towards cancer cells compared to healthy cells.



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HYBRID NANOPARTICLES OF A HISTONE DEACETYLASES INHIBITOR COATED BY HYALURONIC ACID FOR CD44-TARGETING DELIVERY IN CANCER TREATMENT

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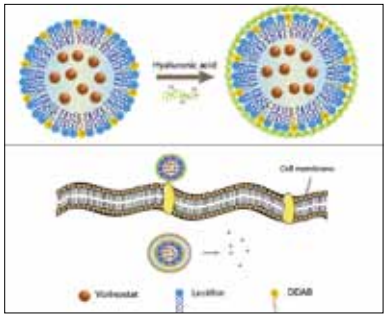


Figure 1: Schematic illustration of LV-HA nanoparticles

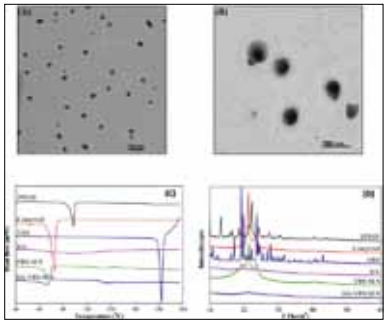


Figure 2: TEM images of (A) VRS-SLNs and (B) HA-VRS-SLNs. (C) Differential scanning calorimetric thermograms and (D) X-ray diffraction patterns of DDAB, Compritol, free VRS, VRS-SLNs, and HA-VRS-SLNs.

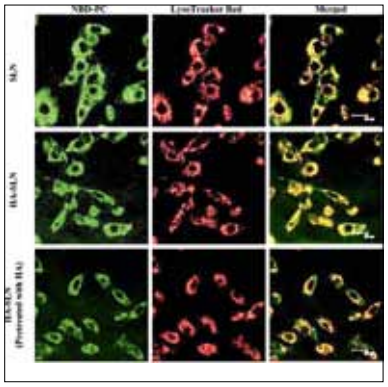
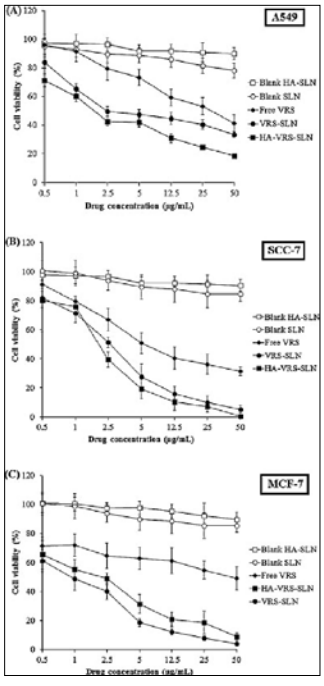


Figure 3: Intracellular uptake of SLNs and HA-SLNs by confocal laser scan microscope images in SCC-7 cells. SLNs and HA-SLNs containing NBD-PC (green) and Lysotracker Red (red) staining lysosome were used for this experiment.



Purpose: Vorinostat is a histone deacetylase inhibitor which shows chemotherapeutic promise in variety hematopoietic and solid tumor treatment. However, the low solubility, low permeability and short half-life properties of this drug have limited the ability of clinical application. Recently, many studies have emphasized to investigate the targeted cancer therapy to enhance the clinical efficacy. In many cancers, there is an up-regulation of CD44, a receptor that specifically binds hyaluronic acid (HA). The aim of this study is to develop hybrid nanoparticles coated by HA as CD44-targeted delivery for vorinostat (HA-VRS-SLNs) to enhance the anticancer activity.

Methods: Vorinostat was incorporated in solid lipid nanoparti-

cles by emulsification method. HA coated NPs for CD44 targeted delivery via electrostatic interaction. HA-VRS-SLNs were evaluated physic-chemical properties, such as particle size, zeta potential, morphology, DSC, XRD and drug loading capacity. In addition, the *in vitro* anticancer activity of NPs was estimated by cytotoxicity study as well as flow-cytometric analysis in different cancer cell lines.
Results: The optimized NPs were spherical, small size (~100nm), narrow distribution and negative charge. The targeting property was confirmed by cellular uptake study with enhanced uptake in A549 and SCC-7 cells (cell lines with high level of CD44 amplification) compared with MCF-7 or blocked-CD44-SCC-7 cells. NPs coated HA also performed better bioavailability by prolonging blood circulation and reducing clearance in rats.
Conclusion: These results indicated the promising of HA coated hybrid nanoparticles application in delivery of vorinostat to tumor side to achieve the anticancer efficacy.
Keywords: Histone deacetylase inhibitor, Hyaluronic acid, CD44, targeting.

COMBINED CHEMO-PHOTOTHERAPY FOR ABLATION OF TUMOR USING INDOCYANINE GREEN AS A PHOTOSENSITIZER

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Purpose: Local hyperthermia has been widely investigated for complete tumor ablation by increasing temperatures to 80oC, combined with chemotherapy or radiation therapy to improve the anticancer effect. Indocyanine green (ICG) is a photosensitizer, which has a spectral absorption maximum at 800 nm and high-intensity fluorescence emission at 820 nm. In this study, we combined a photosensitizer (ICG) and a therapeutic agent (DOC) in a low temperature-sensitive liposome (LTSL/DI) for synergistic effect in treatment of breast cancer.
Methods: LTSL/DI were prepared by lipid thin-layer-hydration-extrusion method. The LTSL system has been evaluated in terms of particle-size distribution, morphology, and release kinetics. In addition, detailed *in vitro* biological evaluation and *in vivo* anticancer effects in SCC-7 xenograft tumor model were performed.
Results: The particle size of LTSL/DI was 130.8 ± 2.3 nm. The release of DOC from LTSL/DI depended strongly on the temperature and pH, which could be beneficial in its potential application for targeted delivery to tumor sites. The *in vitro* anticancer activity of LTSL/DI was significantly enhanced compared with free DOC in SCC-7 and MCF-7 cell lines. In addition, this study demonstrates that *in vivo* efficacy of the combination chemo-hyperthermia therapy in tumor regression was significantly enhanced with minimal side effects.
Conclusions: These results indicate that the LTSL/DI is a promising therapeutic strategy with effectively localized anti-tumor activity and low risk of side effect to non-target organs.
Keywords: combined therapy, photosensitizer, breast cancer, near-infrared laser irradiation, intratumoral injection.

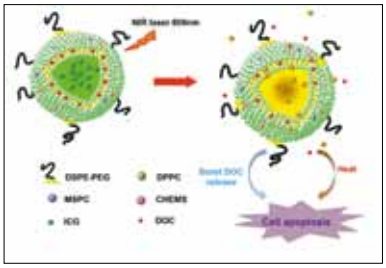

Figure 1: Schematic illustration of the combined anti-tumor activity of hyperthermia therapy and chemical therapy induced by LTSL/DI

Table 1: The low-temperature sensitive formulation characteristics

Formulation	Size (nm)	PDI	Zeta potential (mV)	Loading capacity (%)		Entrapment efficiency (%)	
				DOC	ICG	DOC	ICG
Blank LTSL	92.1 ± 3.4	0.194 ± 0.022	-6.3 ± 0.3	-	-	-	-
LTSL/ICG	121.7 ± 6.4	0.146 ± 0.031	-21.5 ± 1.3	-	9.25 ± 0.17	-	89.68 ± 1.62
LTSL/DOC	129.0 ± 0.9	0.155 ± 0.004	-14.4 ± 1.4	4.94 ± 0.24	-	91.19 ± 4.41	-
LTSL/DI	130.8 ± 2.3	0.153 ± 0.010	-23.2 ± 1.0	4.89 ± 0.17	9.33 ± 0.15	90.27 ± 3.23	92.45 ± 1.50

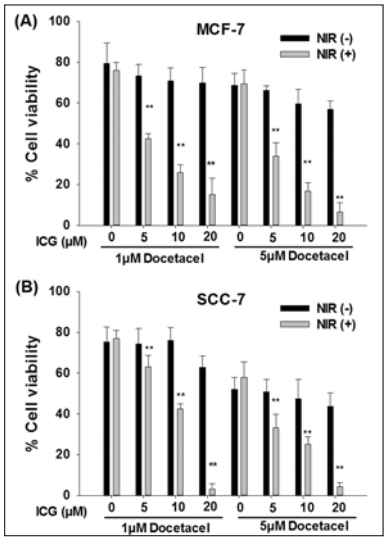

Figure 2. Cell viability of (A) SCC-7 cells and (B) MCF-7 cells bearing the combined treatment of DOC with different ICG concentrations with/without NIR laser irradiation

NANOCARRIER COATED POLYASPARTIC ACID FOR TARGETED DRUG DELIVERY SYSTEM OF DOCETAXEL IN TREATMENT OF RESISTANT CANCER

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Purpose: Docetaxel (DTX), an antimitotic agent, has been clinical applied for several cancer treatments. However, the limitations of this drug have been reported due to the severe side effects as well as multidrug resistance. To overcome this problem, we investigated an unique lipid polymer hybrid (LPH) nanoparticles with a pH-responsive PEG layer for targeting acidic microenvironment in tumor area.
Methods: Docetaxel (DTX) was incorporated into the lipid core of the nanoparticles, which was then shielded with the pH-responsive block co-polymer polyethylene glycol-b-polyaspartic acid (PEG-b-PAsp) using a modified emulsion method (LPH). The pH-responsive property of the nanoparticles was confirmed by measuring zeta potentials and drug release at different pH values. Physico-chemical properties of nanoparticles were characterized including XRD, TEM, AFM, drug release profile. In addition, the *in vitro* anticancer activity of nanoparticles such as cytotoxicity, cellular uptake, Western blotting; *in vivo* pharmacokinetic study and *in vivo* antitumor effect were carried out to confirm the anti-cancer efficiency in resistant cell lines.
Results: The optimized NPs were spherical, small size (~200nm) and narrow distribution. Zeta potential and drug release property were indicated to be pH-dependent. In addition, the improved intracellular uptake due to the charge-reversal characteristics allowed the drug to reach the cytoplasm. DTX-LPH significantly reduced the size of tumors in SCC7 cancer-bearing animals, and its effectiveness was further confirmed by the elevated levels of caspase-3 and PARP in tumor mass.
Conclusion: These results indicated that LPH nanoparticles could provide a new platform for treating cancer.
Keywords: docetaxel, polyaspartic acid, drug delivery systems, antitumor, pH-sensitive

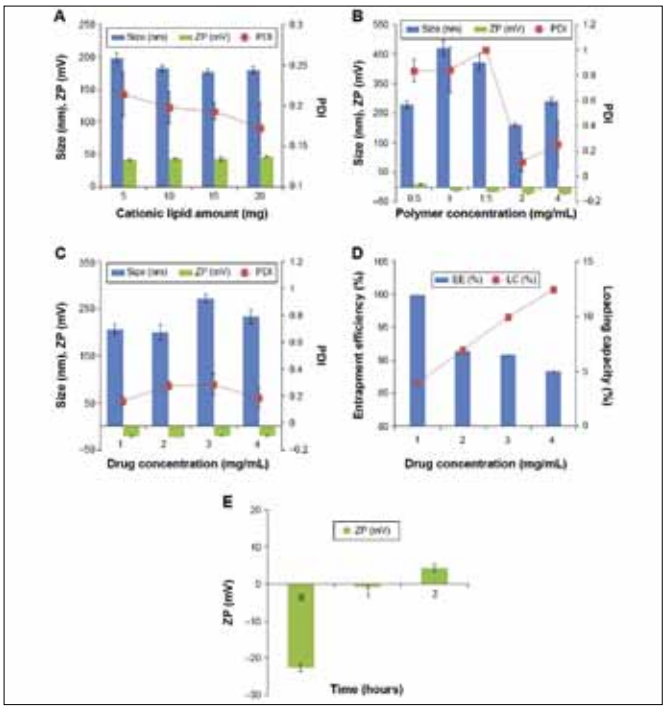

Figure 1: Optimization of DTX-LPH nanoparticles. Effect of (A) cationic lipid amount, (B) polyethylene glycol-b-polyaspartic acid concentration, and (C) drug concentration on formulation parameters: particle size, PDI, ZP. (D) Effect of drug concentration on drug entrapment efficiency and loading capacity. (E) Effect of exposed time on zeta potential at pH 5.5.

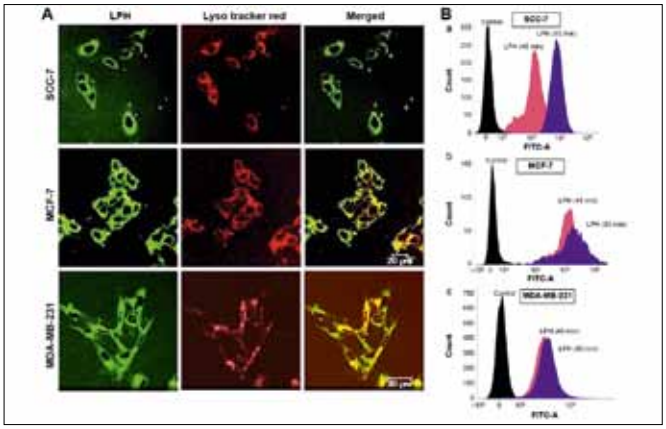

Figure 2: (A) Intracellular uptake of LPH nanoparticles as shown by confocal laser scan microscopy. (B) Uptake of LPH nanoparticles as assessed by flow cytometry.

OPTICAL IMAGING EX VIVO EVALUATION OF PLAQUE PERMEABILITY IN APOE$^{(-/-)}$ MICE USING FLUORESCENT BLOOD POOL AGENT

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Atherosclerosis is a progressive disease characterized by the accumulation of lipids and fibrous elements in the large arteries, causing the formation of atherosclerotic lesions. Dysfunction of the vascular endothelium promotes atherosclerosis through leukocyte and monocyte arterial infiltration, platelet activation and smooth muscle cell proliferation[1]. Under pathological conditions and with aging, open/leaky junctions (diameter > 25 nm) increase endothelial permeability and allow the intake of all solutes and low-density lipoprotein particles and transmigration of leukocytes, that are critical in atherosclerotic plaque formation. With the progression of the disease, plaques can become increasingly complex, with calcifications, ulcerations at the luminal surface and haemorrhages[2]. 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. Currently, several imaging techniques are able to detect atherosclerotic plaques in humans. Despite these advances, these imaging tools are not able to clearly define plaque composition as a predictor of an acute event and thus, it is difficult to define whether and how to treat the lesions. In particular, a diagnostic tool aimed to stratify plaques with respect to different permeability (i.e. different dangerousness) could help clinicians to predict the response to a drug-loaded nanosystem based therapy. 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-/-) develop severe hypercholesterolemia and atherosclerosis with characteristics and distribution similar to those observed in humans[3,4]. The aim of this study was the investigation with Optical Imaging (OI) of the endothelial local permeability in aortic tree atherosclerotic plaques in ApoE$^{(-/-)}$ mice. To this purpose, human serum albumin conjugated with a fluorescent probe (HSA-Cy5) and the albumin binder aminodeoxycholic acid conjugated with IrDye800 (B26170) were administered to ApoE$^{(-/-)}$ mice and fluorescence signal was analyzed ex vivo on the excised aortic trees.

ApoE$^{(-/-)}$ mice were fed with a high-cholesterol diet from 6 weeks of age. HSA-Cy5 and B26170 were intravenously administered to two different experimental groups of ApoE$^{(-/-)}$ mice. After perfusion, arterial tree was removed and imaged with the appropriate filter at 630 nm and 775 nm, respectively on the Axio Zoom V16 microscope system. All the collected images were analyzed with the use of in-house software.

Figure 1. Representative images of OI acquisitions. Brachiocephalic artery (a) bright field and (b) fluorescence image. Abdominal aorta (c) bright field and (d) fluorescence image

For ex vivo fluorescence images (Figure 1) three particular areas were picked out on the sample with the guide of the bright field corresponding image: 1- ROIs on atherosclerotic plaque; 2- ROIs on endothelial area without plaques; 3- ROIs out of sample, representing the image background. OI signal was calculated as follows:

$$OI\left({}^{plaque}/_{no\ plaque\ signal}\right) = \frac{F_p - F_{bkg}}{F_n - F_{bkg}}$$

Fp = Plaque Fluorescence signal, Fn = Fluorescence signal of area without plaques, Fbkg = Background Fluorescence signal.

At the end of OI experiment, the brachiocephalic artery together with the aortic arch and the abdominal aortas were embedded in Optimum Cutting Temperature (OCT) medium. The tissue blocks were frozen in isopentane cooled down in liquid nitrogen and subjected to cyosectioning. For histological analysis, consecutive frozen sections were stained with hematoxylin and eosin (HE). Plaque stenosis was calculated as the percentage of plaque area on total vessel wall area. For each atherosclerotic lesion, a grading of the pathology (grade I, II, III) weighted on the relative stenosis was performed. Due to the heterogeneous morphology and composition of the atherosclerotic lesions, a grading index between 1 and 3, namely grade score, that takes into account the different contribution of the grades, was calculated for each plaque. Moreover, in

order to deeply characterize the tissue composition of atherosclerotic lesions, serial plaque sections were stained and dedicated to specific immunofluorescence staining: the staining of CD68, which is an endogenous intracellular protein expressed by macrophages and foam cells, was evaluated; the endothelial permeability of plaques was studied analyzing the presence of endogenous mouse serum albumin (MSA), the most abundant protein in the plasma.
The ApoE$^{(-/-)}$ mouse model developed plaques in different districts with heterogeneous morphology and composition. Atherosclerotic lesions of each animal were internally classified with respect of the stage of the pathology progression (Figure 2). In order to associate the ApoE$^{(-/-)}$ mice plaques to the human disease, the lesions progression was classified following the literature grading (American Heart Association[5,6], and Virmani[7]). The early atherosclerotic lesions, defined as "intimal xanthoma", rich in fat-filled macrophages (foam cells) accumulated in the intima and separated from the lumen by the overlying endothelium were classified as grade I; "Fibrous cap Atheroma", plaques characterized by the presence of a fibrous cap, composed of smooth muscle cells in a collagenous-proteoglycan matrix, with varying degrees of infiltration by macrophages and lymphocytes were classified as grade II; the fibro-calcific plaques, considered the advanced stage of the pathology progression, were classified as grade III and display extensive accumulations of fibrotic tissue and calcium in the intima close to the media and the reduction of the cellular content.

Figure 2. Classification of atherosclerotic lesions. (a) Grade I: Intimal Xantoma. (b) Grade II: Fibrous cap Atheroma. (c) Grade III: Fibrocalcific plaque.

Atherosclerotic plaques developed in brachiocephalic arteries or abdominal aortas showed different OI signal after treatment with both HSA-Cy5 and B26170. The percentage of MSA positive area correlated with the relative content of macrophages and foam cells, indicating a higher endothelial permeability in lesion rich in inflammatory cells than advanced plaques, constituted of fibrotic and calcified tissue. To evaluate if OI technique with the use of HSA-Cy5 and B26170 enables the stratification of the different type of atherosclerotic lesion, the OI fluorescence signal acquired on the whole plaque was compared with the histological analysis outcomes. Fluorescent signal ratio decreased with the increase of plaque grade, thus with ageing and loss of plaque permeability, with both HSA-Cy5 and B26170. HSA-Cy5 and B26170 signals decreased with the progression of the pathology: the plaque at very early stage, with the lowest grade score, displayed the highest value of fluorescent signal, while the group of advanced lesions, higher grade score with a predominance of grade III, showed a very low signal (Figure 3a and Figure 3b). The percentages of CD68 and MSA staining on plaque sections were compared with the relative plaque grade score to find out a correlation between the inflammatory state and the ageing of lesions. Atherosclerotic plaques at very early stage of development showed the highest value of macrophages content, while the group of advanced lesions displayed a very low amount of macrophages and foam cells (Figure 3c and Figure 3d). Thus, a high inflammatory state correlated with high OI fluorescence signal ratio for both HSA-Cy5 and B26170. Similar findings were achieved with the comparison to the extent of MSA staining, in which the permeability of the lesions to the fluorescent albumin HSA-Cy5 or blood pool agent B26170 followed the accessibility to endogenous albumin.

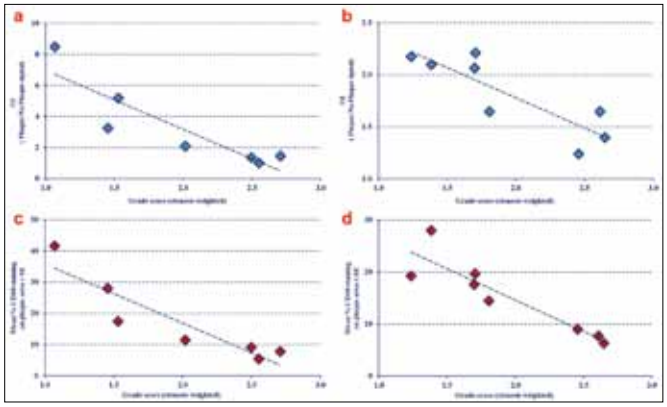

Figure 3. Correlation between OI signal, calculated as fluorescence ratio (Plaque area/No Plaque signal) and the grade score of the corresponding lesions: (a) HSA-Cy5, $R^2 = 0.79$ and (b) B26170, $R^2 = 0.73$. Correlation between the percentage of CD68 staining and the grade score of the corresponding lesions in the two experimental groups: (c) HSA-Cy5, $R^2 = 0.84$, and (d) B26170, $R^2 = 0.83$.

Nano-based systems are suitable tools to probe permeability of atherosclerotic plaques, allowing the stratification on the basis of the local permeability of the lesions. Here we prove the concept with two nanosystems labeled with fluorescent moieties, a Cy5-conjugated albumin and the albumin binder B26170. The high sensitivity of the technique and the possibility to acquire images of the excised vessel samples allowed to obtain different signals in the plaques that correlate with all the examined histological parameters for both the fluorescent probes. It's known that a tool able to predict the detailed nature of the plaques and to identify the high risk ones is extremely important. The method we proposed is able to establish which plaques are suitable for an anti-inflammatory therapy based on drug-loaded nanoparticles. This is a crucial point because in patients with severe stenosis and atherosclerotic lesions at late stage of development the plaque permeability is definitely reduced preventing a potential nano-therapy efficacy. An OI-based method cannot be easily used as clinical tool since there is an intrinsic limitation of this imaging technique related to the reduced ability of penetration of the light in the tissues. On the other hand, the concept can be relatively easily translated into a clinical tool for MRI, replacing the fluorescent probe with a paramagnetic complex, namely B22956/1, that contains the same aminodeoxycholic residue able to bind albumin present in the B26170 molecule. In this sense, a MRI B22956/1 based permeability stratification could allow clinicians to classify the nature of the plaque and to define a proper therapeutic regimen based on drug-loaded nanoparticles.

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CHARACTERISATION OF COMPLEMENT-INDEPENDENT PSEUDOALLERGY CAUSED BY LIPOSOMAL PREPARATIONS IN MICE

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INTRODUCTION

Complement activation-related pseudoallergy (CARPA) is a hypersensitivity reaction triggered by various nanomedicines and is initi-

ated by the complement system. CARPA can be characterized by a cascade of events leading to serious, sometimes lethal side effects including hemodynamic and cardiopulmonary changes, rashes on the skin, arrhythmia, and changes in blood count. We have shown previously that Abelcet, a commercially available amphotericin B containing liposomal drug, known to induce CARPA in man, caused a major increase in systemic arterial pressure (SAP) in anesthetized mice, while zymosan had a biphasic effect on blood pressure, starting with a considerable increase and followed by a hypotensive shock. The goal of the present study was to further characterize the above hypersensitivity reactions in mice.

MATERIALS AND METHODS

Male NMRI mice weighing 28-35g were anesthetized with pentobarbital (60 mg/kg i.p.). The right carotid artery and the left jugular vein were cannulated to measure the blood pressure (BP) and for drug administration. SAP and heart rate (HR) were continuously registered (PowerLab and LabChart, ADInstruments, Budapest, Hungary). Separate groups of conscious animals were treated via the tail vein, followed by exsanguination under deep isoflurane anesthesia from the transected vena cava at set times after treatment (3 or 5 min). Blood count was immediately measured by Abacus cell counter (Budapest, Hungary). The rest of blood was centrifuged at 1500 rpm for 10 min at 4°C, and the plasma was stored at -80°C until further analysis. Complement (C) activation was evaluated by a C hemolytic assay (CHA) on antigen-sensitized sheep red blood cells, by assay of a mouse specific C3a ELISA (TECOMedical, AG, Sissach, Switzerland, and Pan-Specific C3 (Quidel Co. San Diego, CA, USA). Plasma TXB2 concentration (Cayman Chemical, Ann Arbor, USA) was also measured as a sensitive marker of CARPA. The drugs tested were Abelcet, wherein amphotericin B is complexed with two phospholipids, high cholesterol ("HC") multilamellar liposomes, wherein the phospholipid bilayer contained 71% cholesterol and zymosan, a yeast cell membrane extract.

RESULTS

Administration of zymosan (30 mg/kg, n=5) caused a biphasic effect on SAP starting with a 15% increase peaking at 3-4 min, followed by a hypotensive shock in NMRI mice (Fig. 1). Treatment with Abelcet (n=4, 30 mg/kg) resulted in a maximum of 30% increase in SAP that lasted for approximately 10 min, but no decrease in SAP was observed thereafter. HC liposomes (300 mg/kg, n=6) caused a small and transient increase in SAP that was not significant.

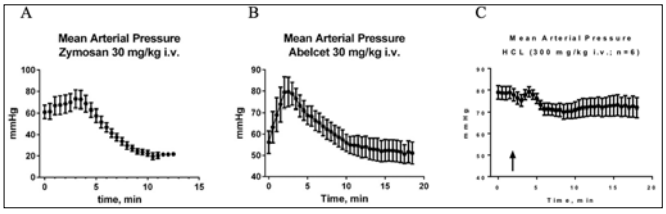

Figure 1: Effects of zymosan (30 mg/kg i.v., A), Abelcet (30 mg/kg i.v. B) and HC liposomes (300 mg/kg i.v. C) on mean arterial pressure in male NMRI mice (n=4-6/group).

Zymosan increased hematocrit (Hct) suggesting plasma extravasation, while neither liposomal preparation altered Hct (Fig. 2). None of the treatments altered white blood cell count. Both zymosan and Abelcet decreased platelet count while HC liposomes caused no effect.

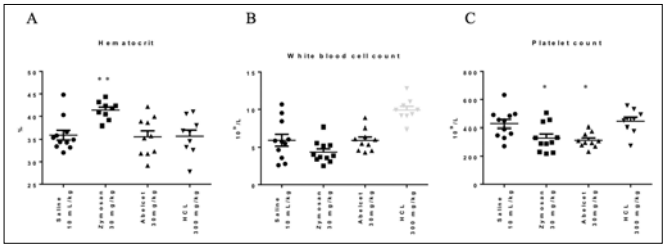

*Figure 2. Effects of saline (10 mL/kg i.v.), zymosan (30 mg/kg i.v.), Abelcet (30 mg/kg i.v.) and high cholesterol liposomes (HCL; 300 mg/kg i.v.) on the hematocrit (A), white blood cell count (B) and platelet count (C) in male NMRI mice. As treatment with HCL interfered with the evaluation of white blood cell count the increase in this parameter seems to be an artefact, and, therefore, the result of statistical evaluation is not marked. *, ** = p<0.05, 0.01.*

Zymosan induced a marked increase in C3a concentration, complement consumption in the PanC3 (Fig. 3) and hemolytic assays (not shown) but no similar effects were observed after treatment with Abelcet or HC liposomes. Zymosan considerably increased plasma TXB2 concentration while Abelcet caused a smaller but significant effect on plasma TXB2 level. There was a tendency for an increase in TXB2 concentration after treatment with HC liposomes but it failed to reach the level of statistical significance.

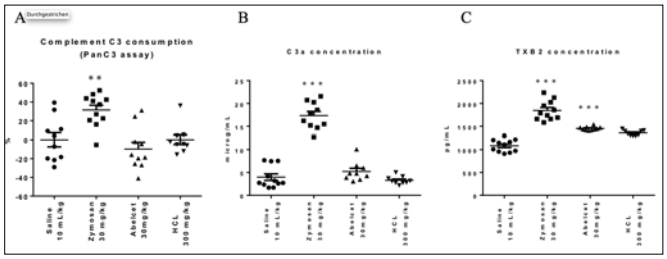

*Figure 3. Effects of saline (10 mL/kg i.v.), zymosan (30 mg/kg i.v.), Abelcet (30 mg/kg i.v.) and high cholesterol liposomes (HCL; 300 mg/kg i.v.) on complement C3 consumption (Pan C3 assay, A) C3a concentration (B) and thromboxane B2 concentration (TXB2, C) in male NMRI mice. ** = p<0.01; *** = p<0.001.*

DISCUSSION AND CONCLUSIONS

As for the practical significance of our study, among the assays used to evaluate complement activation, the mouse specific C3a ELISA proved to be the most sensitive. Although complement consumption measured using Pan C3 and hemolytic assays also indicated complement activation after zymosan administration, the effects were smaller with larger biological variation. Since the effect of zymosan on plasma TXB2 concentration showed a small scatter only, it seems that the CARPA reaction was quite reproducible in our experiments. Therefore, the mouse specific C3a ELISA is a reliable and sensitive assay for the demonstration of complement activation in mice.

The results presented clearly show that zymosan caused complement activation in male NMRI mice as it increased complement C3a concentration and complement consumption in both the PAN C3 and hemolytic assays. Zymosan elicited all responses that are characteristic for CARPA in rodents, such as changes in blood pressure, accompanied by indication of plasma extravasation, as wells as increased plasma TXB2 concentration and decreased platelet count. On the other hand Abelcet and HC liposomes failed to raise plasma C3a or show evidence of complement consumption. However, both Abelcet and HC liposomes induced some changes that are typical of CARPA reaction in other species, including pigs, dogs, rats, and man. Such effects include the increased blood pressure, decreased platelet count and increased plasma TXB2. Therefore, it may be concluded that liposomes can cause some pathophysiological responses in mice that partly resemble CARPA but with a mechanism that is independent of complement activation.

The liposomes, which are potent C activators in humans, rats and pigs, and do cause CARPA in these species, did not cause apparent C activation in mice despite inducing some of the typical CARPA symptoms, implying a new phenomenon, "complement-independent pseudoallergy" or "pseudo-CARPA". The reason for this lack of C activation in mice *in vivo* remains to be established in future studies.

SOFT AND NANOSTRUCTURED ELECTRODES FOR APPLICATIONS IN ARTIFICIAL MUSCLES, NEURO-PROSTHETICS AND WEARABLE ELECTRONICS

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Compliant and soft electrodes are essential components of thin-film dielectric elastomeric actuators alias artificial muscles, neuroprosthetics and wearable electronics [1, 2]. The two main paths to increase the compliance include the manipulation of intrinsic material properties of the electrode, or its structural features, such as the introduction of wrinkles. Wrinkling is a universal phenomenon exhibited by a compressed stiffer film resting on a soft substrate [3]. Many approaches have been presented to control the wrinkling periodicity and amplitude for enhanced stretchability [4]. Here we demonstrate that by tuning oxygen plasma parameters, we were not only able to tune the topology of the Au electrodes on PDMS surfaces but also suppress its stiffness increase, as quantified by atomic force microscopy nanoindentation measurements.

Keywords: Compliant electrodes; oxygen-plasma treatment; nanoindentations; silicone elastomers; PDMS; wrinkles.

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REVERSIBLE PEPTIDE SELF-ASSEMBLY INTO NANOFIBRILS FOR LONG-LASTING FORMULATIONS

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Peptides are highly selective and efficacious therapeutic agents which have demonstrated their ability to treat a broad range of conditions, including cancer, autoimmune, cardiovascular and metabolic diseases. Their clinical potential will be only fully realised once their physicochemical and pharmacokinetic properties have been precisely controlled. To this end, peptide-based nanostructures have emerged as an attractive mode of drug delivery for long-lasting formulations. This approach has been inspired by the discovery that many natural hormones are stored in the form of nanofibrils in specific cellular compartments. Based on this finding, we have developed peptide formulations based on the reversible self-assembly of peptides into nanofibrils as a strategy to control and prolong their activity *in vivo* (Figure 1).

We show that insulino-mimetic peptides self-assemble into nanofibrils which subsequently dissociate under physiological conditions to release active peptide. Notably, the subcutaneous administration of the nanofibrils in rodents resulted in greatly prolonged serum activity as compared to free peptide. This work opens a new area of investigation to assess the clinical application of reversibly self-assembling nanofibrils to prolong the residence time of peptides in serum.

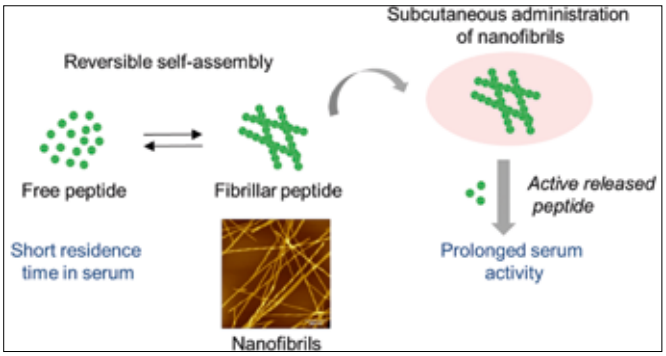


Figure 1. Reversible peptide self-assembly to control and prolong the activity of peptides in vivo.

NANOPARTICLES FOR THE DELIVERY OF PEPTIDES AND OTHER SOLUBLE THERAPEUTICS PRODUCED BY FLASH NANOPRECIPITATION

ROBERT F. PAGELS, Chester E. Markwalter, Prof. Robert K. Prud'homme
Suggested Track 1: Novel Therapeutic and Diagnostic Approaches

Biologics, including protein and peptide therapeutics, are the most rapidly growing segment of the pharmaceutical marketplace. In 2012, the growth rate of biologic sales was seven times higher than the rate of total pharmaceutical sales in the US [1], and by 2019 the market is expected surpass $200 billion [2]. This explosive growth is in part due to their high specificity to disease targets compared to small molecule drugs. Despite this rapid growth, they suffer major limitations in how they are administered to patients. Due to their large size and sensitive structures, biologics have poor oral bioavailability and must be administered by injection. Some proteins, especially antibodies, may have long circulation times on their own. However peptide therapeutics, such as the GLP-1, can be cleared by proteases on the order of minutes. Peptide therapeutics could benefit from new delivery methods which can protect them from proteases, prolong their circulation times, and increase bioavailability. A PEGylated nanoparticle delivery vehicle could meet those requirements. However, many of the most prominent and successful nanoparticle delivery platforms, including polymer, lipid, and even albumin based formulations, are designed to carry hydrophobic cargo. The low solubility of hydrophobic drugs in aqueous biologic environments helps them stick to the delivery vehicle. One such technology is Flash NanoPrecipitation (FNP), an industrially-scalable and controllable continuous precipitation technique which has been used to produce nanoparticles highly loaded with hydrophobic drugs [3]. However, more creative engineering is required for the delivery of peptides and other water soluble molecules.

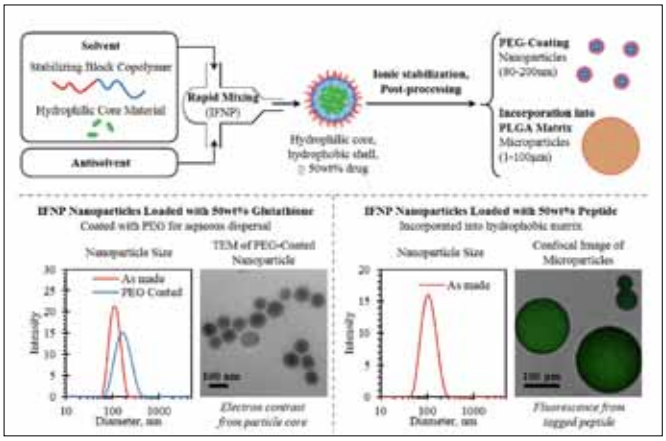
Some strategies to incorporate soluble drugs into nanoparticles have included reversible chemical linkages, the degradation of which releases the drug from the nanoparticle. This is unfavorable for already marketed drugs, because the formulation will need to go through a more vigorous approval process compared to a new formulation in which the drug is not chemically modified. Nano-gel and liposomal formulations do not require any chemical modification, however they generally suffer from poor encapsulation efficiencies and low loadings.

Recently we introduced a novel method called Inverse FNP, or IFNP, which we use to manufacture nanoparticles highly loaded with

water soluble molecules [4]. In this technique a stream of peptide therapeutic and amphiphilic block-copolymer is rapidly mixed with a non-polar antisolvent stream in special mixing geometries. The antisolvent causes the peptide to precipitate, and this precipitation is halted by assembly of the stabilizing block copolymer on the particle surface. The resulting nanoparticles have a low polydispersity, and we can control the size from 50 to 200nm. We have applied the IFNP process to more than a dozen biologic-like molecules, all with loadings of ≥50wt%.

Here we will we will show the technological development of the IFNP process, given in Figure 1. We start with the formulation parameters that can be used to control the nanoparticle size and loading. We then show how these particles can be ionically stabilized so that they may be further processed into biomedically appropriate forms. From here there are two paths we have taken. First, we can coat these particles in PEG for applications in which a circulating nanoparticle formulation is desired (Figure 1, bottom left). Alternatively, we can incorporate these primary nanoparticles into microparticles composed of a hydrophobic matrix for sustained release applications (Figure 1, bottom right). With either of these methods, we have been able to achieve loading ten times higher than most commonly published technologies. Finally, we will show our progress in synthesizing new biocompatible and biodegradable polymers and their application in the IFNP platform.

Figure 1: The IFNP process for encapsulating soluble therapeutics. Top: Block copolymer stabilized nanoparticles are produced by a rapid, continuous mixing process (IFNP). This process is industrially scalable and produces highly loaded particles of a controllable size. These particles may be PEG-coated for circulating applications, or incorporated into a hydrophobic matrix for sustained release applications. Bottom right: An example of glutathione-containing nanoparticles (110nm) which were coated with PEG for aqueous dispersion (130nm). Electron contrast is from metal ions in the particle core. Bottom left: An example of peptide containing nanoparticles (100nm, proprietary peptide provided by Merck) which were incorporated into microparticles. Fluorescence for confocal imaging was provided by tagging the peptide with AF488.



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DIFFERENTIATING HUMAN MYOBLASTS AND UROTHELIAL CELLS AS A MODEL FOR STUDY LONG-TERM NANOTOXICITY IN VITRO

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For both industrial and biomedical nanoparticles (NPs) it is crucial that we assess potential short-term and long-term toxicity. NPs can by several mechanisms cause cell stress expressed as oxidative stress lower proliferation rate, changes in cell metabolism, damages of DNA, lysosomal dysfunctions and hindered cell differentiation. NPs can also trigger pro-inflammatory reaction through different mechanisms like NF-κB, inflammasome or other. The mechanisms of NPs toxicity are complex and any new type of NP has to be appropriately characterized which is also one of the main reasons for the difficult implementation of NPs into biomedicine. Moreover, cytotoxicity strongly depends on the selected *in vitro* model [1-3].

Short-term and acute exposure *in vitro* nanotoxicity experiments are routinely performed, while there are not many suitable protocols enabling to access long-term cytotoxic effects of NPs [4,5]. Clearly, some specific harmful effects of NPs can be observed only for long-term exposures. Therefore, we implemented two *in vitro* models for assessing long-term nanotoxicity; biomimetic *in vitro* urothelium model and L6 rat myoblasts cells, both with differentiation capability. Acute and continuous exposure to selected types of NPs was analysed. We analysed potential effects of i) two types of magnetic nanoparticles used for biomedical applications - polyacrylic acid (PAA) [2,3] and polyethileneimine (PEI) coated magnetic NPs), and ii) two commonly used industrial types of NPs: SiO_2 and TiO_2. We further analysed induction of ROS, and activation of NF-κB factor with western blot. Also expression of genes related to myoblast differentiation was determined by qPCR. DLS and zeta potential were determined in water and in relevant physiological media.

In general there was vey little effect on cell viability of the urothelial PU cells, both for short-term and long-term exposures. Cell viability did not drop significantly even in the case of 31-day of continuous exposure of urothelium cells to NPs, except for PEI NPs, that are know to be toxic already at lower concentrations [3].

In contrast, myoblast L6 cells were much more sensitive to exposure to NPs with dose-dependent response to PAA, PEI and TiO_2. For short-term incubations (96h) PEI NPs killed all the cells while TiO_2 at 50 μg/ml reduced cell viability for 30%. Myoblast cells were not sensitive when treated with silica NPs, while TiO_2 showed concentration dependent cell survival for short-term and long-term exposure, cell viability dropped to zero for continuous exposure to 50 μg/ml TiO_2 concentrations. In general the selected silica NPs and PAA NPs exhibited very little toxicity, since some effects were observed above 50 μg/ml which presents extremely high concentration.

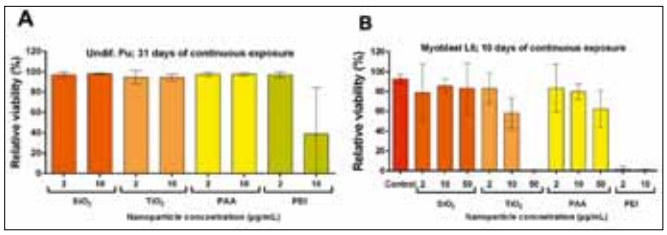

Figure 1: Viability of myoblasts after long-term exposure to SiO_2, TiO_2, Co-ferrite-PAA and Co-ferrite-PAA-PEI NPs analysed on PU urothelial cells (A) and on L6 myoblasts (B).

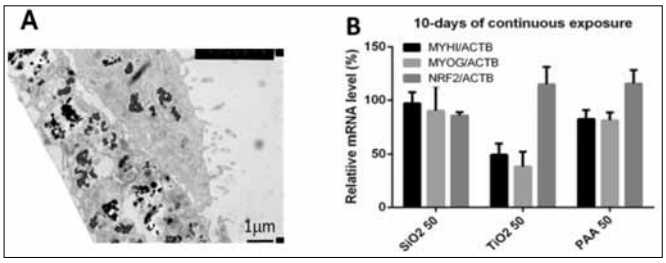

Figure 2: A) TEM images of primary human myoblasts after 10 days continuous exposure to TiO_2 NPS (50 µg/ml). qPCR measured levels of mRNA for myosinHI, myoG and NRF2, normalized to ACTB, after treating L6 cells for 10 days with SiO_2, TiO_2, Co-F-PAA NPs (50 µg/ml).

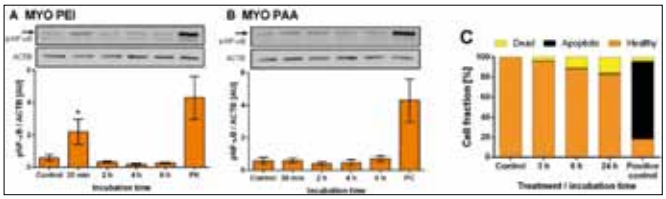

Figure 3: Phospho NF-κB, normalized to actin in primary human myoblasts after increasing incubation time with 4 µg/ml A) PEI coated magnetic NPs and for PAA coated magnetic NPs. C) Time dependent percentage of apoptosis/necrosis for 4 µg/ml PEI NPs determined on myoblasts cells. Percentage of apoptotic/necrotic cells was determined using Annexin/PI differential assay, staurosporine (1 µM, 6h) was used as a positive control.

Interestingly, there was a transient increase in ROS levels after 24h for all NPs for the highest concentration of NPs (200 µg/ml), while after 10 days no increase was observed. In addition, transient activation of nuclear factor NF-κB was observed for PEI magnetic NPs on primary human myoblast cells but not for PAA NPs.

In general, the dose dependent toxicity was observed only on myoblast L6 cells, while on porcine urothelial cells only PEI NPs were toxicity. The differences in the viability between both cell types are expected as urothelial barrier is the strongest barrier in our body and as such almost impermeable for majority of substances. This was confirmed with TEM microscopy where almost no NPs where found in urothelial cells in contrast to high intracellular loading in L6 cells for PAA and TiO_2 NPs. The selected industrial silica NPs did not induce cytotoxic effects, which can be explained mostly with large aggregation under physiological conditions (aggregates sizes in range of micrometres). The most stable NPs – the magnetic PAA nanoparticles (hydrodynamic radius below 100 nm in culture media with 10%FBS) had some moderate toxic effect, but only at extremely high concentrations despite very high intracellular loading. As expected the rat myoblasts L6 cells were much more sensitive than porcine urothelial cells are. Interestingly, elevated levels of mRNA for nuclear factor (erythroid-derived 2)-like 2 (Nrf2) were observed. Nrf2 is a transcriptional factor involved in regulation of expression of antioxidant proteins indicating induced stress response for both TiO_2 and PAA NPs after continuous exposure. The transient increase of ROS levels in myoblasts L6 cells indicates that production of ROS could be one of the main mechanisms behind the reduction of rat myoblasts L6 cell proliferation. The effect of some NPs on differentiation level of L6 cells was additionally confirmed by measuring mRNA levels of myogenin (MyoG) and myosin I (MyhI), characteristic biomarkers of differentiated rat myoblasts L6 cells. While for PAA NPs no effect was observed, TiO_2 NPs clearly decreased MyoG and MyhI. However, it is important to stress that 50 µg/ml is still very high concentration that is difficulty to be achieved *in vivo*, therefore further experiments are needed to asses potential stress repose at lower concentrations.

Altogether we show that presented *in vitro* models of differentiating myoblast and urothelial cells can contribute to field of nanotoxicology and testing of newly designed NPs.

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MILD HYPERTHERMIA COMBINED WITH THERMO-RESPONSIVE LIPOSOMES: A DUAL APPROACH TO ENHANCE THE THERAPEUTIC EFFICACY OF DOXORUBICIN-PSA CLEAVABLE PEPTIDE PRODRUGS IN CASTRATION-RESISTANT PROSTATE CANCER

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Prostate cancer (PC) is the most common cancer, and the second cause of cancer-related deaths in men in the UK. Early stages of PC, where cancer cells are confined to the prostate, have been successfully treated with radiotherapy and/or androgen deprivation therapy. However, overtime androgen-independent cancer cells develop which can spread beyond the prostate, resulting in poor prognosis and limited treatment options. A strategy to target antitumor drugs to prostate tumours is to use prostate specific antigen (PSA), an extracellular peptidase, solely secreted in prostate tissue and prostate carcinoma in PC patients. Doxorubicin-PSA (Dox-PSA) prodrug is currently in clinical trials. The intravenous administration of a doxorubicin prodrug, which would only be active at the tumour site, could reduce systemic exposure to the active drug. Mild hyperthermia (HT) improves drug bioavailability at the tumour sites, and it has been shown to synergise with several anticancer agents. In this study, we aim to combine Dox-PSA selectivity with temperature-sensitive liposomes (TSL) and mild HT to improve the delivery and the therapeutic efficacy of Dox-PSA prodrug in castration-resistant PC cells.

METHODS:

Dox-PSA prodrug was synthesized and loaded into TSL using remote-loading methods. A series of androgen-dependent and independent PC cell lines, which represent different stages of PC, were evaluated for their PSA expression levels by Western blot and RT-qPCR. Free PSA levels in LNCaP and C4-2B cell lysates and supernatants were also quantified by ELISA. Mild HT effect on the cell viability was assessed using resazurin cell viability assay. The effects of HT on the expression of PSA were also evaluated by Western blot and RT-qPCR. Finally, the cytotoxicity of liposomal Dox-PSA prodrug in combination with mild HT was determined in PSA positive and negative 2D and 3D PC models.

RESULTS:

Dox-PSA prodrugs (Fig. 1a) were successfully loaded into TSL (Fig. 1b). HT exposure (42°C) up to 1h did not significantly affect cell vi-

ability, but it influenced mRNA levels of PSA, with no significant down regulations in the final protein observed. Dox-PSA prodrugs demonstrated dose- and time-dependent toxicities in PSA-expressing PC cells, whereas in PSA-negative cells toxicity was minimal up to 10 µM of Dox-PSA, and no time-dependence was observed. On the contrary, free Dox exhibited dose-dependent toxicities in both PSA-positive and negative cells, suggesting that Dox-PSA prodrugs were effectively being activated by PSA present in these cells. Interestingly, HT did not improve the cytotoxicity of free Dox-PSA prodrugs in PSA-expressing cells, however, a significant toxicity was observed in 2D and 3D tumour models following encapsulating

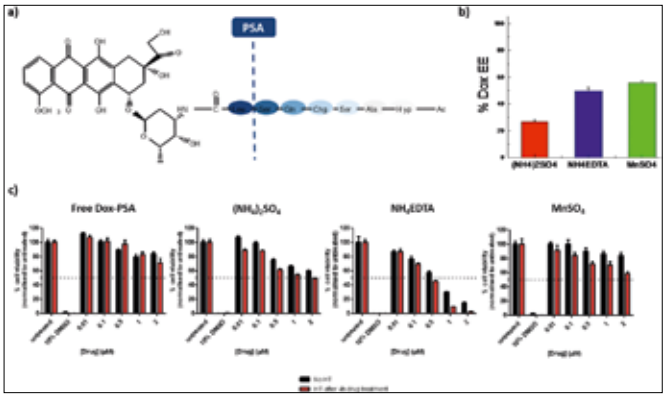

Figure 1| Dox-PSA prodrug structure, encapsulation studies and in vitro cell viability ± HT. a) Dox-PSA structure and PSA cleavable site, b) Dox-PSA encapsulation in TSL using remote loading techniques, c) Cell viability of C4-2B monolayers after treatment with 0.01-2 µM of free or liposomal Dox-PSA for 72h. Effect of hyperthermia-combined treatment was also assessed by incubating cells for 1h at 42°C.

CONCLUSIONS:

This is the first study to investigate the effect of mild HT on the cell viability and PSA expression in PC cells, where HT showed no significant impact on PC cell viability and PSA expression. Our results also showed that mild HT could be a promising approach to enhance the therapeutic efficacy of liposomal-Dox-PSA formulations in castration-resistant PC cells.

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CYTONANOHEAL: ENGINEERED NANOTOOLS FOR ADVANCED CELL THERAPIES IN OSTEOARTHRITIS

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Osteoarthritis (OA) is a degenerative disease characterised with pain, stiffness and loss of function in the weight-bearing joints, occurring as a consequence of mechanical and biological destabilisation of the articular tissues. It affects cartilage, subchondral bone, ligaments, synovial membrane and periarticular muscles as a result of the inflammatory biological molecules that disturb the balance between the tissue synthesis and degradation. The persistent inflammation alters the function of the synovial membrane that releases the breakdown products to stimulate further degradation. The disruption of the joint homeostasis induces profound phenotype modifications in cells that, in turn, synthesise more catabolic mediators, mainly pro-inflammatory cytokines. Maintaining the composition of this physiological lubricant by restoration of homeostasis is thus a necessary step to retard the OA progression and provide a suitable environment for tissue remodelling.

Current pharmacological treatments are only minimising the disability of patients. These are directed to relieve pain, decrease joint swelling and maintain its function to preserve the patients' quality of life, but are not capable to cease the cartilage loss or the disease progression.

In turn, novel therapeutic concepts rely on biochemical stimuli to counteract the chronic inflammation. They share a common approach to extract biological entities from the same patient (autologous formulation) or a donor (allogenic formulation), preparation of a product (e.g. by aggregation of bio-entities), and its administration into the patients' joints. For example, the platelet-rich plasma (PRP) therapy increases proliferation of meniscus cells, reduces pain and improves the joint function. Although showing some improvements compared to the conventional interventions, the PRP therapy is not standardised for specific tissue (cartilage, skin, bone, ligaments, etc.), and the product preparation has no selection criterion.

Retarding the disease progression and at the same time providing a suitable environment for tissue remodelling are still unmet medical prerequisites for the efficient treatment of OA. CytoNanoHeal project aims at engineering of delivery systems that enable long-term efficiency of biological entities in cell-based therapies. A patent-protected anti-inflammatory cytokine cocktail (ACC) , formulated from human blood plasma will be injected together with in-situ gelating hydrogels peptide into the OA-affected joints to delay the disease progression and boost the anabolic pathways for recovery of the damaged cartilage (Fig.1). The ACC is a synergistic combination of bioentities that inhibit the chronicity of OA pro-inflammatory factors. The novel delivery systems will be used to protect the unstable cytokines and boost the therapeutic effect. Additionally, mesenchymal stem cells from adipose tissue (ASCs) will be supplied using the engineered nanoconstructs to initiate the cartilage regeneration by differentiation of ASCs into chondrocytes.

As a proof-of-concept in regenerative medicine, the nanoconstructs will be used to provide optimal spatial and functional support for bioentities in a combined therapy for osteoarthritis (OA) comprising:

- Cytokines for retarding the disease and pain relief, and
- Stem cells for cartilage regeneration.

The ACC formulation is a technology patented by consortium partners.[1] This product is already being exploited as a stand-alone injectable formulation for the treatment of grade II-III OA (non-degenerative). Administration with the CytoNanoHeal nanotools will broaden the application potential of the cytokine therapy not only to grade IV OA, but also to other degenerative diseases. Inspired by the native environment of proteins, the extracellular matrix (ECM), and targeting higher stability of cytokines, new delivery systems based on glycosaminoglycan (GAG) have been developed. The ACC cytokines have been firstly incorporated into nanoparticles for stabilisation and prolonged their activities, in order to attain the homeostasis niche necessary for ASCs differentiation into chrondrocytes and cartilage regeneration. Besides, hydrogels will be engineered to house simultaneously cytokines and stem cells in order to provide their long-term efficiency and enhanced activity for cartilage regeneration.

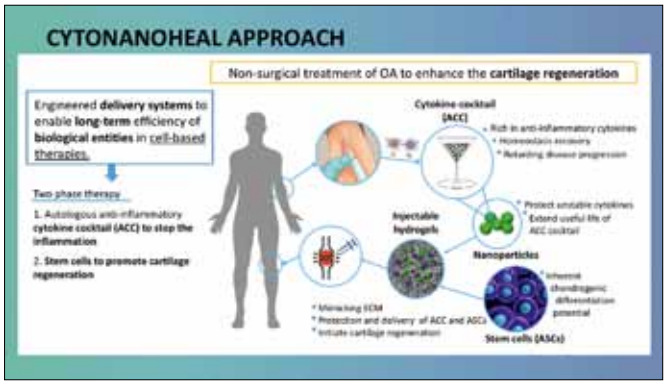

Figure 1. Schematic representation of CytoNanoHeal project approach

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MAGNETIC TISSUE ENGINEERING OF THE VOCAL FOLD: GENERATION OF 3D CELL CONSTRUCTS USING SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES

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INTRODUCTION

The principles of biology and engineering are combined in tissue engineering to generate functional replacement for damaged tissue. Growing interest and new approaches in tissue engineering are promising solutions for overcoming the rejection of transplanted organs. Tissue engineering comprises the isolation of autologous cells from healthy tissue or stem cells, cultivation and proliferation of these cells and finally generation of 3D structures resembling natural tissue structures. Even though expertise of these methods in tissue engineering has been established, still there is scope for enhancement. *In vivo* tissue has complex cellular organization and defined arrangements of cells need to be established. Therefore, techniques to manipulate and remotely control cellular behavior can deliver a powerful tool for tissue engineering. Such a tool bargains Magnetic Tissue Engineering (MTE). As the voice is the most important instrument of oral communication, tissue defects in this region lead to serious aggravation in quality of life. Hitherto, no satisfactory possibilities for vocal fold (VF) transplantation exist. We aim for establishment of a functional VF transplant in a rabbit model by MTE using superparamagnetic iron oxide nanoparticles (SPION).

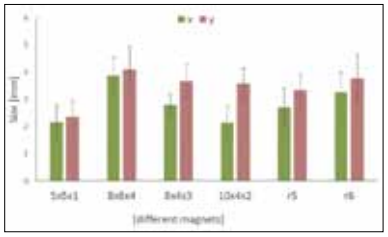
MATERIAL AND METHODS

Rabbit vocal fold fibroblasts were incubated for 24 h with 20 µg/cm2 superparamagnetic iron oxide nanoparticles (SPION). After trypsinisation, cells were counted and 2 x 106 cells per well were placed in a 48- well plate coated with 500µl 1% agarose in 600 µl full medium. The lid of the 48- well plate was closed immediately, so as the magnet, which is on the lid, is able to induce magnetic cell levitation. After 24 h, 3D vocal fold fibroblast (VFF) constructs were formed in this matter. Different magnets were used for 3D- VFF construct formation. These were investigated for altered shape formation measuring x- and y- dimensions via Image J software. Flow cytometry analysis using live/dead staining was performed after 24 h to identify cell viability within 3D- VFF constructs formed by different magnets. Furthermore, 3D constructs were analyzed for their viability in a time dependent manner. Immunohistochemically analysis was performed after 24 h to elucidate if 3D- VFF constructs bear a necrotic core and to investigate if stable cell- cell interactions are established. Isolation of rabbit vocal fold epithelial cells was performed to start co- cultures together with VFF 3D- constructs.

RESULTS

VFF loaded with 20µg/cm2 SPIONs formed stable 3D constructs. To optimize this process we used different magnets (square, rectangle and round), which were able to induce variances in 3D cell construct shapes. 3D- VFF constructs formed by square magnets (5x5x1 mm and 8x8x4 mm) have nearly the same width and length, while with longish magnets (8x4x3 mm and 10x4x2 mm) they are longer and have a smaller width. The biggest constructs can be obtained with the biggest magnets, the 8x8x4 mm square magnet; sized 4mm x 4mm. The cylindrical magnets (r5 mm and r6 mm) reveal a small difference between length and width. (Figure 1)

Figure 1: X- and y- dimensions of 3D- VFF constructs obtained by different magnets.



Flow cytometry analysis revealed no significant difference between the viability of VFFs after 24 h when comparing different shaped magnets. Average viability of 3D- VFF constructs was 83%, with only low percentage in apoptotic or necrotic cells (17%). The best viability was observed in 3D- VFF constructs assembled by magnets with radius 5 mm (88.9%), whereas lowest viability was observed in constructs built with the square magnets 5x5x1 mm (79.3%) and 8x8x4 mm (79.0%). (Figure 2)

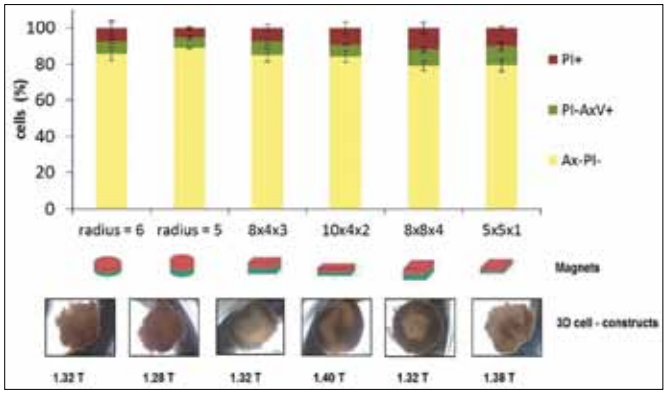
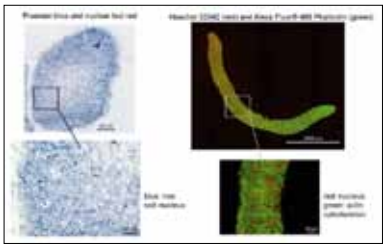


Figure 2: Flow cytometry analysis of single cell solutions of constructs established by different magnets stained for AnnexinV (AxV) and Propidium Iodide (PI). Viable cells are AxV-PI- (yellow), early apoptotic cells are PI-AxV+ (green) and late apoptotic as well as necrotic cells are AxV+PI+ (red). Below in each column different magnets as well as the 3D- VFF constructs are presented.

Immunohistochemistry was used to analyze if 3D- VFF constructs bear a necrotic core. When establishing the 3D- VFF constructs the gap between magnet and medium surface shall not be bigger than 3mm, otherwise a necrotic core will be present, because the construct generated will be much thicker. Staining for actin- cytoskeleton (Phalloidin) revealed dense cell-cell interaction. (Figure 3)

Figure 3: Upper left: Cryosections were stained for Prussian blue to identify iron in the cells and nuclear fast red. Lower left: magnification of tissue structure. Upper right: Hoechst 33342 and Alexa Fluor®

488 Phalloidin staining of cryosection. Lower right: Magnifaction indicating dense F-Actin structure.



CONCLUSION AND DISCUSSION

The VF is a very complex tissue and a prerequisite for voice production. VF scarring is non-reversible and has a severe impact on patients' life quality. Due to the complex structure of the VF no satisfactory transplant could be developed yet. Therefore, we aim to generate 3D- VF transplants which are able to model the complex structures. Our results demonstrate the possibility to generate distinctive shaped 3D- VFF structures, relating to the applied magnets. Since these engineered tissues are viable in culture for at least 7 days, this enables the possibility of individual and personalized preparation of a VF transplant. Necrotic areas within the tissue can be avoided by controlling the thickness.
Next steps include the establishment of 3D co-cultures together with epithelial cells, as well as the proof of functionality in a flow channel model of the rabbit larynx. These results generate a solid foundation for successfully transferring this method into humans, in order to provide an individual and personalized VF transplant.

Acknowledgement
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TUMOR STROMA-CONTAINING 3D SPHEROID MODEL: A TOOL TO STUDY NANOMEDICINE

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Nanomedicine provides an excellent avenue for enhancing therapeutic index of anti-cancer therapies. Recent studies have shown that penetration of nanoparticles through tumor tissue after extravasation is considered as a key issue for tumor distribution and their accumulation and thereby the therapeutic effects. Many tumors are enriched with stroma, a fibrotic tissue composed of cancer-associated fibroblasts (CAFs) and extracellular matrix (ECM), co-exist with endothelial cells, inflammatory immune cells, adipocytes in the tumor microenvironment which acts as a barrier for nanoparticle penetration. However, there is a lack of suitable *in vitro* systems to study the tumor stroma penetration of nanoparticles, while animal models are expensive, time consuming, and could fail to reflect human tumor biology.

AIMS

In the present study, we aim to develop and thoroughly characterize a 3D co-culture spheroid model to mimic tumor stroma and investigate the penetration and efficacy of nanomedicines in these spheroids.

METHODS

We cultured homospheroids of 4T1 mouse breast cancer cells or 3T3 mouse fibroblasts alone as well as heterospheroids of 3T3 and 4T1 cells in different ratios (1:1 and 5:1) using microwell array platform. The spheroids were characterized for their size and cellular reorganization. We also examined the presence of CAF biomarkers using immunohistochemical staining and qRT-PCR. To study the penetration of nanoparticles, we observed the penetration of high and low negatively charged fluorescent silica nanoparticles (30 nm; red and 100 or 70 nm; green; zeta potential: -40 mV and -20 mV) and as well as Cy5-conjugated pegylated PLGA nanoparticles (200 nm, -7 mV) in both homo- and heterospheroid models. Furthermore, we also developed human homospheroids (MDA-MB-231 or Panc-1 tumor cells) and heterospheroids (MDA-MB-231/BJ-htert and Panc-1/pancreatic stellate cells) and performed silica nanoparticle penetration studies. The efficacy study was performed using paclitaxel-loaded polymeric micelles and paclitaxel-loaded PLGA in homospheroids and heterospheroids of 3T3 and 4T1 cells compared to 2D monolayer culture model.

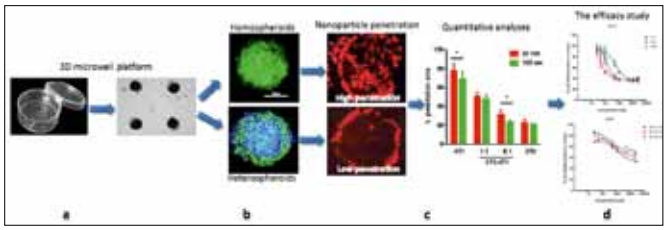

Figure 1. Graphical abstract of nanomedicine study in 3D homo- and heterospheroids

RESULTS AND DISCUSSIONS

Spheroids were formed within 48 h using microwell-array platform (Figure 1a). Confocal live imaging of formed spheroids revealed that fibroblasts distributed and reorganized within 48 h in heterospheroids (Figure 1b). Furthermore, immunohistochemical staining and gene expression analysis showed a proportional increase of α-SMA and collagen in heterospheroids with higher fibroblast ratios attaining 35% and 45% positive area at 5:1 (3T3:4T1) ratio, in a good match with the clinical breast tumor stroma, in our preliminary study of human breast tumor patient biopsies. Subsequently, we studied the penetration of high and low negatively charged fluorescent silica nanoparticles (30 nm; red and 100 or 70 nm; green; zeta potential: -40 mV and -20 mV) and as well as Cy5-conjugated pegylated PLGA nanoparticles (200 nm, -7 mV) in both homo- and heterospheroid models. Fluorescent microscopy on spheroid cryosections after incubation with silica nanoparticles showed that 4T1 homospheroids allowed a high penetration of about 75-80% within 24 h, with higher penetration in case of the 30 nm nanoparticles. In contrast, spheroids with increasing fibroblast amounts significantly inhibited nanoparticle penetration (Figure 1c). Subsequently, similar experiments were conducted using Cy5-conjugated pegylated PLGA nanoparticles and confocal laser scanning microscopy live imaging showed that an increased nanoparticle penetration was found in 4T1 homospheroids until 48 h, but significantly lower penetration in heterospheroids. The penetration study in human spheroids also revealed heterospheroids had significantly a lesser penetration of the nanoparticles compared to homospheroids. Furthermore, the efficacy study of paclitaxel-loaded nanomedicines derived from 3D-spheroids showed higher cell viability of about 9-folds than that from 2D cultured monolayer cells (Figure 1d). In addition, tumor stroma provides resistance to tumor cells as revealed by 3D heterospheroids compared to homospheroid model.

CONCLUSIONS

Our data demonstrate that tumor stroma acts as a strong barrier for nanoparticle penetration. Furthermore, the herein proposed 3D co-culture platform mimicking the tumor stroma, is ideally suited to systematically investigate different features of newly developed nanomedicines for their optimal design.

FLASH NANOPRECIPITATION ENABLES THE PRODUCTION OF NANOPARTICLE DRUG COCKTAILS AND MULTIVALENT TARGETED NANOPARTICLES FOR IMAGING AND DRUG DELIVERY

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We have developed a block-copolymer-directed, kinetically-controlled self-assembly process called Flash NanoPrecipitation (FNP) to produce 50-400 nm nanoparticles. The process involves controlling micromixing to effect supersaturations as high as 10,000 in 1.5 ms, and then controlling nucleation and growth rates to match block copolymer assembly rates. The rapid assembly enables the encapsulation of multiple drugs and imaging agents into the same nanoparticle, and the production of multivalent targeted nanoparticles. We will give examples of nanoparticle assembly and focus on targeting and imaging applications. Imaging using long wavelength fluorphores, MRI contrast agents, and SPEC agents will be presented. An example of TB treatment combining multiple drugs and targeting made in a single-step process shows the versatility of the FNP assembly. The FNP process enables the production of nanoparticle libraries with variable ligand density. Examples of small molecule (mannose for TB), folate ligand, and antibody targeting will be presented. For the mannose targeted nanoparticles we show a maximum ligand density for uptake into macrophages, and a reduce uptake beyond this maximum value. The ability to tune ligand density on the nanoparticle surface affords the possibility of create strong and specific binding using lower cost targeting constructs than in traditional antibody targeting. This has strong implications for the cost and effectiveness of nanoparticle constructs for targeted delivery. Finally, the block copolymer makes a dramatic difference in the circulation and clearance times *in vivo*. Even with the same 5K PEG steric stabilizing layer, the presentation on the nanoparticle surface can change clearance half times between 2 hours to 24 hours.

THE CORE OF SELF-ASSEMBLING PEPTIDE NANOFIBER BESIDE OF ITS BIOLOGICAL MOTIF WILL DEFINE OSTEOGENESIS; AN IN-VITRO AND IN-VIVO STUDY

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Bone is a real nanocompsite of nanofibers and nano ceramics and approximately 600,000 suffers from craniofacial deficits in US. RADA as a core of self-assembling peptides exhibits an acidic pH while the pH of KSL is higher than RADA. The acidic pH of RADA usually is an obstacle in tissue engineering but by regards to the acidophilic nature of bone, it was investigated for the first time. In the present investigation, for the first time the BMHP motif was bound to the RADA and KSL as a core of self-assembling peptide nanofiber and was evaluated its cell viability, ROS, NO and LDH release on MG-63 cell line as a cell line of bone osteosarcoma and then its effects was evaluated as a gene expression of apoptotic and integrin. Then, they were implanted in a critical size bone defect in rats for 2 month and densitometry of bone defects were analyzed and compared.
Results showed that KSL core due to higher cell viability, BCL2 gene over-expression and less intracellular ROS production was more effective than RADA ones in bone regeneration. However, KSL showed higher cell membrane damage and BAX gene over-expression than RADA. These data were in good agreement with X-ray radiographic data that disclosed higher bone density in KSL nanofiber than RADA. Based on the presented data since KSL induced higher nerve regeneration (not shown) and bone regeneration it is a good candidate for spine repair that its biodegradation will improve motor neuron recovery, as well.

Today, Spine and craniofacial disorders are a broad term that describes malformations of the face and skull that may be resulted from birth defect, disease or trauma and accidental injuries. In the US, approximately 600,000 individuals have been diagnosed with craniofacial deficits. There has been different ways of treatment such as surgical applications, bone cement, fillers, bone grafts and etc. However, defects typically have complex three-dimensional structural needs which are difficult to restore. Self-assembling peptide nanofibers are a newly type of hydrogel based scaffolds with promising application in spine and craniofacial disorders, however, they have poetical to encapsulate drugs as a sustain release drug nanocarrierthe. It is valuable to note that the acidic amino acids induce higher osteogenesis and also, the bone marrow homing peptide (BMHP) motif has been confirmed as an osteogenic motif (1). RADA as a core of self-assembling peptides exhibits an acidic pH while the pH of KSL is higher than RADA (2). The acidic pH of RADA usually is an obstacle in tissue engineering but by regards to the acidophilic nature of bone, it was investigated for the first time. In the present investigation, for the first time the BMHP motif was bound to the RADA and KSL as a core of self-assembling peptide nanofiber and was evaluated its cell viability, ROS, NO and LDH release on MG-63 cell line as a cell line of bone osteosarcoma and then its effects was evaluated as a gene expression of apoptotic and integrin. Then, they were implanted in a critical size bone defect in rats for 2 month and densitometry of bone defects were analyzed and compared.

MATERIAL AND METHODS

RADA-BMHP and KSL-BMHP were synthesized via solid phase synthesis method and then revers phase HPLC and GC-MAS was performed. Then, cell viability, cell membrane damage, NO and kinetic of intracellular ROS were investigated with MTT assay, LDH release, and Elisa reader. Then, to investigate BAX, BCL2 and integrin, real time PCR was performed. Calvaria defect model in rat weighting 220 g was made in male Wistar rats. The oligopeptides that are self-assembled in face to in-vivo environment and form hydrogel based scaffold with nanofiber structure less than 50 nm were implanted in two bilateral defect (n=4). Then, rats were scarified via CO2 asphyxiation after 2 months and X-ray was performed to investigate bone density and bone regeneration derived from nanofiber with two different of self-assembling peptide core.

RESULT AND DISCUSSION

Based on MTT assay and intracellular ROS kinetic, it might be said that KSL-BMHP exhibited higher cell viability and less intracellular ROS than RADA-BMHP while KSL-BMHP induced significantly higher cell membrane damage and NO production than RADA-BMHP. BAX/BCL2 data were in good agreement with LDH release and NO production and it seems that KSL ones over-expressed higher BAX as a apoptotic genes than RADA-BMHP and cell membrane damage eventually influence BAX genes while BCL2 was significantly over-expressed in KSL-BMHP than RADA ones and seems that the amount of ROS production and cell mitochondrial function affects on BCL2 gene. Besides, it seems that the nanofiber, RADA-BMHP, with less harmful effect on cell membrane significantly over-expressed higher integrin however, due to higher intracellular ROS and afterwards less BCL2 as an anti-apoptotic gene induces less cell viability in in-vitro and bone regeneration in in-vivo.

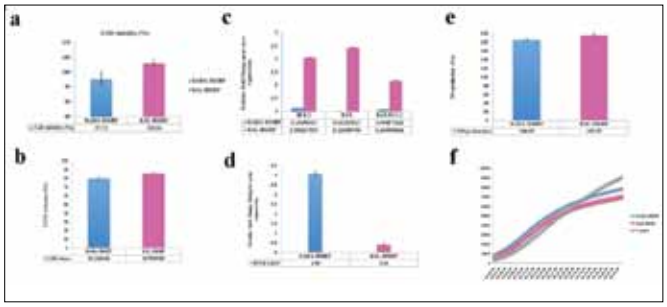

Fig. 1 a) MTT assay b) LDH release c) apoptotic genes d) integrin fold change gene expression e) NO production f) Intracellular ROS kinetic

Bone densitometry of critical sized defects in rats showed stronger bone regeneration in rats implanted with KSL-BMHP than RADA-BMHP after two months.
Our study reported here has far reaching implications beyond the current study. The simple addition of short, biologically active peptide motifs can significantly use for tissue repair, tissue engineering and regeneration medicine. In fact we are going to develop our *in vivo* researches on the bigger animals such as sheep.

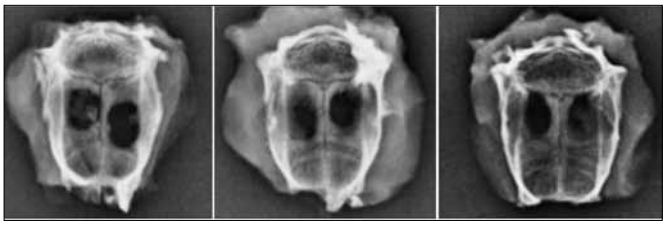

Fig. 2. X-ray radiographic photos from left to right, Control model, RADA-BMHP, KSL-BMHP

According to the X-ray radiographic photos they were determined the percentage of bone regeneration of RADA-BMHP, KSL-BMHP as a self-assembling peptide nanofibers and were compared to the control group. X ray results of the animal models which have been sacrificed two months post-implantation, it was disclosed that KSL with higher cell viability and BCL2 gene over-expression and higher amount of NO production and less intracellular ROS production conducted .57.5± 4.5 % bone density than RADA-BMHP with 45±2.2%. However, both of them were significantly higher than control group with 20% density.
Based on presented data it might be said that although the acidic amino acids same as glutamic acids has conductive role in bone regeneration but the biocompatibility of nanofiber has more effective roles and the amount of NO production increases bone regeneration, as well. It is worthy of value to be mentioned that not only the biological motif but also the core of self-assembling peptide nanofiber will determinative in bone regeneration.

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DISCOIDAL POLYMERIC NANOCONSTRUCTS: TUNING SIZE, SHAPE, SURFACE PROPERTIES AND MECHANICAL STIFFNESS

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Over the last decade, a variety of nano-sized drug delivery platforms – nanomedicines – have been realized with the primary objective of enhancing biodistribution, bioavailability, blood longevity and on-site controlled release of therapeutic and imaging agents as well as for protecting biological drugs from rapid degradation. However, drug loading efficiency, sequestration by phagocytic cells and poor tumor accumulation limit the therapeutic and imaging efficacy of blood-borne nanoparticles.[1]
Here, a novel fabrication strategy is presented for the realization of polymeric nanoconstructs with tunable size, shape, mechanical stiffness and surface properties – the 4S design parameters. The precise and independent control of these 4 features during the fabrication process allows us to generate theranostic nanoconstructs with unprecedented blood longevity and accumulation within the tortuous tumor neovasculature. These polymeric nanoconstructs are slightly smaller than blood platelets, discoidal in shape and deformable.[2]

MATERIALS AND METHODS:

Discoidal Polymeric Nanoconstructs are synthetized through a top-down fabrication approach involving five main steps schematically illustrated in Figure.[1]: a. realization of a silicon template resembling the geometrical properties of the nanoconstruct; b. PDMS replica molding of the silicon template; c. PVA replica molding of the PDMS template; d. deposition of a PLGA/PEG mixture in the PVA template; e. dissolution of the PVA template in aqueous solution and collection of nanoconstructs. DPN physico-chemical features are extensively characterized using different methods, namely electron, optical and atomic force microscopy as well as multisizer and dynamic light scattering analyses.

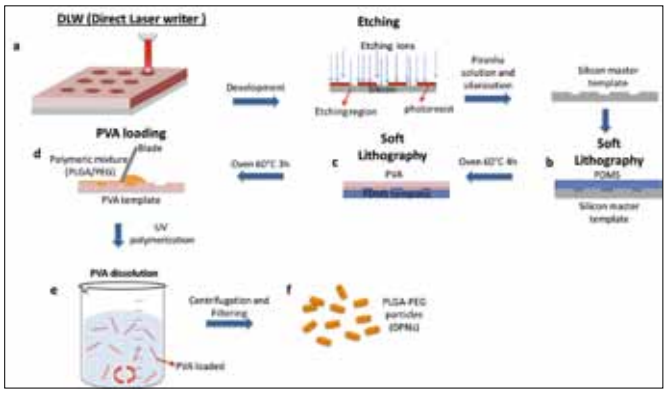

Figure 1: Fabrication steps for DPNs. a. realization of a silicon template; b. PDMS replication; c. PVA replication; d. loading of the PLGA/PEG mix. e. DPN collection.

Results and Discussion: Direct laser writing and electron beam lithography parameters have been optimized to realize silicon templates with various size and shape combinations. The optimization process focused on the template design; fine tuning of the defocusing and dosing parameters in laser writing and litography; and PDMS replica molding. Seven different configurations are currently available in the Laboratory of Nanotechnology for Precision Medicine presenting three different shapes (circular, elliptical and square) and a characteristic size ranging from 1.0 to 20.0 µm. Specifically, the Laboratory routinely fabricated 1.0 and 2.0 µm circular nanocostructs; 1.5×2.0 µm elliptical nanoconstructs; 1.0, 5.0, and 20.0 µm square nanoconstructs. Figure.[2] shows representative images for the smaller and systemically injectable DPNs.

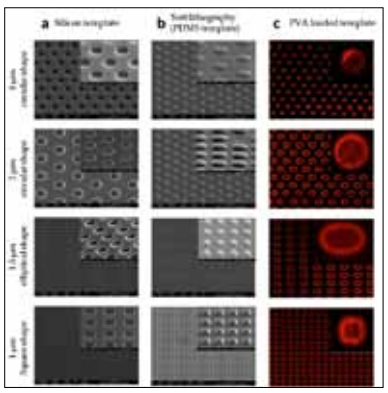

Figure 2. a. SEM images of the silicon templates. b. SEM images of the PDMS templates. c. Confocal microscopy images of the PLGA/PEG mix-loaded into PVA templates.

The resulting DPNs are made out of polymeric chains of poly(lactic-co-glycolic acid) (PLGA) and polypropylene glycol (PEG2000) entangled together.[2] In this study, four different DPN configurations are characterized for their physico-chemical properties, namely 1,000 × 400

nm circular DPNs, 2000 × 600 nm circular DPNs, 1500 × 2000 × 400 nm elliptical DPNs; and 1000 × 1000 × 600 nm square DPNs. Rgardless of the size and shape, the surface electrostatic charge is similar and equals about -30 mV. Figure.[2] displays also higher magnification confocal fluorescent images of individual DPNs. By changing the volume and ratio between PLGA and PEG, DPNs with different mechanical stiffness are realized. Three different configurations are here considered – soft (s-DPNs), rigid (r-DPNs); and rigid-rigid (rr-DPNs) – corresponding to Youngs' moduli of 100 kPa, 1 MPa and 10 MPs, respectively. Indentation analyses conducted with atomic force microscopy document the DPN Young's modulus in Figure.[3].

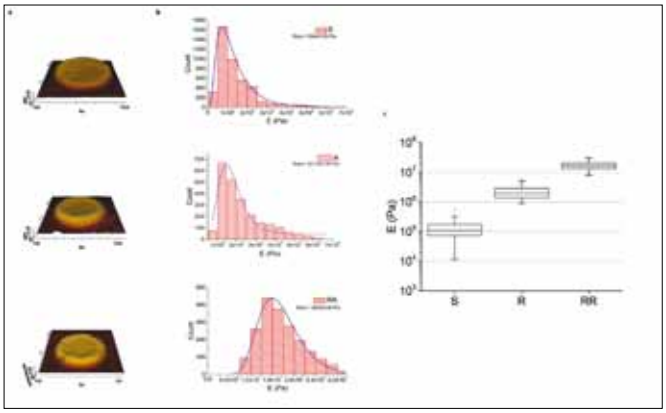

Figure 3. a. 3D AFM images from s-DPN, r-DPN and rr-DPN; b. Young's modulus distribution; c. Young's moduli for the three different DPN configurations.

CONCLUSIONS:

Over 20 different configurations of DPNs with varying size, shape and mechanical stiffness have been demonstrated. The flexible fabrication strategy has been described and optimized for increasing the production yielding as well as the absolute number of DPNs per template. Currently, discoidal polymeric nanoparticles for therapeutic studies on 10 mice can be fabricated in 1 single day. These different DPNs are currently being tested *in vitro* and *in vivo* to elucidate the mechanisms regulating particle biodistribution, blood longevity and therapeutic efficacy in cancer and cardiovascular diseases.

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LAYER-BY-LAYER ASSEMBLY OF HIERARCHICAL NANOARCHITECTURES TO ENHANCE THE SYSTEMIC PERFORMANCE OF NANOPARTICLE ALBUMIN-BOUND PACLITAXEL

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Although protein-bound paclitaxel, a Cremophor EL1-free formulation of 130nm PTX-bound albumin (PTX, Abraxane®) has been approved by United States Food and Drug Administration (USFDA), and established as a standard PTX-based therapy against multiple cancers, its clinical success is limited by unfavorable pharmacokinetics, suboptimal biodistribution, and acute toxicities. Layer-by-layer (LbL) nanoparticles (NPs) fabricated by the deposition of polycations and polyanions on the colloidal surface in an alternative manner are becoming increasingly important in cancer-targeting applications. LbL NPs offer some unique benefits, including controlled drug release, improved stability of the nanocarrier in circulation, increased storage stability, and robustness. In the present study, we aimed to apply the principles of a layer-by-layer (LbL) technique to improve the poor colloidal stability and pharmacokinetic pattern of nanoparticle albumin-bound paclitaxel (nab-PTX). LbL-based nab-PTX was successfully fabricated by the alternate deposition of polyarginine (pARG) and poly(ethylene glycol)-block-poly (L-aspartic acid) (PEGb-PLD) onto an albumin conjugate as seen in the Fig.1 a.

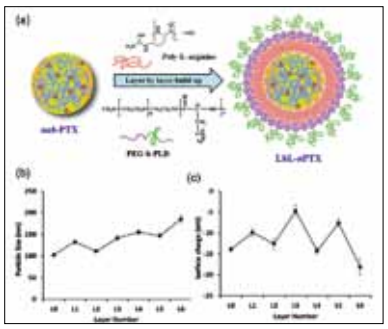

Fig. 1. (a) Schematic illustration of the layer-by-layer (LbL) assembly of polyarginine (pARG) and poly(ethylene glycol)-block-poly (L-aspartic acid) (PEG-b-PLD) onto nanoparticle albumin-bound paclitaxel (nab-PTX), (b) changes in mean particle size, and (c) changes in surface charge resulting from LbL-nPTX formation.

The excellent biocompatibility and antifouling properties of pARG and PEG-b-PLD prevented unnecessary adsorption of blood proteins and potentially prolonged blood circulation. Albumin allows sequential deposition of oppositely charged polyamino acids, attributable to its definite primary structure and high amino acid content. Adding the first layer of pARG increased the particle size from 100 nm to 140 nm, indicating the presence of complementary material on the surface of the albumin conjugate (Fig.1 b).

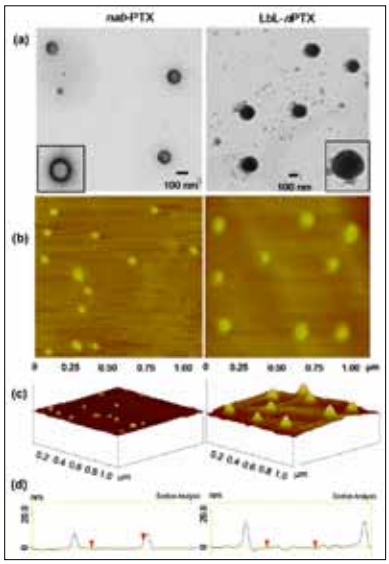

Fig. 2. Morphological analysis of nanoparticle albumin-bound paclitaxel (nab-PTX) and layer-by-layer (LbL)-nPTX. (a) Transmission electron microscope (TEM) images of nab-PTX and LbL-nPTX, (b) 2-D atomic force microscopy (AFM) nanoparticle images, (c) 3-D images, and (d) height profile analysis of nanoparticles.

Adding PEG-b-PLD, however, resulted in the formation of a thin layer surrounding the particles and decreased the overall particle size to 110 nm. This phenomenon is likely attributable to the electrostatic interaction of two polyamino acids that formed a dense material mesh on the particle surface. This trend continued with the addition of 6 layers until the final particle size was reached (180 nm), which remained in the optimal range. The presence of protective entanglement by polyamino acids prevented the dissociation of nab-PTX and improved its colloidal stability even at a 100-fold dilution. Several morphological characterizations like TEM and AFM was performed to check its polydispersity and morphology of the nanoparticle (Fig 2a and b). Also, the height profile analysis performed by AFM reveals the deposition of several layers around the nabPTX (Fig 2,c and d). The release data clearly reveal significant retardation of drug release upon coating the nab-PTX with polypeptide materials; a Higuchi type of diffusion-based controlled release pattern was predicted.

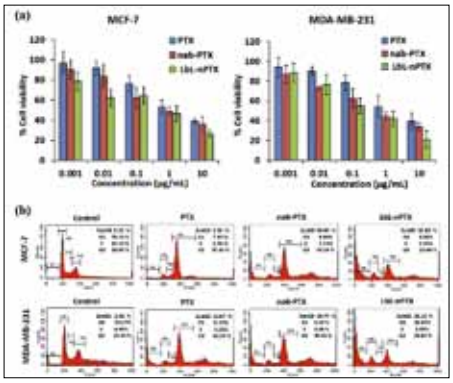

Fig. 3. (a) In vitro cytotoxicity potential and of free paclitaxel (PTX), nanoparticle albumin-bound (nab)-PTX, and layer-by-layer (LbL)-nPTX in MCF-7 and MDA-MB-231 breast cancer cells. (b) Effect of formulations on the cell cycle distributions of MCF-7 and MDA-MB-231 cells at 1 mg/mL concentrations and incubated for 24 h. Cell cycle phases were analyzed using FACS. G0/G1, S and G2/M show the cell cycle phase, and subG1 refers to the proportion of apoptotic cell.

The combined effect of high nanoparticle internalization and controlled release of PTX from LbL-nab-PTX increased its cytotoxicity in MCF-7 and MDA-MB-231 breast cancer cells. LbL-nab-PTX consistently induced apoptosis in approximately 52% and 22% of MCF-7 and MDA-MB-231 cancer cells, respectively (Fig 3). LbL assembly of polypeptides effectively prevented exposure of PTX to the systemic environment and thereby inhibited drug-induced hemolysis. Most importantly, LbL assembly of polypeptides to nab-PTX effectively increased the blood circulation potential of PTX and improved therapeutic efficacy via a significantly higher area under the curve (AUC). We report for the first time the application of LbL functional architectures for improving the systemic performance of nab-PTX with a view toward its clinical translation for cancer therapy.

ENGINEERING OF POLYPEPTIDE NANOVEHICLE CO-DELIVERY OF A SYNERGISTIC COMBINATION DOXORUBICIN AND QUERCETIN FOR EFFECTIVE CHEMOTHERAPY IN SOLID TUMORS

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In this study, we report a facile method to construct a bioactive and biodegradable polypeptide nanovehicles as an advanced platform technology for application in cancer therapy. (poly(phenylalanine)-b-poly(L-histidine)- b-poly(ethylene glycol) polypeptide nanoconstruct to co-load doxorubicin (DOX) and quercetin (QUR) (DQ-NV) (Fig. 1).

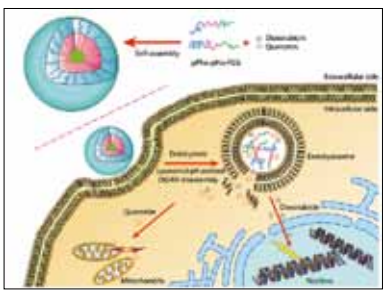

Fig. 1. Schematic representation of the fabrication of DQ-NV and the possible anticancer mechanism of action of DQ-NV.

The smart pH-sensitive nanovehicle was fabricated with precisely tailored drug-to-carrier ratio that resulted in accelerated, sequential drug release (Fig. 2C). The blank NV had a uniform, spherical shape with typical coreshell morphology (Fig. 2E). A closer look by TEM revealed a dark core, and a greyish outer shell corresponding to Phe, His, and PEG. The DQ-NV maintained the same morphology, although the overall size increased (Fig. 2F). AFM further showed a circular flat structure adhered to the mica surface.

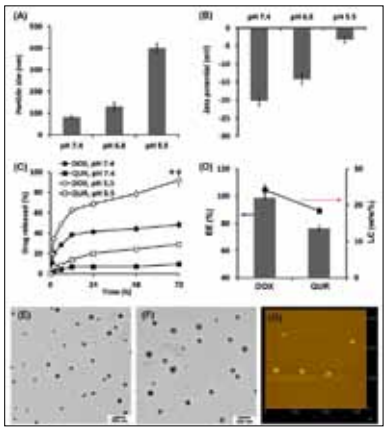

Fig. 2. Influence of pH on (A) hydrodynamic particle size and (B) zeta potential surface charge of DQ-NV. (C) pH-responsive release profile of DOX and QUR from DQ-NV. The released study was carried out in PBS (pH 7.4) and acetate-buffered saline (ABS, pH 5.0). (D) Encapsulation efficacy (EE) and loading capacity (EC) of DQ-NV. TEM images of (E) blank NV and (F) DQ-NV. (G) AFM images of DQ-NV.

As a result of ratiometric loading, QUR could significantly enhance the cytotoxic potential of DOX, induced marked cell apoptosis; change cell cycle patterns, inhibit the migratory capacity of sensitive and resistant cancer cells. In particular, pro-oxidant QUR from DQ-NV remarkably reduced the GSH/GSSG ratio, indicating high oxidative stress and damage to cellular components.

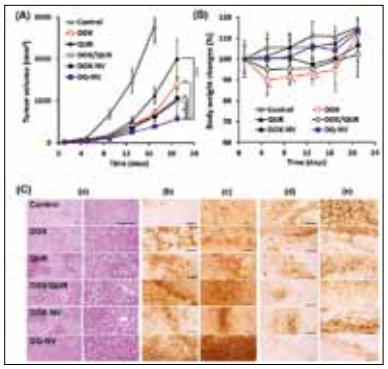

*Fig. 3. In vivo antitumor efficacy of DQ-NV. (A) Changes in tumor volume and (B) body weight in nude mice bearing SCC-7 xenografts after treatment with different formulations. The formulations were administered via the tail vein at a fixed dose of 5 mg/kg on days 1, 4, and 7. Data are presented as the mean ± S.D. (n = 7). (C) Histological and immunohistochemical analysis following different treatments. (a) H&E staining, (b) caspase-3, (c) PARP, (d) CD31, and (e) Ki67. *p < 0.05 and ***p < 0.01.*

DQ-NV induced tumor shrinkage more effectively than the single drugs in mice carrying subcutaneous SCC-7 xenografts. DQ-NV consistently induced high expression of caspase-3 and PARP and low expression of Ki67 and CD31 immunomarkers (Fig. 3). In summary, we demonstrate the development of a robust polypeptide-based intracellular nanovehicle for synergistic delivery of DOX/QUR in cancer chemotherapy.

MOLECULARLY TARGETED CO-DELIVERY OF A HISTONE DEACETYLASE INHIBITOR AND PACLITAXEL BY LIPID-PROTEIN HYBRID NANO-PARTICLES FOR SYNERGISTIC COMBINATIONAL CHEMOTHERAPY

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In this study, a transferrin-anchored albumin nanoplatform with PEGylated lipid bilayers (Tf-L-APVN) was developed for the targeted co-delivery of paclitaxel and vorinostat in solid tumors. We contemplated the design of lipid bilayer encapsulation of APVN, followed by the loading of second drug (VOR) and Tf conjugation (Fig. 1). The main objectives of the present study were to (a) improve the colloidal stability and systemic performance of albumin NP; (b) investigate the synergistic activity of combinational drugs; (c) contemporaneously target solid tumors with high specificity.

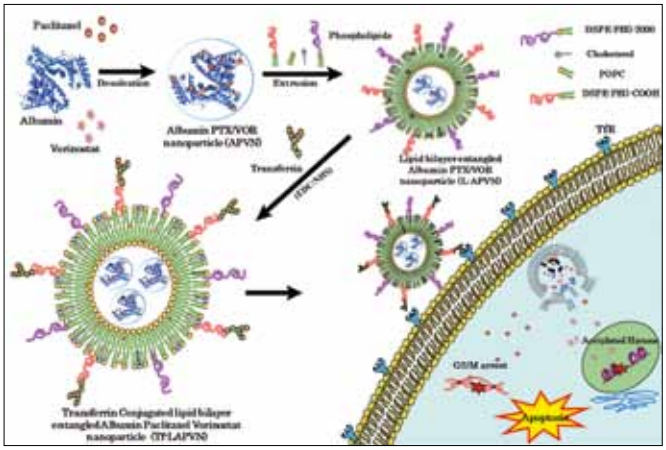
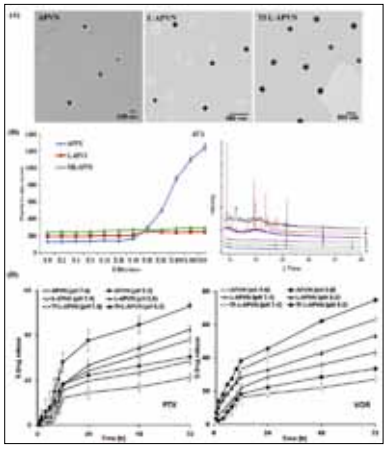


Fig. 1: Schematic illustration of preparation of ligand-directed albumin conjugate-supported lipid bilayer for combinational co-delivery of paclitaxel and vorinostat. The PTX/VOR (PV) loaded albumin conjugate (APVN) was prepared and supported with PEGylated lipid bilayer (L-APVN). The lipid bilayer-supported albumin nanocarrier was covalently conjugated with transferrin ligand (Tf-L-APVN) to design an actively targeted delivery vehicle.

TEM revealed the presence of distinct, discrete, and spherical particles, which are uniformly dispersed in the copper grid (Fig. 2A). Consistent with the DLS analysis, particles were nanosized and showed incremental addition upon Tf conjugation. The colloidal stability of Tf-L-APVN in systemic circulation is one of the foremost requirements for cancer targeting applications. The colloidal stability of nanoparticles was evaluated by DLS (Fig. 2B). As expected, particle size of APVNs immediately increased upon dilution by a factor of 20 due to the aggregation or disassembly of albumin carriers. In contrast, L-APVN and Tf-L-APVN maintained the same particle size even when diluted in phosphate-buffered saline (PBS) by a factor of 100, indicating their excellent colloidal stability. Tf-L-APVN exhibited a sequential and controlled release profile of paclitaxel and vorinostat, with an accelerated release pattern at acidic pH (Fig. 2D).

Figure 2: Physicochemical characterization of Tf-L-APVN. (A) TEM images of APVN, L-APVN, and Tf-L-APVN. (B) Colloidal stability of APVN, L-APVN, and Tf-L-APVN upon multi-fold dilutions with buffer. (C) X-ray diffraction patterns of free PTX (a), free VOR (b), BSA (c), APVN (e), L-APVN (f), and Tf-L-APVN (g). (D) In vitro release profile of PTX and VOR from APVN, L-APVN, and Tf-L-APVN in PBS and ABS. The release was carried out at 37°C and data are shown as mean ± SD (n = 3).



At cellular levels, Tf-L-APVN significantly enhanced the synergistic effects of paclitaxel and vorinostat on the proliferation of MCF-7, MDA-MB-231, and HepG2 cancer cells. Vorinostat could significantly enhance the cytotoxic potential of paclitaxel, induce marked cell apoptosis, alter cell cycle patterns, and inhibit the migratory capacity of cancer cells.

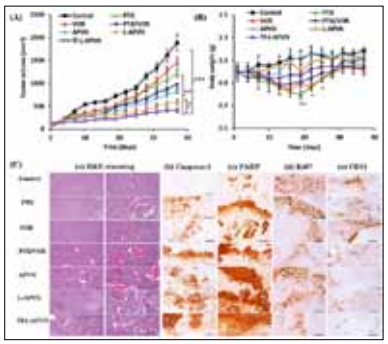


*Fig. 3: In vivo antitumor efficacy of combinational nanoparticles. (A) Changes in tumor volume and (B) body weight in nude mice bearing HepG2 xenografts after treatment with different formulations. The formulations were administered via the tail vein at a fixed dose of 5 mg/kg on days 1, 4, 7, and 10. Data are presented as the mean ± SE (n = 7). (C) Histological and immunohistochemical analysis following different treatments. (a) H&E staining, (b) caspase-3, (c) PARP, (d) Ki67, and (e) CD31. Caspase-3 and PARP, markers for apoptosis; CD-31 and Ki-67, markers for angiogenesis; scale bars = 120 µm. *p < 0.05, **p < 0.001, ***p < 0.0001.*

In addition, Tf-L-APVN showed prolonged circulation in the blood and maintained an effective ratio of 1:1 (for paclitaxel and vorinostat) throughout the study period. In HepG2 tumor-bearing mice, Tf-L-APVN displayed excellent antitumor efficacy and the combination of paclitaxel and vorinostat significantly inhibited the tumor growth (Fig 3). Taken together, dual drug loaded Tf receptor-targeted nanomedicine holds great potential in chemotherapy of solid tumors.

A NOVEL INTEGRIN-ALPHA11 TARGETING PEPTIDE TO TARGET THERAPEUTIC OLIGONUCLEOTIDES TO PANCREATIC CANCER

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The development of pancreatic ductal adenocarcinoma (PDAC) is promoted by its highly abundant tumor stroma. [1]. A major compo-

nent of tumor stroma, stromal myofibroblasts or cancer-associated fibroblasts (CAFs) were previously shown to support PDAC progression by enhancing tumor cell growth, invasion and metastasis. The collagen binding transmembrane receptor integrin-alpha11-beta1 (ITGA11) is known to be overexpressed by cancer-associated fibroblasts (CAFs), making it a potential target in pancreatic cancer. Therapeutic oligonucleotides, e.g. microRNAs can show promise to be applied as therapeutics to diminish the pro-tumorigenic effects of the pancreatic tumor stroma when encapsulated into targeted nanoparticles [1].

RESULTS AND DISCUSSION

In this study we have for the first time stained ITGA11 in human PDAC specimens. We found that ITGA11 was highly expressed in the stromal myofibroblasts of PDAC patients, as shown by co-localization with the CAF marker alpha smooth muscle actin (α-SMA). Interestingly, there was no expression in healthy human pancreas and various other tissues from human organs, making ITGA11 a great target for the delivery of nanomedicines to PDAC. To enable active targeting of ITGA11 we have selected a novel peptide (AXI), specifically binding to ITGA11, using phage display. Using microscale thermophoresis, we found that AXI was binding to human recombinant ITGA11 with a binding constant (Kd) of 1.8 +/- 0.3 uM while there was no binding to human recombinant Integrin-alpha4-beta1. This proves specificity of the peptide to the alpha11 subunit of ITGA11.

We used quiescent pancreatic stellate cells (PSCs) and TGF-β activated PSCs, which present a CAF-like phenotype, for quantitative cellular binding studies. ITGA11 protein levels are induced in PSCs upon treatment with TGF-β (Figure 1 A). Binding studies performed with flow cytometry showed a significant relation in AXI-FITC binding (Figure 1A) on PSCs and the protein expression of ITGA11 (Figure 1 A, B). Additionally, the binding of fluorescently-labeled AXI to PSCs could be blocked using excess of AXI, demonstrating the specific binding to ITGA11 on PSCs (Figure 1 C).

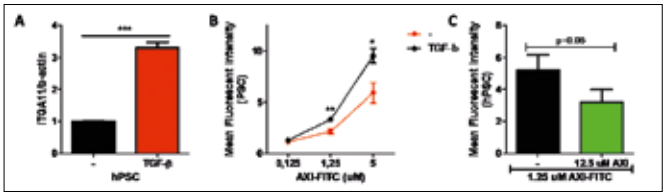

Figure 1.

To evaluate the ability of AXI to be used in nanomedical applications for oligonucleotide delivery, we conjugated AXI to microRNA loaded PLGA-Peg nanoparticles. The resulting PLGA-Peg-AXI nanoparticles had a size of 180.5 +/- 4.2 nm and a charge of -8.5 mV. Cellular binding studies on PSCs showed that non-modified PLGA-Peg nanoparticles were not binding to PSCs, while binding was increased for PLGA-Peg-AXI nanoparticles. As for the binding of free AXI, binding of PLGA-Peg-AXI nanoparticles to PSCs increased, when PSC ITGA11 levels were induced with TGF-β.

CONCLUSION

This study presents ITGA11 as a specific target in the tumor stroma of pancreatic cancer. We have developed a novel ITGA11 specific targeting peptide (AXI) with the potential to target nanoparticles loaded with therapeutic oligonucleotides to the tumor stroma of pancreatic cancer.

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ABUNDANCE AND ORIENTATION OF MYELIN SHEATHS IN PARTS OF HUMAN BRAINS

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For human brain tissues histology is so far the gold standard for the differentiation of the structures on the sub-cellular level. Using small-angle X-ray scattering (SAXS), which is a reciprocal space technique with an inverse relationship between the size of the inspected particles and scattering angle, nanostructures within the human brain (e.g. myelin with a periodicity of 16.46 nm) can be detected [1]. But it is impossible to relate the results to established histology because of the lack of localization. Combination of SAXS with a spatial resolution of a few micrometers in real space (scanning SAXS at cSAXS beamline, SLS, PSI, Switzerland [2]) provides information on the abundance and orientation of the nanostructures present [3]. The result is a more detailed understanding of the nanoanatomy of human brain tissue.

Keywords: small-angle X-ray scattering, nanostructures in human brain, myelin, orientation

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WAY TOWARDS NANOMETRE-THIN POLYMER FILMS FABRICATED BY ELECTROSPRAY DEPOSITION

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Polydimethylsiloxane (PDMS) as FDA approved polymer is widely used for medical implants because of its biocompatibility and chemical stability. Electrospray deposition (ESD) is a fast and well-controlled technique to fabricate nanometre-thin PDMS membranes [1]. The thickness and morphology of the PDMS films produced by ESD can be easily adapted for the corresponding medical application by changing the spray parameters, such as PDMS concentration in the sprayed solution and its flow rate [2]. In sum, ESD illustrates a cost-effective method and is predestinated to realize multilayer dielectric nanostructures as intended for e.g. artificial muscles and skin.

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CANCER CELL GROWTH INHIBITION BY MAGNETIC NANOPARTICLES EXPOSED TO STATIC MAGNETIC FIELDS

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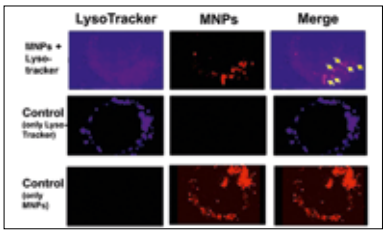

Figure 1. Co-localization of endocytosed MNPs with lysosomes.

Magnetic nanoparticles, as a versatile platform, enable new interdisciplinary approaches in experimental design in basic and translational research such as the development and application of multimodal therapy strategies against cancer. In the field of cancer research, several studies suggest that inhibition of tumor growth in experimental tumor models can be achieved also by mechanical effects. It is becoming evident that mechanical stimulation is an effective biological tool, based on which, we could develop novel approaches and even therapeutic biomedical technologies. Since the scale of biological systems is inherently nanometric, the mechanical cues to which cells are being exposed can be generated by nano-tools, such as nanoparticles. Magnetic nanoparticles (MNPs), which are stimuli-responsive, allow for highly controllable application of magneto-mechanical forces when being exposed to magnetic fields. Internalized MNPs, can apply intracellular forces that could cause growth inhibitory effects in cancer cells and thus, open the way for innovative cancer treatment.

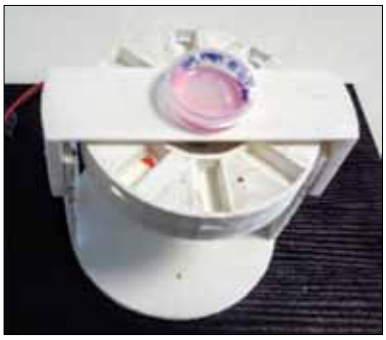

Figure 3. 3D printout polymer holder for the subjection of cells to magnetic fields.

Herein we examined the effect of magneto-mechanical stress induced by internalized single-domain magnetic nanoparticles subjected to static magnetic fields of increasing strength (40 mT-200 mT), on the proliferation rate of the human colon cancer cell line HT29. We confirmed the endocytosis of MNPs by confocal fluorescence microscopy. A 3D printout of a polymer holder was manufactured, for keeping the exposure parameters consistent between experiments. We calculated the applied force per cell by extrapolating from the estimated maximum magnetic force on a single nanoparticle. ICP-OES was used to quantify the total number of internalized nanoparticles per cell.

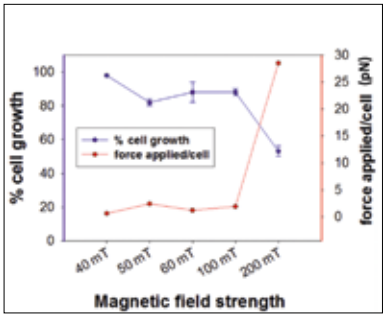

Figure 3. HT29 cell growth dependence on forces induced by different setups of static external fields on endocytosed MNPs.

We observed a force-dependent cell growth inhibition in MNPs-treated cells by SRB analysis. Moreover, magnetic field application, attenuated formation and growth of 3D tumor spheroids formed with the hanging drop method. Interestingly, as revealed by flow cytometry and fluorescence microscopy, MNPs co-localized with lysosomes and the magnetic field induced lysosomal damage in MNPs-treated cells. We provide evidence that we can externally control movement of internalized MNPs inside living cells by generating magnetic field of specific characteristics, and that the generated magnetic fields and magneto-mechanical stress affect cell growth. Further research on the underlying molecular mechanisms that are being triggered, extended on different cell types as well as nanoparticles of different sizes and multiple magnetic fields, could reveal the relevant parameters that influence the growth inhibitory effect of static magnetic field exposure on MNPs-treated cancer cells. This is of utmost importance for developing this approach towards a new cancer treatment modality.

DISPERSION OF NANOPARTICLES IN DIFFERENT MEDIA IMPORTANTLY DETERMINES THE COMPOSITION OF THEIR PROTEIN CORONA

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Despite numerous advances of nanotechnology in the area of biomedicine in recent years, the technology itself did not completely fulfil high expectations in the field of medical applications [1]. Although early NPs-based formulations like Doxil® were very successful [2], some of the later nanomedicine products were not so successful and others were even retracted from the market (e.g. Endorem® and Sinerem®) [3]. Several studies showed that problems with reproducibility and scaling up are sometimes tightly connected with insufficient characterization of the nanomedicine formulations. To understand the effects of nanomedicines *in vitro* or *in vivo*, we need to consider several physico-chemical parameters, such as size, size distribution, surface area, charge and surface chemistry which forms synthetic identity [4]. The second level of characterization is performed when nanomedicines interact with biological systems [5]. This biological identity is defined also by adsorption of biomolecules, mainly proteins, to the surface of nanoparticles (NPs) and it is generally referred to as their protein corona [6–10]. Interaction between NPs and biological environment such as tissue and cells is importantly mediated also by this outmost layer of NPs, one of the frequently overlooked factors in the nanomedicine.

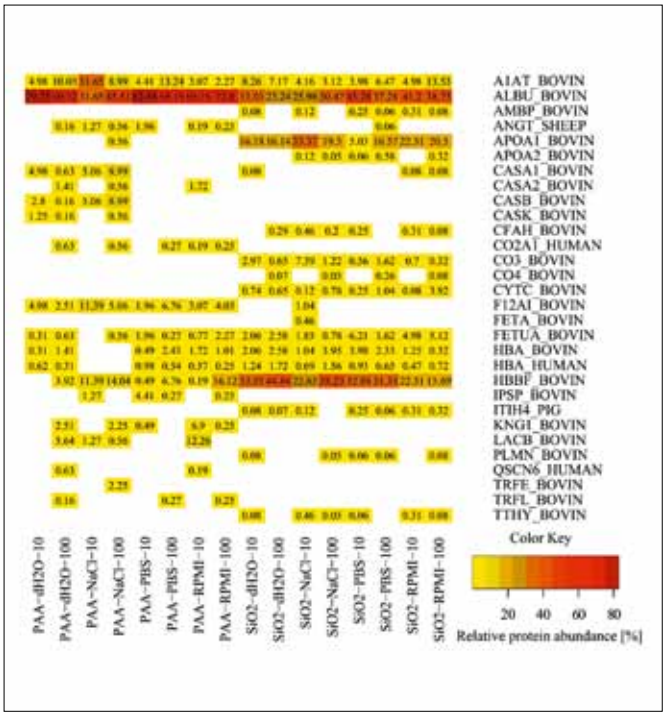
The main question addressed in the present work was, whether different "principal" dispersion media used for preparation of NPs affects the resulting protein corona composition or not. Even though several studies demonstrated the importance of the "secondary" medium [e.g. growth medium, serum] the effect of primary medium (e.g. water, physiological saline) was not analysed yet. To answer this question, we have chosen two examples of nanoparticles: i) physiologically stable polyacrylic acid (PAA) coated cobalt ferrite NPs (PAA NPs; 55 nm, -59 mV) developed in our group for further use in biotechnology and biomedicine [11] and ii) commercially available silica NPs (22 nm), used in cleaning products for everyday use. Since there is a multitude of different nanoparticle systems and since the interaction with the dispersion media is very much dependent also on the properties of the NPs itself, we have selected these two NPs suspensions as an example of the potential media effects on protein corona composition.

Dispersions of NPs were prepared in four different biologically relevant media: Dulbecco`s phosphate buffered saline with $CaCl_2$ and

$MgCl_2$ (PBS), 0.9% (m/v) NaCl, ATCC modified RPMI-1640 cell culture media (RPMI) and distilled water (dH2O). Protein corona was formed in low or high fetal bovine serum (FBS). Our results demonstrated that the medium in which NPs were initially dispersed had significantly affected NPs protein corona composition and could have an important implication on potential biological effects of NPs. Moreover, there was a clear difference in protein corona composition between dispersion of PAA NPs and silica NPs in complex media where macromolecular corona was formed (e.g. RPMI) compared to media without macromolecules (e.g. dH2O).

NPs were dispersed in four different media (Table 1) and subsequently incubated in fetal bovine serum. Followed by centrifugation and washing steps, proteins of hard protein corona were detached from NPs, separated on SDS-PAGE and identified by mass spectrometry analysis. Relative abundance of individual proteins found in protein coronas is shown in Figure 1.

Figure 1: Relative abundance of proteins identified in individual NP samples. PAA and silica NPs were dispersed in different media and incubated for 1h in 10% or 100% FBS. Proteins were separated from NPs, analysed on SDS-PAGE and identified by MS. Spectral counts were used as a measure of individual protein in a sample. White space indicates the absence of a protein in a sample. Protein accessions are ordered alphabetically. Accessions are further explained in Table 1. NPs formulations are coded as: type of NPs—dispersion media—% of FBS (e.g. PAA—NaCl—100 designates PAA NPs prepared in NaCl and incubated in 100% FBS).



Dispersion media	pH	Ionic strength [mM]	Osmotic concentration [mOsm/L]	Conductivity [mS/cm]
dH2O	7.0 ± 0.4	0.0	0.0	0.1
PBS	7.3 ± 0.3	166.0	287-309	15.6
NaCl	7.0 ± 1.1	154.0	308	15.1
RPMI	7.2 ± 0.2	NA	246-306	12.5

Table 1: Characteristics of the media used to disperse PAA NPs and silica NPs

Additionally, effects of PAA NPs and silica NPs on viability of differentiated THP-1 cells were studied. Only minor cytotoxicity was noticed at very high NP-concentrations. Moreover, the effects of protein corona composition on cytokine production (ELISA; IL-6, TNF-α) were determined. Again, only minor effects were noticed.

To conclude, in this study we analysed different factors that affect the composition of the protein corona of NPs. We demonstrated that the type of the dispersion media in addition to the selected NPs type very importantly determines binding of proteins to NPs surface. The type of the dispersion media also importantly dictated the relative abundancies of the individual proteins in NPs corona: patterns were similar for NPs dispersed in dH2O and NaCl or for NPs dispersed in PBS and RPMI. It is important to note that the protein corona of silica NPs contained three complement system-related proteins: complement factor H, complement C3 and complement C4 [12]. Although abundancies of those proteins were sometimes relatively small, they could play an important role in the context of immune response. We believe that dispersion media is an important factor to consider in further studies of the protein corona and may also be acknowledged in retrospective for the studies already performed.

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CHARACTERIZATION OF NANOPARTICLES IN AQUEOUS MEDIUM BY USE OF ATOMIC FORCE MICROSCOP

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Size control of nanotechnology-based drug products is crucial for the successful development since their *in vivo* properties such as pharmacokinetics behavior, or bioavailability are size-dependent.[1] Several techniques are currently used to measure the size of nanoparticles; however, interpretation of the obtained results is challenging.[2,3]

Here, we evaluate atomic force microscopy (AFM) for size measurement of nanoparticles in aqueous medium (Figure 1); 120-nm sized polystyrene latex (PSL) nanoparticles (certified reference material 5701-a, the National Metrology Institute of Japan) and liposomes are used as test samples. Liposomes were prepared by modified extrusion method.[4]

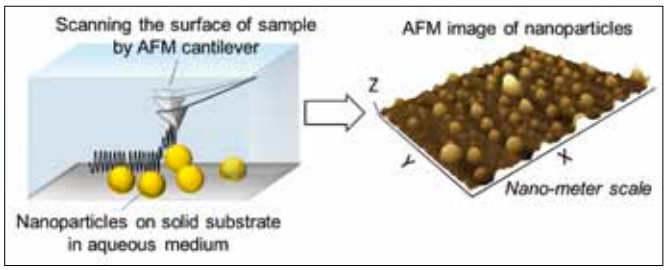

Figure 1. Schematic view of AFM imaging for nanoparticles in aqueous medium.

Dynamic light scattering (DLS) to characterize the hydrodynamic diameters of nanoparticles was performed at 25 °C by using a Zetasizer Nano-ZS instrument equipped with Zeta Sizer Software v.6.01 (Malvern Instrument, UK). DLS provides intensity-weighted diameter (ID), which is calculated based on light scattering intensity autocorrelation function of particles.[4] Number-weighted diameter (ND) was also obtained from the ID data using the Zeta Sizer Software. Before the AFM measurements, the OLYMPUS cantilever (BL-AC40TS-C2; nominal spring constant 90 pN/nm) was calibrated by thermal fluctuation method. AFM imaging of nanoparticles on a Neo glass cover glass (Matsunami Glass Ind., Japan) in aqueous medium was carried out at 26 ± 1 °C by using a JPK Nanowizard Ultra Speed microscopy with JPK Data Processing Software v.6.0 (JPK Instruments AG, Berlin, Germany). In the case of liposomes, the glass substrate was coated with bovine serum albumin.[5] AFM images (256 × 256 pixels per image) were recorded in QI mode. Area-equivalent diameter (AD) obtained from AFM image, which is beneficial for size measurement of nanoparticles, 6 was adopted.

Figure 2 shows analysis of AD of PSL nanoparticles in an AFM image. As a result, AD of PSL nanoparticles was similar to the ID obtained by DLS (120 ± 29 nm vs 119 ± 24 nm). In the case of liposomes, however, the AFM analysis exhibited lower AD than ID (118 ± 45 nm vs 45 ± 12 nm). We found that the AD of the liposomes by AFM is close to the number-weighted diameter, ND obtained by DLS (64 ± 21 nm). There is a possibility that larger liposomes are less likely adsorb on to the AFM substrate.

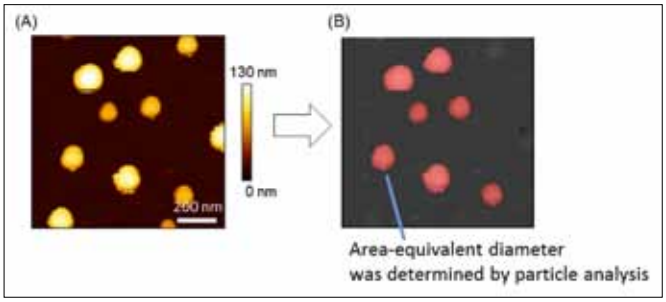

Figure 2. Size measurement of PSL nanoparticles by AFM. (A) AFM image of PSL nanoparticles in aqueous solution. Scale bar in lower right, 200 nm. The z-scale, 130 nm. (B) Extraction of nanoparticles above the threshold based on the height of pixel data in (A). The area-equivalent diameter of nanoparticles was calculated from the area of pixels.

In summary, we could successfully obtain the AFM images of PSL and liposomes in aqueous medium and demonstrated that AFM and DLS provided similar results of PSL nanoparticle sizes. However, the liposome sizes obtained by AFM were smaller than those by DLS. Further researches are required to study the characteristics of these measurement techniques.

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THE SIZE OF NANOPARTICLES IMPROVES NEUROGENESIS AND INHIBITS ASTEROGLIOSIS

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Spinal cord injury (SCI) in humans stayed a ruining and healless disorder. At the first line of recovery, the most important factors in motor neuron recovery is decrease of inflammation and astrogeliosis and then neurogenesis improvement. There is no reports to investigate the effect of particle size on neurogenesis so it was evaluated in the present investigation. Encapsulated drug nanocarriers at the same concentration of oil, surfactant and co-surfactant were synthesised by stirrer and sonication at the size of 60±1 nm and 190±10 nm. Then pH, cell viability, cell membrane damage, NO and ROS production was investigated by pH meter, MTT assay, PI flow-cytometry, LDH release, Elisa and flow-cytometry respectively. Then, its neurognesis potential was investigated by real time PCR. To evaluate its efficacy in motor neuron recovery, FTY nano carriers injected in site in acute model of SCI in rats. MTT and PI results showed that smaller NPs at two concentrations of ethanol increased cell viability while increase of ethanol decreases NO production and relative fold change nNOS genes expression and the larger NPs increased the NO production and nNOS gene at low ethanol concentration. ROS kinetic measurements showed that increase of ethanol and decrease of particle size induced higher interacellualr ROS production. Interestingly, the data related to nNOS gene expression was in good agreement with Tuj-1, NF and MAP2 genes expression. GFAP as a marker of reactive astrocyte increased by the particles size while MAP2 as a marker of mature neurons was over-expressed in small nanocarriers and interestingly ethanol got worsen both of them in favour of motor neuron recovery in in-vivo. It means that the ethanol will increases astrogenesis and decreases neurogenesis.

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. Fingolimod (FTY) has been FDA approved in MS patients due to decrease of inflammation and neurogenesis improvement in patients [(1)]. There are some reports on the motor neuron recovery of drug nanocarriers [(2)]. Beside of FTY, ethanol at low concentration has neuroprotective effect but there was no findings to investigate its effect at the concentrations of 1 and 3 percent. So, in the presented study, the drug was encapsulated into the nanocarriers at two different particle sizes and the effect of particle size and ethanol concentration was investigated in-vitro and in-vivo.

MATERIAL AND METHODS

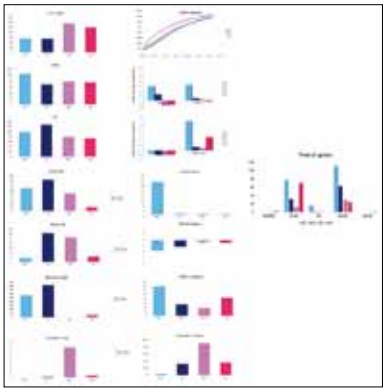
Nanoemulsions of FTY were synthesized using stirrer and ultrasound at equal size. Then, to investigate the cell viability, cell membrane damage, NO production, dead cells, intracellular ROS flow-cytometry and kinetic, and the effect of nanoparticle size on cell cycle, MTT assay, LDH release, NO production, PI and DCFA flow-cytometry were performed on BE2M17 cell line. Then, cells treated with nanocarries were differentiated to mature neural cells for 4 days. Real-time PCR and NO production were performed to investigate the fold change gene expression of Nestin, Tuj-1, NF, MAP2, GFAP, GDNF. nNOS, BAX and BCL2 gene over-expression were done to investigate the apoptotic and cytotoxic genes. Also, integrin 5 and focal adhesion kinase (PTK2) were investigated as genes involve in signal transduction. Then, to investigate its influence in in-vivo, an acute model of SCI was created and the nano-

carriers implanted in them and follow up for 35 days as a weigh and BBB score.

RESULTS AND DISCUSSION

Encapsulated drug nanocarriers at the same concentration of oil, surfactant and co-surfactant were synthesised by stirrer (S1, S3) and sonication (H1 and H3) at the size of 60±1 (nm and 190±10 nm. The number of 1 and 3 shows the percentage of ethanol in nano-formulations.

MTT and PI results showed that smaller NPs at two concentrations of ethanol increased cell viability while increase of ethanol decreases NO production and relative fold change nNOS genes expression and the larger NPs increased the NO production and nNOS gene at low ethanol concentration. ROS kinetic measurements showed that increase of ethanol and decrease of particle size induced higher interacellualr ROS production. Based on MTT and ROS data it might be said that ROS did not have influence on cell viability while NO production was more effective on cell mortality.



The profile of BAX was in good agreement with nNOS production after 24 h while they were not in good agreement during differentiation. The relative fold change Bcl2 gene expression was in concomitant of Tuj-1 in which disclosed that H1 and S3 induced higher Tuj-1 and Bcl2 genes expression. However, the H1 induced higher neurogenesis thatn others. Interestingly the gene expression of MAP2 and NF versus of GFAP were higher in small nanocarriers than larger ones at both less and high concentrations. It showed that size of nanoparticles have directly influence on neurogenesis and astrogenesis. Interestingly the NPs with less cell membrane damage (LDH release) resulted in increase of integrin and then transmitted from the cell membrane protein (integrin) into the cells and directly influence FAK2. Afterwards, the amount of focal adhesion kinas (FAK2) gene expression affect on the beta tubulin III gene expression and cells with more signal transduction using FAK2 transmitted the signals to the nuclous to encourage them to over-express higher Tuj-1. It seems that the NPs with higher inductive effect on transmembrane cell adhesion protein that activate intracellular signaling pathways induces higher FAK2 and then Tuj-1 over-expression. In conclusion it might be said that the size of NPs influence neurogenesis and astrogenesis.

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SIGNAL TRANSDUCTION INFLUENCE BY THE SIZE OF NPS IN A NANO DRUG FORMULATION

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Todays, nano-pharmaceutics as a major branch of nanomedicine has developed and there are some nanoformulations in market with less side effect and high efficacy and bioavailability in patients. Although, the basic investigation in this field has critical importance to design of safer nanoformulation but there is no reports that investigate in parallel the effect of ethanol concentrations as a major co-surfactant in nanoemulsions and some other type of nanoformulations along with the particle size. So, for the first time, NPs with equal and different particle size at same and different concentrations of ethanol were prepared and their influence on cell behavior and inflammatory and apoptosis genes were investigated. The results disclosed that the effect of ethanol on nanoformulations with equal size and larger is significant and based on the cell therapy as a tissue engineering or drug delivery to the cancers, the particle size and ethanol concentration must be optimized and will be different.

INTRODUCTION

Todays, scientists are going to develop sustain release nanocarriers for the pharmaceutical industries to decrease the side effects, dosage and increase bioavailability and hydrophilicity of conventional drugs. Nanoemulsion is one of the most famous form of nanocarriers as a sustain release drug. Although, these dosage form are frequently prepared and investigated by researchers but there are some drawbacks and gaps for their studying. One of the main concerns in designing of nanoemulsions is the concentrations of co-surfactant and the optimal size of nanoparticles that influence cell behavior. Despite of the efforts to use more biocompatible surfactant and co-surfactant in nanaoemulsion preparation but this concern has remained till now and there is no report that in one time investigate the effect of co-surfactant and particles size of a nano-formulation in cell viability, cell membrane damage, ROS and NO production, cell cycle phases, apoptosis and inflammatory genes and at the final its effects on integrin over-expression as a transmembrane cell adhesion protein that activate intracellular signaling pathways. Since one the nanoemulsion preparation method is using of temperature, so in the present investigation, it was compared to the non-temperature method via L929 cell line as a standard ISO (10993) cell line for nanotoxicology [1].

MATERIAL AND METHODS

nano-curcumin formulations have been prepared at different concentrations of co-surfactant (Ethanol) 0, 3 and 5 % and also in low (25 º C) and high (60 º C) temperature. Then, thermodynamic and chemical stabilities, pH, viscosity, entrapment efficacy and particle size of NPs were studied by centrifugation, thin layer chromatography, pH meter, viscometer, ultrafiltration and dynamic light scattering, respectively. To investigate the cytotoxic effects of NPs in variable particle size and ethanol concentration, MTT assay, LDH release, NO and ROS production, NP up-take, PI staining, cell cycle phase analysis were performed. To assesses the effect of theses variables on gene expression of BAX, BCL and NFKB, iNOS real-time PCR was performed. Besides, to investigate the influence of nano particle' s size on triggering of transmembrane cell adhesion proteins as a cell signaling activator, integrin gene expression was performed and then, its influence on focal adhesion kinas 2 gene along with cell migration assay were studied.

RESULTS AND DISCUSSION

The NPs with no ethanol were nominated 3.10 and at the ethanol concentrations of 3 and 5 % were nominated 3.10.3 and 3.10.5 and if the temperature was applied they were nominated 3.10T, 3.10.3T and 3.10.5T. the particle size of 3.10 and 3.10.3 were same and had an mid diameter of 56.8 nm, however temperature applying resulted in larger particle size in both group, 78.9 and 77.7 nm, respectively. Increase of ethanol concentration from 3 to 5 % decreased particle size in non-temperature and temperature formulations to 47.9 and 70.7 nm, respectively. Thermodynamic stability showed no sign of biphasic and physical appearance in the formulations and TLC disclosed that all the nano-formulations had equal Rf of 0.14. Based on the mentioned results it might be said that temperature, surfactant, co-surfactant and the preparation method not damage to the curcumin as a drug substitute. However, the temperature had significantly decreased drug entrapment efficacy in 3.10T for-

mulation and non-significant in other groups. Increase of ethanol in temperature formulations unlike the non-temperature ones had gradually non-significantly increased the EE%. The drug release profile of non-temperature formulations showed it is dependent to the particle size than co-surfactant concentration. However, the particle size its self influenced by the ethanol concentration. Based on the presented data, it might be said that in non-temperature formulations, the smaller particle size released higher drug volume as compared to the larger ones.

Based on MTT assay (Fig. 2a) and PI flow-cytometery data (Fig. 2b), the increase of particle size in temperature formulations as compared to the non-temperature ones had significantly decreased cell viability. However, the increase of ethanol in nano-formulations with the same size (56.8 nm) and (77.7-78.9 nm) formulations resulted in less cell viability while in higher ethanol concentration with larger particle size (70.7 nm), cell viability was higher than all groups. It seems that particles size has been dominant effects than ethanol and has increased cell viability than larger particle with less ethanol. However, 10.3.5T has induced higher cell viability as compared to smaller ones with less and equal concentration. Unlike the MTT assay and PI flow cytometery data, the BAX/BCL2 ratio increased in small particles as compared to the larger ones (Fig.2c). Based on LDH release data, the increase of particle size in poor ethanol formulations induced higher cell membrane damage while addition of ethanol to the nano-formulations induced higher cell membrane damage with smaller NPs as compared to the larger ones (Fig. 2a). These data were in good agreement with BCL2 gene expression in which small particle with poor of ethanol over-expressed higher BCL2 genes as compared to the larger ones while when ethanol was added to the formulations, the larger ones induced BCL2 over-expression as an anti-apoptotic gene.

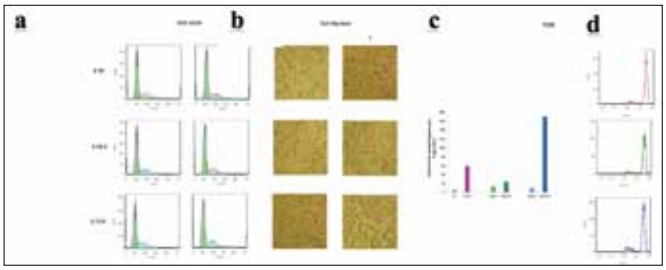

Fig. 1a) cell cycle phases by nanoemulsions, b) cell up-take, c) fold change integrin gene expression, d) ROS flowcytomety of nanoformulations.

Gene expression results showed that NFκB over-expression as an inflammatory gene is in good agreement with BAX over-expression. Small NPs induce higher expression of apoptosis and inflammatory genes of BAX and NFκB while addition of ethanol at the concentration of 3 % decreases expression of BAX and NFκB and increase of ethanol along with decrease of particle size will increase apoptosis and inflammatory genes (Fig. 2d). So, we were going to curious that the provocation of apoptosis and inflammatory genes is related to the ROS and NO production derived from NPs or not (Fig. 1c and 2a). Based on the NO production data, it might be said that it had been increased by the particle size increment and ethanol concentration decrement. It is valuable to mention that NO concentration was significantly higher in control group than larger ones. Larger NPs in poor ethanol formulation over-expressed higher iNOS genes as compared to the smaller ones while when ethanol was added to the formulations, the smaller ones over-expressed higher iNOS gene. In fact ethanol in both large and small NPs induces lower iNOS gene expression and was in good agreement with NO production. iNOS data was in good agreement with BCL2 gene expression. ROS flowcytometery showed that increase of ethanol and particle size decreases ROS production by cells. These data was in good agreement with the expression of BAX and NFκB. So, small NPs induced higher ROS production. However, the ROS production by 3.10.5 and 5T were in parallel by BAX/BCL2, as well. Besides, it seems that the increase of ROS production might be due to higher potential of cell up-take in 3.10.5T that it has been disclosed with flowcytometery and microscopic imaging. It has been demonstrated that increase of G2/M phases is in good agreement with NP up-take in cells (Fig 1a). However, cell migration was higher in 3.10 and 3.10.5T groups as compared to other, as well.

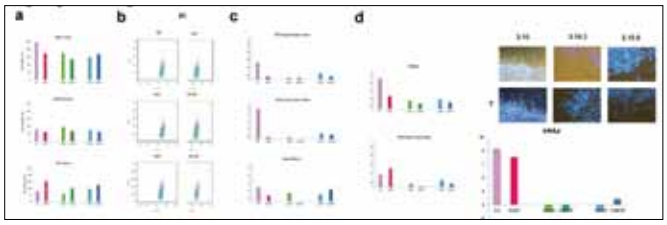

Fig. 2 a) from up to bottom: MTT assay, LDH release, NO production. b) PI flow cytometery. c) Up to bottom: BCL2, BAX and BAX/BCL2 gene expression. d) Fold change NFKB and iNOS genes expression. e) Cell migration assay in face to nanoemulsions f) Focal adhesion kinas 2 gene expression

Based on integrin 5 gene expression data and micrsocopic images, larger particles around 70-78 nm were more up-taken in cells and induces higher integrin 5 gene expression as compared to the smaller ones (49-56 nm). Interestingly, 10.3.5 T (70.7 nm) formulation induces the most integrin 5 gene expression, cell viability and less dead cells (PI positive) (Fig. 1c). Earlier, it has been reported that the space of integrin clustering is less than 70 nm so eventually 3.10.5T NPs with a hydrodynamic diameter of 70.7 nm stimulate cells to over-express integrin and then through the its clustering, the interaction of NP with integrin increases and transduce this signal to F actin and then focal adhesion kinas and the cell migration increases as seen in this report by prolonged mechanotrasduction signal derived from binding [2].

In conclusion, it might be said that the particle with average diameter of 70-78 nm were more up-taken by cells than smaller ones and induce more integrin gene expression and larger ones exhibit less damaging effects on cell membrane and induces less intracellular ROS production and afterwards iNOS gene expression and inflammatory gene of NF-KB. However, smaller nanoformulation around 49-56 nm exhibited higher cell viability by the MTT assay, BCL2 gene expression and less NO production. Based on our findings, it seems that cell viability FAK2gene expression have more direct effect than integrin gene over-expression and cell up take on cell migration and the 3.10, 3.10T and 3.10.5T induce higher cell migration than others (Fig.2f), respectively that they were in good agreement with the FAK2 gene over-expression (Fig. 2f).

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THE CORE OF SELF-ASSEMBLING PEPTIDE NANOFIBERS WILL INFLUENCE NEUROGENESIS POTENTIAL OF ITS ATTACHED BIOLOGICAL MOTIF

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To date, spinal cord injury (SCI) has remained an incurable disaster. The use of self-assembling peptide nanofiber containing bioactive motifs such as bone marrow homing peptide (-BMHP1) as an injectable scaffold in spinal cord regeneration has been suggested and investigated earlier. Although, in all the investigation the effect of biological motifs have been investigated but the influence of self-assembling core of peptide nanofibers in tissue engineering has been neglected. In the present investigation for the first time, the influence of two major core of self-assembling peptide nanofibers attached to the famous neurogenic biological motif of BMHP1 was

assessed. BE2M17 one of the reference cell line (human Neuroblastoma cell line) in neurological investigation was choose and cells were treated with the peptide nanofibers and cell behavior investigated. Then cells were differentiated for 4 days and neural genes were assessed by real time PCR. To investigate the spinal cord recovery potential of nanofibers, they were implanted into a chronic model of SCI in rat. Results showed that the core of KSL-B induced higher cell viability and LDH release while the core of RADA-B exhibits higher acidic environment and induced more ROS, NO production and higher amount of PI positive cells (Dead cells) with higher percentage of Sub-G1 in cell cycle characterized by Flow-cytometery. However, these results were in good agreement with BAX/BCL2 ratio indicated higher BAX/BCL2 ratio for cells treated by RADA-B. Interestingly, the RADA-B induced higher gene expression of nNOS in agreement with NO production. Results showed that although RADA-B induced higher gene expression of Integrin 5 as a cell membrane signaling receptor but KSL-B over-expressed higher gene of focal adhesion kinase 2 as a downstream of integrin and resulted in higher gene expression of Nestin, MAP2, TH, GFAP and GDNF while the over-expression of NF and GABA was higher in RADA-B. Interestingly, RADA-B induced over-expression of GABA while, KSL induced over-expression of TH. The BBB score of Spinal cord injury model in rat disclosed that KSL-B induced higher motor recovery in rats. In conclusion, it might be said that based on the targeted tissue the core of self-assembling peptide nanofiber must be choose. In a bone with acidic friendly environment the RADA core would be better (data not shown) while in the neural tissue, KSL with less inducible acidic environment would be preferred.

INTRODUCTION

One of the most serious incurable and divesting condition that is associated to paralysis is spinal cord injury (SCI). Although a number of investigations have been deeply performed but thus far, successful treatment has not achieved. Earlier studies indicated that physical entrapment of adhesive motifs into scaffold enhances its biological efficacy. However, scaffold nanotopography influences cell signal transduction such as adhesion, proliferation and differentiation. Scientists are looking for the best biological motif to increase neurogenesis and decrease astrogliosis, meanwhile the importance of self-assembling core' s type has been neglected. Usually, researchers used RADA core but the acidic pH derived from it, is a problematic issue and should not be neglected especially when it is implanted to human and animal. In the in-vitro environment, the acidic pH will be manipulated with the medium exchange but what will be happened in in-vivo when it can not exchange with the medium to optimize the acidic pH around 3 especially in a scar site of SCI. Based on the investigations, it seems that bone marrow homing peptide (-BMHP-1) is a favorable biological motif in neurogenesis [1, 2]. In fact, -BMHP-1 nanofiber acts as bio-functional material and mimics the matrix structure of underlying network. In the earlier investigations, it was bound to the RADA as a self-assembling core (1) but this core induces acidic environment that has the ability to damage neural cells so in the present investigation for the first time BMHP was bound to the KSL as a self-assembling core, as well and its influence on a well-known neuroblastoma cell line (BE2M17), confirmed for neurotoxicity investigation, was assessed. Then, it was implanted in a chronic model of spinal cord injury in rat and their motor neuron recovery were investigated by BBB score.

MATERIALS AND METHODS

The peptides were synthesized using solid phase synthesis method and for purity, reverse phase HPLC were performed. Then, to investigate the cell viability, cell membrane damage, NO production, dead cells, intracellular ROS flow-cytometry and kinetic, and the effect of peptides on cell cycle, MTT assay, LDH release, NO production, PI and DCFA, cell cycle flow-cytometry were performed on BE2M17 cell line. Then, cells treated with nanofibers were differentiated to mature neural cells for 4 days. Real-time PCR and NO production were performed to investigate the fold change gene expression of Nestin, Tuj-1, NF, MAP2, TH, GABA, GFAP, GDNF and nNOS, BAX and BCL2 gene over-expression were done to investigate the apoptotic and cytotoxic genes. Also, integrin 5 and focal adhesion kinase (PTK2) were investigated as genes involve in signal transduction. Then, to investigate its influence in in-vivo, a chronic model of SCI was created and the KSL-B and RADA-B nanofibers implanted in them and follow up for 35 days as a weigh and BBB score.

RESULTS AND DISCUSSION

Based on MTT assay (Fig. 1a), PI flow-cytometry (Fig.1 b) and BAX/BCL2 ratio (Fig .1 d) data it might be said that the KSL core induced higher cell viability as compared to the RADA core. Bax/BCL2 ratio was higher in KSL-BMH group by 24 h but then it was gradually decreased during differentiation as such seen in LDH release profile)Fig. 1 c). NO production (Fig. 1 e)and fold change expression of nNOS (Fig. 1 e) in BE2M17 cells showed higher extent of NO production by cells treated with RADA-B as compared to KSL ones. Although, the amount of LDH was higher in cells treated by KSL-B after 24 h but its amount was not higher than control group after 48 h.

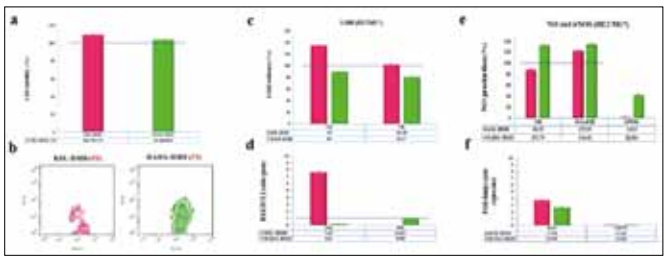
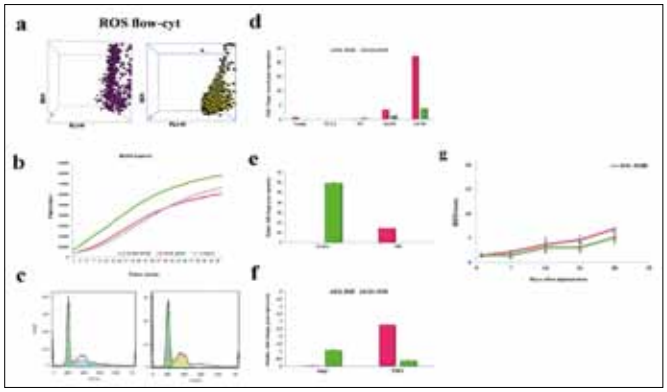


Fig 1 a) MTT assay, b) PI flow cytometry, c) LDH release 24 and 48 h, d) BAX/BCL2 ratio (at the level of gene), e) NO production and nNOS gene expression, f) Relative fold change BCL2 and GDNF genes expression.

LDH release data from neural differentiated human endometrial stromal cells showed there was no significant difference between them. Fold change GDNF and BCL2 gene expression disclosed that KSL core had more protective role in neurons than RADA, however, the relative fold change of GDNF was not significant (Fig. 1 f). Bcl2 not only is an anti-apoptotic gene but also, is a marker of neurogenesis through β- catenin pathway. Beside, intracellular ROS flow-cytometry and its kinetic by Elisa fluorescence showed that RADA-BMH induced higher amount of ROS as compared to the KSL-BMH and the control group (Fig.2a and b). The cell cycle analysis also was in good agreement with other cytotoxic genes and the sub G1 percentage of cells treated by RADA-BMH as a marker of dead cells was higher than KSL ones (Fig. 2 c). Based on the presented findings, it might be said that KSL core makes a more favorable scaffold and environment for neural cells than RADA with an acidic moiety despite of integrin over-expression (Fig. 2f).

Fig. 2 a) ROS flow-cytometry b) ROS kinetic c) cell cycle flow-cytometry, d) Neural genes expression, e) Relative fold change TH and GABA gene expression, f) Relative fold change Integrin and focal adhesion kinase gene expression, g) BBB score of rats implanted with nanofibers in a chronic model of SCI.



Neural genes analysis showed that neural genes such as Nestin, MAP2, TH, GFAP were over-expressed in cells treated by KSL-BMH

as compared to the RADA-BMH. However, the GABA and TUJ-1 were over-expressed in RADA ones. Interestingly, focal adhesion kinase 2 as a downstream of signal transduction over-expressed in cells treated by KSL-BMH and then, it increases neurogenesis. Since, the motor neuron recovery is in good agreement with the anti-apoptosis and cell viability, so the KSL nanofibers improved motor neuron recovery as well (Fig. 2g). In conclusion, it might be said that the type of self-assembling nanofiber core same as biological motif has critical importance and it can change the fate of neurogenesis and recovery and based on type of tissue it must be selected. The osteogenesis is more favorable with acidic environment and the RADA core has shown higher efficacy (data not shown).

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TISSUE-LIKE SILICONE/GOLD NANO-MEMBRANES WITH MICRO-STRUCTURED MORPHOLOGY FOR BIOMIMETIC IMPLANTS

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Polydimethylsiloxane (PDMS) is well known for its biocompatibility and together with its stress-strain behaviour close to human tissue it is the material of choice for biomimetic medical implants. Organic molecular beam deposition (OMBD) of PDMS [1] combined with in situ spectroscopic ellipsometry, illustrates a unique method to reliably deposit compliant PDMS/gold thin films with nanometre precision and long-term stability [2]. In situ ultraviolet (UV) irradiation enables to tailor the polymers elasticity [3] and surface morphology – the key parameters for tissue-to-implant interaction. Stacked as multi-layered silicone/gold nano-membranes they are proposed as artificial muscles or skin implants [4].

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SYNTHESIS AND CHARACTERIZATION OF TISSUE PLASMINOGEN ACTIVATOR FUNCTIONALIZED SUPERPARAMAGNETIC IRON OXIDE NANO-PARTICLES FOR TARGETED FIBRIN CLOT DISSOLUTION

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Superparamagnetic iron oxide nanoparticles (SPIONs) have attracted great attention in many technological fields. Especially in pharmaceutical applications, new nanoparticulate based strategies can provide novel solutions for cancer treatment, autoimmune diseases, implantable materials, medical imaging and diagnosis and thrombolytic therapy [1]. According to the world health organization (WHO) over 30 % of global deaths and disabilities are attributed to cardiovascular diseases [2]. Intravascular thrombosis, such as acute myocardial infarction, ischemic stroke, pulmonary embolism and deep vein thrombosis can be a direct consequence of these diseases. In all cases, a fast recanalization of the occluded vessel is essential in order to minimize negative outcomes for the patients. Clinical thrombolytic therapy with recombinant tissue plasminogen activator (rtPA) induces plasmin formation, which enables fibrinolysis of the clot in the obstructed blood vessel and hence restores blood flow. However, efficacy of modern thrombolysis is often limited by systemic side effects. Poor convey to the site of the thrombus, because of reduced blood flow, and a high risk of internal bleedings reduces the number of patients who can be treated with this method [3]. To overcome these drawbacks and potential risks, and at the same time to augment the local concentration and enhance drug stability during storage and in blood circulation magnetic drug targeting (MDT) systems have been deployed.

In this study, biocompatible magnetite drug carriers, stabilized by a dextran shell were developed to carry tissue plasminogen activator (tPA) for targeted thrombolysis driven by an external magnetic field. Different concentrations of active tPA were immobilized on carboxymethylated nanoparticles (SPIONDex-COOH) through carbodiimide-mediated amide bond formation. Evidence for successful functionalization of SPIONs with carboxyl groups was shown by Fourier transform infrared spectroscopy (FTIR). The altered surface properties after tPA crosslinking could be demonstrated by pH titration and corresponding ζ potential measurements as well as dynamic light scattering (DLS). The enzyme activity of the bound tPA, as determined by fibrin containing agarose gels, was about 74% of that of free tPA. Particles could be stored for up to three weeks, before a slight decrease of activity could be observed. tPA-loaded SPIONs (SPIONDex-COOH-tPA) could be navigated into thrombus-mimicking gels by external magnets (see figure 1), proving effective drug targeting without stripping off the protein. As all synthesized nanoparticles revealed no cytotoxic behavior in cell culture experiments with human umbilical vein endothelial cells (HUVECs), they will be promising candidates for future therapeutic applications in thromboembolic diseases.

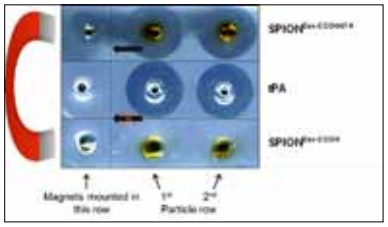

Figure 1: SPIONs with covalently immobilized tPA could be directed into the fibrin matrix. They were able to diffuse into the gel and dissolve fibrin fibers under the influence of a magnetic field. Control samples without tPA were also attracted by the magnets but did not show any activity.

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DEVELOPMENT OF TARGETED CIS-PLATINUM CARRYING NANOFORMULATIONS FOR GLIOBLASTOMA MULTIFORME

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Glioblastoma multiforme(GBM), is the most aggressive type of brain cancer; survival after diagnosis averages at 14 months. GBM is reputable for its intra- and inter- heterogeneity meaning that from tumor to tumor and within the same tumor subpopulations or clones of tumor cells evolve independently under genetic and epigenetic cues leading to recurrence and resistance to standard care treatment. In this context there is a need for a potent therapy which can eradicate multiple tumor subtypes. We have shown[1] that there is a synergistic effect between cis platinum chemotherapy delivered via gold nanoparticles and radiotherapy in arresting tumor cells growth. The further advancement of this formulation is to target it to the site of the tumor by means of an iRGD internalizing peptide which homes to GBMs due to high expression of neuropilin-1. This facilitates the delivery of the nanomedicine across the blood brain barrier and localization to those subpopulations of cells responsible for the relapse of disease.

METHODS

Multiple approaches to implement this nanotechnology construct are discussed. Three custom internalizing peptide sequences ER23, ER2prime and ER24 are employed as targeting moieties. The chemical composition of the peptides also allows for conjugation of the chemotherapy cis platinum via elimination of water between carboxyl groups and the aquated complex of cis-platinum.
Then, the first construct relies on the robust self-assembled monolayers of these individual peptides with spherical gold nanoparticles of various sizes up to 50nm. The second approach, explores the self-assembly of peptides at a selected pH, in buffer, and their ability to reduce gold salt in situ giving rise to porous superstructures. Characterization was typically done via SEM and TEM imaging(as well as EDS spectroscopy for mapping of the elemental composition) to determine the size of the particle core, DLS and zeta potential measurements to extract the hydrodynamic radius and surface charge information(stability parameter), UV-Vis spectrum acquisition to confirm the position of the surface plasmon resonance peak and correlate with size and concentration, amino acid analysis to confirm the peptide conjugation and quantify the amount in the formulation, XPS data was recorded in order to calculate the amount of cis-Platinum attached during the conjugation(i.e. the drug loading) and this was also qualitatively confirmed by FT-IR, as well as XRD and TGA data to infer the average size of the core and composition of the non-metallic shell.
In vitro experiments were carried out using patient-derived tumour cell populations at low passages which ensures is close to the clinical setting. The expression of neuropilin-1 was confirmed via immunofluorescence and Western Blot. GBM cell populations were treated with the candidate formulations and 10Gy dose of radiation from a Cs-137 source. Cell toxicity was verified using an XTT viability assay. Nanoparticle uptake *in vitro* was studied qualitatively by confocal microscopy in reflectance mode, flow cytometry and quantitatively by ICP-MS.

RESULTS

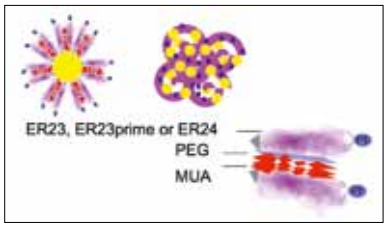


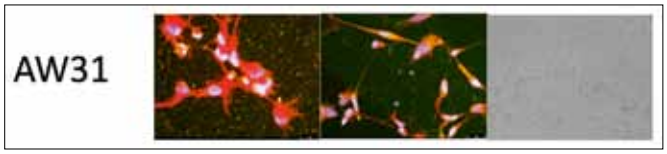
Fig 1. Schematic representation of constructs of nanoformulations.

3 generations of spherical gold nanoparticles termed D1, D2 and D3 with core sizes of approximately 18, 33 and 43 nm in diameter were synthesized by citrate reduction of gold salt and subsequent growth. All were negatively charged and upon conjugation of peptides a shit in charge was observed. Upon conjugation of cis platinum a further reduction in charge was noted.

Figure 2. Detailed sequence of custom internalizing peptides used in this work.
EEEENNLACCALNNEGEGEGRGDR = ER23
EEEENNLACCALNNKGGKGGRGDR = ER24
EEEENNLACCALNNGGEGGRGDR = ER23prime
E facilitates conjugation of cis-Platinum
K/RXXK/R motif is characteristic to internalizing iRGD
C facilitates binding to the gold surface

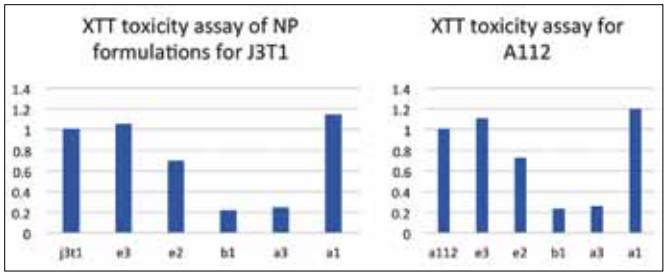
Patient derived, using the Cambridge protocol2, cell populations were shown to express neuropilin-1.

Figure 3. AW31 cells expression of neuropilin-1 is shown in the red channel, integrin is green and the nuclei are blue.



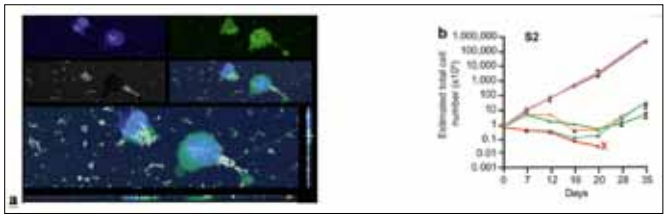
ER23, Er23prime and ER24 targeted nanoparticles do not affect cell viability as shown by XTT viability assay.

Figure 4. D3 core ER23(e3), ER24(e24) and ER23prime(a1) targeted nanoparticles do not affect the viability of GBM cells in vitro



ER23, Er23prime and ER24 targeted nanoparticles show increase internalization demonstrated qualitatively by confocal microscopy in reflectance mode and flow cytometry experiments and this is confirmed quantitatively by ICP-MS: 7-8% of bare particles, 50% of MUA coated nanoparticles and 70% of ER23 targeted D3 core nanoparticles were internalized.

Figure 5. a) Image showing qualitatively the internalization of nanoparticles acquired in confocal reflectance mode. b) Growth curve showing enhanced killing of GBM cells.



When 10Gy radiation dose is subsequently applied, we observed an enhancement in tumour cell death leading to arrest of tumour cell populations growth.

CONCLUSIONS

We have shown the development of targeted nanoformulations based on gold nanoparticle constructs for the multimodal therapy of glioblastoma multiforme. Candidate formulations exhibit potent action *in vitro*. It is essential to validate nanofomulations *in vivo* to assess the targeting potential in order to have proof of concept for a phase 0 clinical trial.

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SIMVASTATIN NANOPARTICLES INDUCE PROLIFERATION AND ACTIVATION OF MURINE OSTEOBLASTS

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Simvastatin, a cholesterol-lowering drug, has shown potential effects in bone metabolism by stimulating BMP-2 gene expression. The controlled release of the statin might be beneficial to this pleiotropic effect and lead to numerous applications in bone tissue engineering, especially in odontology (Padhye and Nagarsenker 2013, Ezirganli, Kazancioglu et al. 2014, Shah, Werlang et al. 2015).

Due to its low oral bioavailability (only 5% of the oral dose), systemic effects might be limited. For this reason, alternative routes of administration of statins might be necessary in order to achieve sufficient local concentrations able to promote bone repair/regeneration. However, the poor aqueous solubility of the drug, along with the mechanisms of the healing process lead to inflammatory reactions, causing damage to the bone tissue, especially in more sensitive areas, such as facial bones (Stein, Lee et al. 2005, Benoit, Nuttelman et al. 2006, Calixto, Lima et al. 2011).

Nanostructured polymeric drug carriers represent a potential solution to overcome the limited aqueous solubility of simvastatin, along with other drawbacks of local administration of the drug. (Musumeci, Ventura et al. 2006, Tai, Fu et al. 2013) .

The purpose of this study was to develop, characterize and evaluate the effect of biodegradable polymeric carboxyl-terminated Poly(D,L-lactide) (PDLLA) nanoparticles entrapping simvastatin on the proliferation and activation of murine osteoblasts (OFCOL II).

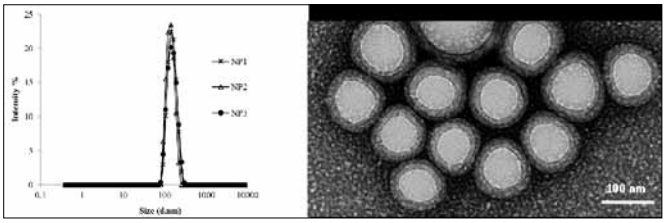
METHODS

Simvastatin-loaded poly(D,L-lactide), carboxyl-terminated (PDLLA) nanoparticles (SIM-NP) were prepared by solvent displacement/nanoprecipitation method. Particle size and polydispersity index was determined by dynamic light scattering - DLS, and zeta potential, determined by electrophoretic mobility. Morphology of NP was examined using a transmission electron microscopy, the samples were negatively stained with 2% (w/v) uranyl acetate solution for observation. Simvastatin was quantified by High Performance Liquid Chromatography (HPLC) with UV detection at 238 nm. Entrapment efficiency (EE%) was determined by ultrafiltration – centrifugation and drug release was assessed by dialysis in a bio-relevant media. Immunostaining and fluorescence microscopy were used to investigate the ability of simvastatin PDLLA nanoparticles fluorescently labeled with rhodamin B to be internalized by a murine osteoblast cell line. Mineralization was performed using Von Kossa assay and the expression of BMP-2 and Rank-L was determined by Western Blot, using alpha-tubulin as standard. Bone Alkaline Phosphatase variation in 3 days was measured via alkaline phosphatase activity assay, were the absorbance was measured at 405 nm in a plate reader and the ALP activity was quantified.

RESULTS

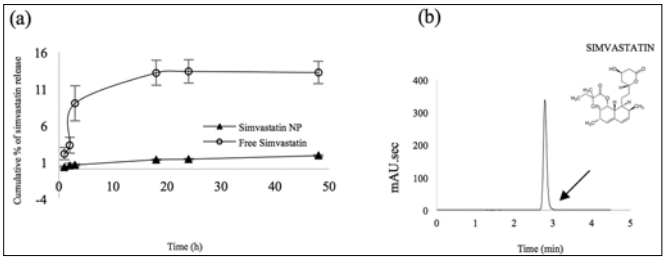
Nanoparticles exhibited an average diameter of 120 nm with a narrow size distribution (PdI 0.06), also confirmed by TEM analysis as presented in Figure 1. Zeta potential (ζ) was -27.21 ± 4.27 mv.

Fig. 1 Size distribution profiles by intensity obtained by DLS for simvastatin PDLLA nanoparticles from three independent batches (a) and TEM micrograph following negative staining of nanoparticles with uranyl acetate (b).



Simvastatin exhibited a well-resolved peak at 2.80 min under the HPLC conditions (Fig. 2b). Entrapment efficiency (EE%) was approximately 100% for simvastatin, and the total drug concentration in the formulation was approximately 500 µg/mL.

Figure 2 - Simvastatin cumulative release profiles from nanoparticles and free drug (a). Values expressed as mean±SD, n=3. (b) HPLC chromatogram of simvastatin 100 µg/mL.



Similarly to previous studies with mesenchymal stem cells shows, the internalization of negatively charged carboxyl-functionalized nanoparticles is dependent of their size and composition (Lohmann, Schwartz et al. 2000, Jiang, Musyanovych et al. 2011, Ernsting, Murakami et al. 2013). Figure 3 shows that a large fraction of SIM-NP was distributed around the cell nucleus. Following SIM-NP particle uptake by osteoblasts gaining intracellular access, effects on cellular viability, proliferation and mineralization were observed.

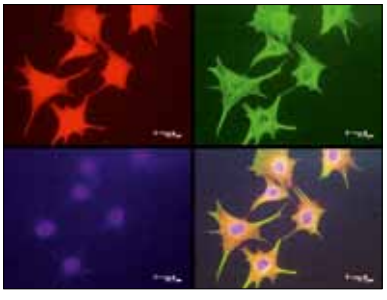
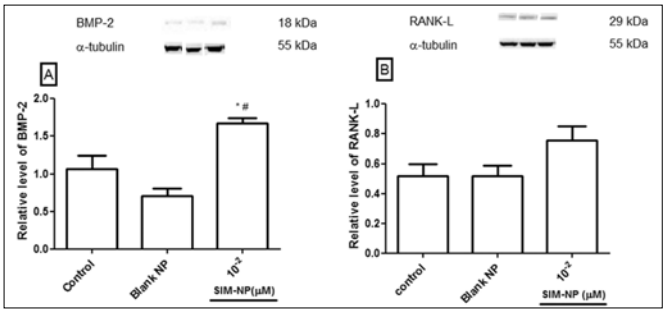
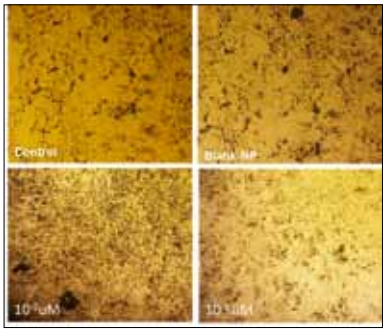


Figure 3 – Cellular internalization of simvastatin nanoparticles. Images obtained by immunofluorescent distribution of nanoparticles (red) in osteoblast cell cytoplasm. Nuclear (blue) and cytoskeletal (green) counterstains are overlaid to increase the contrast of intracellular localization. Bar = 31.7 µm.

Activation of osteoblasts was confirmed by the Von Kossa mineralization assay and the expression and release of alkaline phosphatase (ALP), measured by Western Blot and Elisa, respectively. Von Kossa mineralization assay (Figure 4C), performed 21 days after the incubation period with Blank-NP, SIM-NP in two concentrations (10-2 and 10-1 µM) and complete medium (control), showed that all four evaluated groups had the potential to form mineral nodules *in vitro*, with increased response to the concentration of 10-2 µM. The evaluation of protein expression through Western Blot in the 48h incubation period with SIM-NP, when compared to the control group and blank NP, showed that simvastatin loaded nanoparticles increased significantly ($p < 0.05$) the protein expression of BMP-2 (Figure 4A), as well as of RANK-L (Figure 4B).

Figure 4: Effect of simvastatin loaded nanoparticles on the concentration of bone morphogenetic protein (BMP-2) (A); RANK-L (B) protein expressions in cultures of osteoblasts incubated for 48h using Westen blot analysis. (C) Representative images of Von Kossa staining in OFCOL II cells cultured for 21 days following exposure to simvastatin (10-2, 10-1 µM), Blank NP or plain media (control). Mineralization is seen as black dots. Images were obtained from an inverted microscope with a 40x magnification.





From the evaluation of the protein expression of ALP by Western Blot method, after 48h of incubation with SIM-NP (10-2 µM), it was observed that SIM-NP (10-2µM) increased significantly ($p < 0.05$) the expression of ALP compared to the control and blank NP.

ALP release was monitored using complete culture medium on the third day after incubation. The comparison of 4 groups (control; Blank-NP; SIM-NP (10-2 µM) and SIM-NP (10-1 µM) showed that SIM-NP (10-2µM) increased the release of ALP in the third day, significantly ($p < 0.05$), compared to the control and Blank-NP.

CONCLUSIONS

Our results suggest that biodegradable PDLLA nanoparticles can efficiently entrap simvastatin. Following nanoparticles uptake by osteoblasts, simvastatin-NP were able to promote a stimulatory effect on bone metabolism and mineralization. Activation of osteoblasts, cell proliferation, mineralization and enhancement of alkaline phosphatase activity resulted from the exposure of the cells to the simvastatin nanostructured drug delivery system.

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ENHANCED ANTI-GLIOMA EFFICACY OF ULTRAHIGH LOADING CAPACITY PACLITAXEL PRODRUG CONJUGATE SELF-ASSEMBLED TARGETED NANOPARTICLES

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Glioblastoma multiforme (GBM) presents one of the most lethal brain tumor with a dismal prognosis. And nano-drug delivery system (nano-DDS) have raised a lot of concern, while the conventional nanoformulations addressed many limitations, especially the low drug loading capacity and poor stability *in vivo*. Herein, we proposed PTX prodrug (PTX-SS-C18) conjugate self-assembled nanoparticles (PSNPs) functionalized with glioma homing peptides (Pep-1) to overcome the Blood Brain Tumor Barrier (BBTB) via interleukin 13 receptor α2 (IL-13Rα2) mediated endocytosis for targeting GMB (Figure 1). This nanocarrier was with ultrahigh drug loading capacity (56.03%) and redox-sensitivity to the up-expression of glutathione in glioma cells.

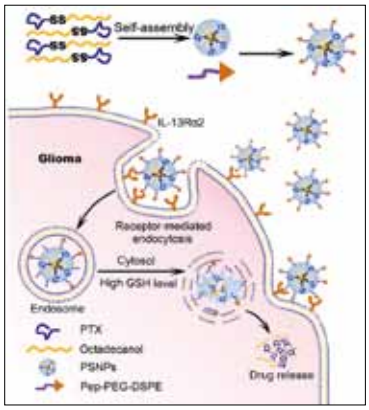
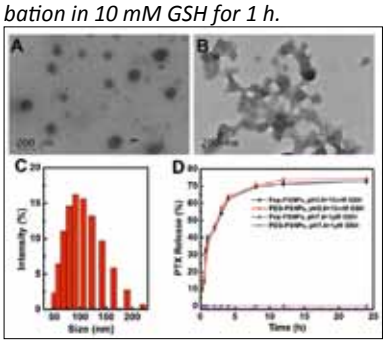


Figure 1. Design of Pep-1 conjugated PEGylated PTX-SS-C18 conjugate self-assembled nanoparticles (Pep-PSNPs) for glioma treatment. In vitro release assay showed that Pep-PSNPs could release PTX rapidly in the presence of GSH (Figure 2).

Figure 2. (A) TEM images of Pep-PSNPs and (B) Pep-PSNPs after incubation in 10 mM GSH for 1 h.



(C) Particle size and size distribution of Pep-PSNPs. (D) Reduction-triggered release of PTX from PSNPs in PBS (pH 7.4) with 1 μM GSH or HAc-NaAc buffer (pH 5.0) with 10 mM GSH (n = 3). Compared with non-targeting PSNPs, Pep-PSNPs could significantly enhance cellular uptake in U87MG cells via IL-13Rα2 mediated endocytosis (Figure 3).

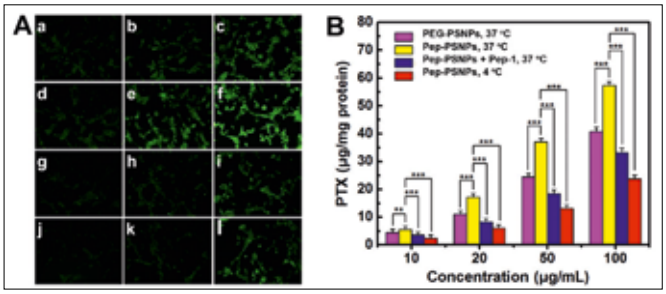


*Figure 3. (A) Cellular uptake of coumarin-6-labeled PEG-PSNPs (a, b, c) and Pep-PSNPs (d-l) at 37ºC (a-i) and 4ºC (j-l) after incubation for 1 h at the coumarin-6 concentration of 5 ng/mL (a, d, g, j), 10 ng/mL (b, e, h, k) and 30 ng/mL (c, f, I, l) in U87MG (a-f, j-l) and Pep-1 pretreated U87MG cells (g, h, i) was examined by fluorescent microscopy. Original magnification: ×20. Bar: 100 μm. (B) U87MG uptake of PEG-PSNPs and Pep-PSNPs at different conditions after incubation for 1h at the PTX concentrations from 10 to 100 μg/mL (n = 3). ***P<0.001, **P<0.01. Enhanced cytotoxicity of Pep-PSNPs against U87MG cells and BCEC cells pretreated with 10 mM of glutathione monoester (GSH-OEt) confirmed that this nanosystem was sensitive to reduction environment, and there was significant difference between targeting and non-targeting groups in MTT assay (Figure 4).*

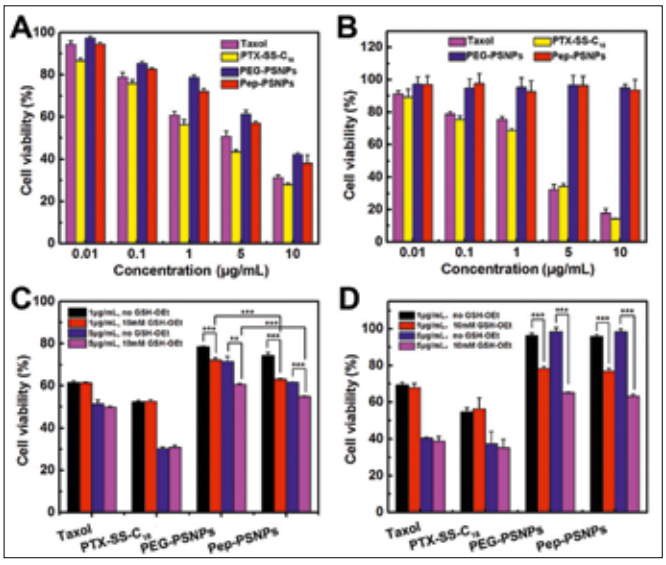


*Figure 4. Cytotoxicity studies (n = 6) of Taxol®, PTX-SS-C18, PEG-PSNPs and Pep-PSNPs in U87MG cells (A) or BCEC cells (B) after incubation for 48h. Viability of GSH-OEt-pretreated or non-pretreated U87MG cells (C) or BCEC cells (D) incubated with Taxol®, PTX-SS-C18, PEG-PSNPs and Pep-PSNPs for 48h. ***P<0.001, **P<0.01.*
Real-time fluorescence image of intracranialU87MG glioma-bearing mice revealed that Pep-PSNPs could more efficiently accumulate at tumor site and improve the penetration (Figure 5A). Furthermore, the ex vivo fluorescence imaging and corresponding semi-quantitative results displayed that the glioma fluorescence intensity of Pep-PSNPs group was 1.74-fold higher than that of non-targeting group (Figure 5B-D). Pep-PSNPs exhibited remarkable anti-glioblastoma efficacy with an extended median survival time (Figure 5E).

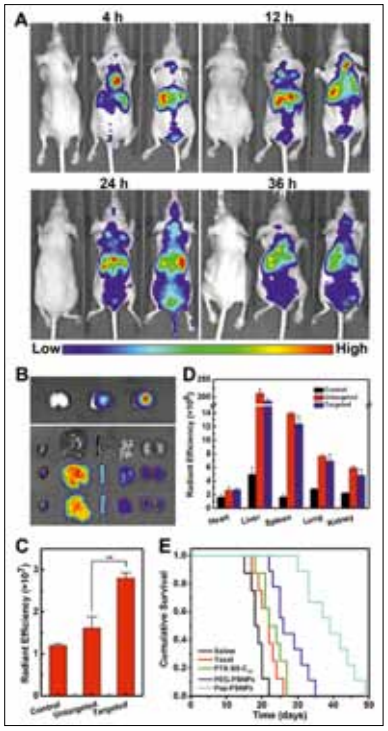


*Figure 5. (A) In vivo fluorescence imaging of U87MG glioma-bearing nude mice administrated with Saline (left, Control), DiR-labeled PEG-PSNPs (middle, Untargeted), and DiR-labeled Pep-PSNPs (right, Targeted) at different time points (4 h, 12 h, 24 h, 36 h). (B) Ex vivo fluorescence imaging of brains and organs sacrificed 36 h after intravenous injection of Saline, DiR-labeled nanoparticles; Brains: Control (left), Untargeted (middle), and Targeted (right); Organs: Control (up), Untargeted (middle), and Targeted (down). The corresponding semi-quantitative radiant efficiency of brains (C) and organs (D). (E) Kaplan-Meier survival curve of U87MG glioma-bearing mice treated with different PTX formulations at a dose of 10 mg/kg PTX on day 2, 4, 6 and 8 post implantation (n = 8). **P<0.01. In conclusion, Pep-PSNPs had a promising perspective as a targeting drug delivery system for glioma treatment of PTX.*

EFFECT OF PROTEIN CORONA ON THE INTERACTIONS OF LIPOSOMES WITH CELLS

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Nanosized materials have been proposed as targeting carriers for drugs, genes and therapies to treat various diseases in nanomedicine. However, once introduced to biological fluids, nanosized objects absorb numerous proteins and biomolecules on their surface forming a layer known as “protein corona”, which critically affects the nanomaterials’ biological identity and targeting properties [1, 2]. Strategies such as PEGylation can help to reduce corona formation but haven’t been proven fully successful. At the same time it is emerging that corona proteins can be actively recognized by cell receptors thus constitute the real biological unit interacting with cells 3. Therefore, rather than trying to avoid corona formation, our aim is to explore how the corona can be used to modulate nanoparticle-cell interactions and assess whether the corona can be used to control nanoparticle uptake and targeting.
For this purpose, liposomes have been chosen as a model nanomedicine: by changing their lipid composition nanosized carriers with tailored surface properties can be designed in a systematic

way. This allows us to tune the resulting coronas once they are exposed to biological fluids such as human serum and plasma, thus to investigate how the corona affects their interaction and behavior on cells.

Liposomes of different charge and composition are prepared by rehydrating lipid film and extrusion. The size and stability of liposomes in biological fluids are characterized with DLS and zeta potential measurements. Once exposed to cells, liposomes of different composition show very different uptake behavior. Different methods have been optimized in order to isolate hard corona coated liposomes. The corona proteins are then identified by SDS-PAGE and mass spectrometry, and the results show that the protein amount and identities change for the different lipid compositions.

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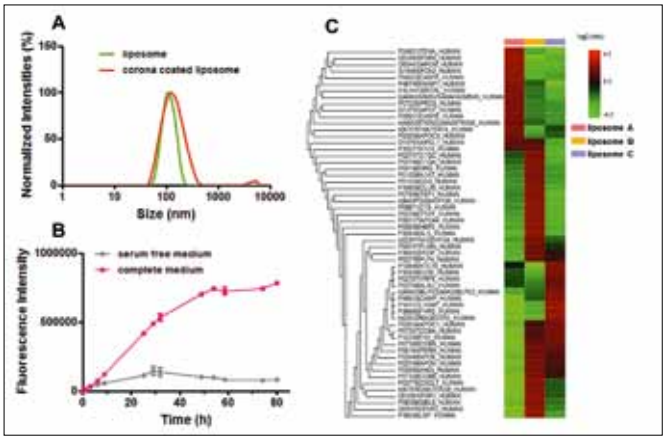

Fig. 1 (A) The size distributions of liposome and hard corona coated liposome. (B) Cell uptake kinetics of liposome in serum free medium and complete medium. (C) Heat map of relative abundance of corona proteins on different liposome surface.

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