CLINAM
European Foundation for Clinical Nanomedicine

nanomedicine
EUROPEAN TECHNOLOGY PLATFORM

CLINAM 5/12 with ETPN

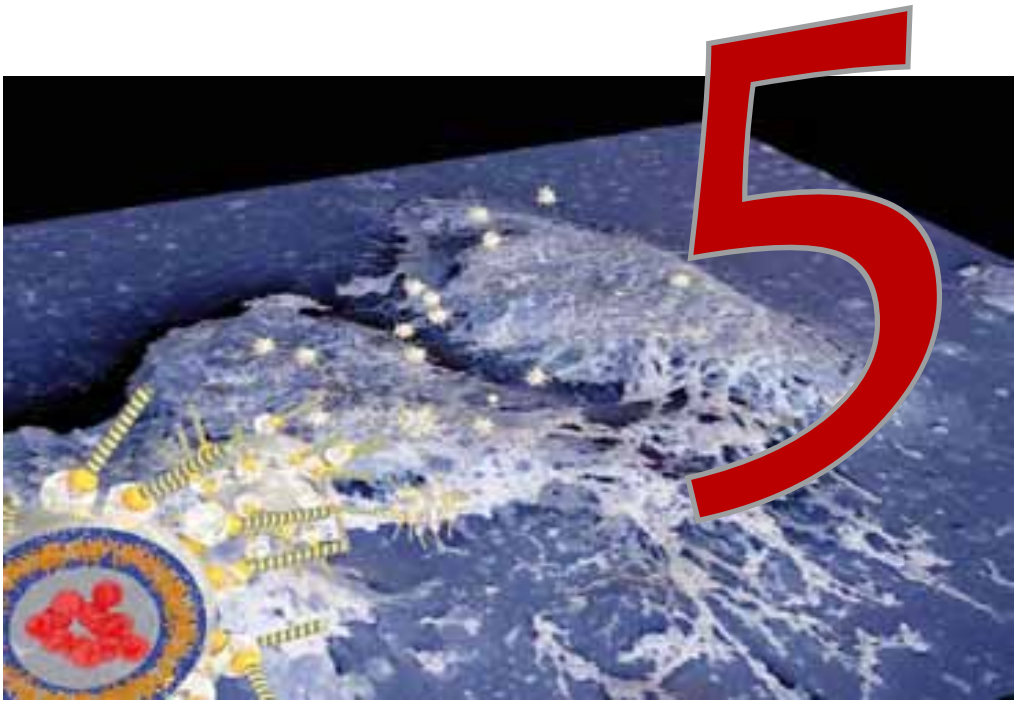
European Summit for Clinical Nanomedicine

Basel, Switzerland, Monday, May 7, 09.00 h – Wednesday, May 9, 2012, 17.00 h

Main Sections of the Summit

- **5th European CLINAM Conference for Clinical Nanomedicine:**
  Focus Day: "The Interplay of Molecular Imaging and Diagnostics with Targeted Therapies"
- **3rd ETP Nanomedicine Brokerage Session**
- **2nd ETP Nanomedicine Policy – Industry Table**
- **3rd EU FP7 Nanomedicine Projects Meeting**
- **5th Nanomedicine University Village & Foyer Exhibition**

CONFERENCE PROCEEDINGS



Editors: Beat Löffler, MA and Prof. Dr. med. Patrick Hunziker

European Foundation for Clinical Nanomedicine
Alemannengasse 12, P.O. Box, CH-4016 Basel, Switzerland
Phone: + 41 61 695 93 95 , Fax: + 41 61 695 93 90
clinam@clinam.org ¦ www.clinam.org

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Conference Proceedings - Table of Content

POSTER ABSTRACTS

INTRODUCTION

Welcome to the European Summit for Clinical Nanomedicine, CLINAM 5/12, the key Nanomedicine event in Europe.

Every emerging technology begins with the pioneers, who set out to investigate the field. Then, with dedicated research and development, they further and further enlarge the discipline. This year, we see growing interest from industry because Nanomedicine now has a profile that deserves to be evaluated for its value in the real world of industrial manufacture for clinical application. Investors and market opinion leaders feel the sweet smell of first chances. Yet novel technologies are expensive and investments are risky despite of the great promise of high return. It is the small startup companies arising at universities that take these risks, invest their time, and show enthusiasm in the new field. Large, potent companies then take a good look at the developments of the startups before diving into the novel technology.

The emerging application of nanotechnology in Health is not just “another technology”. The capability to visualize biologic events at the nanoscale gives medicine a new perspective.

Now is the time for the whole community to interact and debate all facets of Nanomedicine. CLINAM is the platform for clinicians, academic researchers, development engineers from the device and the pharmaceutical industries, material scientists, strategy developers, and regulators to share and to discuss information about the most recent developments, clinical trials and late breaking news. Many excellent experts agree to highlight the advancements and novel results in all fields of this fascinating discipline in Basel, every year.

Looking back five years, we see that Nanomedicine is on its track to pharmaceutical development and already provides solutions and demonstrates results not seen until today. We are convinced that thorough research and responsible development will bring Nanomedicine to the market for the benefit of the patient and all mankind. A well-balanced discussion that respects the opportunities and the risks and considers ethical and regulatory questions is meant to become a valuable barometer for the value of the ongoing nanomedical revolution as a key shaping force worldwide for the medicine of the future.

Beat Löffler
CEO of the CLINAM Foundation

Patrick Hunziker
CSO of the CLINAM-Foundation

Basel, May 2012

CURRICULA VITAE OF SPEAKERS AT CLINAM 5/12

Christoph Alexiou

Dr. Christoph Alexiou, born 2nd of March 1967, received his Ph.D. in 1995 from the Technical University of Munich, Medical school. After finishing his internship in the Gastroenterology Department at the Universityhospital of the Technical University he started as a physician and researcher at the Department of oto-rhino-laryngology, head and neck surgery and founded a research group working on the field of local chemotherapy with magnetic nanoparticles (Magnetic Drug Targeting). In the year 2000 he received his degree as an ENT-Physician and 2002 he changed to the ENT-Department in Erlangen, Germany, where he performed his postdoctoral lecture qualification (Habilitation). He is working there as an assistant medical director in the clinic and leads the Section for Experimental Oncology and Nanomedicine (SEON). Since 2009 he owns the Else Kröner-Fresenius-Foundation-Professorship for Nanomedicine at the Universityhospital Erlangen. The aim of his resarch focus on the translation of Magnetic Drug Targeting into human trials and he received for his research several national and international awards.

David Allsop

PERSONAL DETAILS:

David Allsop, born 05/11/1955
Telephone Number: 44-1524-592-122
E- MAIL: d.allsop@lancaster.ac.uk

CURRENT POSITION:

Professor of Neuroscience, Division of Biomedical and Life Sciences, Faculty of Health and Medicine, Lancaster University, UK.

EXPERIENCE

• 1998-2002 Senior Lecturer in Biomedicine, Department of Biological Sciences, Lancaster University, UK.
• 1994-1998 Assistant Director, Molecular Neuropathology Research, SmithKline Beecham Pharmaceuticals, New Frontiers Science Park, Third Avenue, Harlow, Essex, UK.
• 1990-1993 Lecturer in Biochemistry, Division of Biochemistry, School of Biology and Biochemistry, The Queen's University of Belfast, Northern Ireland.
• 1988-1990 Visiting Scientist, Psychiatric Research Institute of Tokyo, 2-1-8 Kamikitazawa, Setagaya-ku, Tokyo 156, Japan.
• 1986-1988 Recipient of a Fellowship from The John Douglas French Foundation for Alzheimer's Disease to work in the laboratory of Dr. G.G. Glenner, Department of Pathology, The University of California at San Diego, USA.
• 1980-1986 Postdoctoral Research Fellow, Department of Biochemistry, The University of Nottingham Medical School, Queen's Medical Centre, Nottingham, UK.

BIOGRAPHY

David Allsop has a long-standing interest in the role of protein aggregation in neurodegenerative disease. He started this line of research at the Queen's Medical Centre, Nottingham, where he was the first person to isolate senile plaque amyloid from frozen post-mortem brains of patients with Alzheimer's disease (AD) and the first to raise monoclonal antibodies to Aβ. As a post-doctoral researcher, he subsequently worked with George Glenner (University of California, San Diego) and at The Tokyo Research Institute of Psychiatry. He obtained his first academic position at The Queen's University of Belfast, where he co-authored a highly cited review article which sets out the basis of the 'amyloid cascade' hypothesis (Hardy & Allsop (1991) Trends Pharmacol. Sci. 12: 383-388). He then moved to industry (with SmithKline Beecham) before accepting an academic position at Lancaster University. His current research is focussed on detection of amyloidogenic proteins in body fluids as potential biomarkers, and on development of drugs to inhibit protein aggregation. Other recent work has identified the generation of reactive oxygen species from aggregating proteins as a potential common mechanism of cell death in neurodegenerative disease.

SOME RECENT PUBLICATIONS:

• Moore S.A., Huckerby T.N., Gibson G.L., Fullwood N.J., Turnbull S., Tabner B.J., El-Agnaf O.M.A. & Allsop D. (2004) Both the D-(+) and L-(-) enantiomers of nicotine inhibit Aβ aggregation and cytotoxicity. Biochemistry 43, 819-826.
• El-Agnaf O.M.A., Paleologou K.E., Greer B., Abogrein A.M., King J.E., Salem S.A., Fullwood N.J., Benson F.E., Hewitt R., Ford K.J., Martin F.L., Harriott P., Cookson M.R. & Allsop D. (2004) A strategy for designing inhibitors of α-synuclein aggregation and toxicity as a novel treatment for Parkinson's disease and related disorders. FASEB J. 18, 1315-1317.
• Tabner B.J., El-Agnaf O.M.A., Turnbull S., German M.J., Paleologou K.E., Hayashi Y., Cooper L.J., Fullwood N.J. & Allsop D. (2005) Hydrogen peroxide is generated during the very early stages of aggregation of the amyloid peptides implicated in Alzheimer's disease and familial British dementia. J. Biol. Chem. 280, 35789-35792.
• Tokuda T., Salem S., Allsop D., Mizuno T., Nakagawa M., Qureshi M.M., Locascio J.J., Schlossmacher M.G. & El-Agnaf, O.M.A. (2006) Decreased α-synuclein in cerebrospinal fluid of aged individuals and subjects with Parkinson›s disease. Biochem. Biophys. Res. Commun. 349, 162-166.
• Austen B.M., Paleologou K.E., Sumaya A., Ali E., Qureshi M.M., Allsop D. & El-Agnaf O.M.A. (2008) Designing peptide inhibitors for oligomerization and toxicity of Alzheimer's β-amyloid peptide. Biochemistry 47, 1984-1992.
• Kasaia T., Tokuda T., Ishigamia, N., Sasayama H., Foulds P.G., Mitchell J.D., Mann D.M.A., Allsop D. & Nakagawa M. (2009) Increased TDP-43 protein in cerebrospinal fluid of patients with amyotrophic lateral sclerosis. Acta Neuropathol. 117, 1293-1298.
• Paleologou K.E., Salem S.A., Al-Shami R., Mann D.M.A., Allsop D., Ali S.A.E., Hassan A.H., Petersen C.L., Jensen P.H. & El-Agnaf O.M.A. (2009) FILA-1 antibody detects elevated levels of soluble α-synuclein oligomers in brain extracts from patients with dementia with Lewy bodies. Brain 132, 1093-1101.
•Matharu B., Gibson G., Parsons R., Huckerby T.N., Moore S.A., Cooper L.J., Millichamp R., Allsop D. & Austen B. (2009) Galantamine inhibits β-amyloid aggregation and cytotoxicity. J Neurol. Sci. 280, 49-58.
• Foulds P.G., Davidson Y., Mishra M., Hobson D.J., Humphreys K.M., Taylor M., Johnson N., Weintraub S., Akiyama H., Arai T., Hasegawa M., Bigio E.H., Benson F.E., Allsop D. & Mann D.M.A.M. (2009) Plasma phosphorylated-TDP-43 protein levels correlate with brain pathology in frontotemporal lobar degeneration. Acta Neuropathol. 118, 647-658.
• Fukumoto H., Tokuda T., Kasai T., Ishigami N., Hidaka H., Kondo M., Allsop D. & Nakagawa M. (2010) High-molecular weight β-amyloid oligomers are elevated in cerebrospinal fluid of Alzheimer patients. FASEB Journal 24, 2716-2726.
• Taylor M., Moore S., Mayes J., Parkin E., Beeg M., Canovi M., Gobbi M., Mann D.M.A. & Allsop D. (2010) Development of a proteolytically stable retro-inverso peptide inhibitor of β-amyloid oligomerization as a potential novel treatment for Alzheimer's disease. Biochemistry 49, 3261–3272.
• Foulds P., Mann D.M.A., Mitchell D.M. & Allsop D. (2010) Progress towards a molecular biomarker for Parkinson disease. Nature Reviews Neurol. 6, 359-361.
• Taylor M., Moore S., Mourtas S., Niarakis A., Re F., Zona C., Ferla B., Nicotra F., Masserini M., Antimisiaris S.G., Gregori M. & Allsop D. (2011) Effect of curcumin-associated and lipid ligand functionalised nanoliposomes on aggregation of the Alzheimer's Aβ peptide. Nanomed: Nanotech. Biol. Med. 7, 541–550.
• Foulds P.G., Mitchell J.D., Parker A., Turner R., Green G., Diggle P., Hasegawa M., Taylor M., Mann D.M.A. & Allsop D. (2011) Phosphorylated α-synuclein can be detected in blood plasma and is potentially a useful biomarker for Parkinson's disease. FASEB J. 25, 4127-4137.
• Foulds P.G., Yokota O., Thurston A., Davidson Y., Ahmed Z., Holton J., Thompson J.C., Akiyama H., Arai T., Hasegawa M., Gerhard A., Allsop D. & Mann, D.M.A. (2012) Post mortem cerebrospinal fluid α-synuclein levels are raised in multiple system atrophy and distinguish this from the other α-synucleinopathies, Parkinson›s disease and Dementia with Lewy bodies. Neurobiol. Dis.45, 188-195.

Mario Amacker

Date of birth: June 5, 1969 Place of birth: Visp (VS), Switzerland

EXPERIENCE

• 2003 - Pevion Biotech AG, Ittigen / Bern
R & D Scientist for virosomal formulation development (2003 – 2005)
Group leader for development of virosomal vaccines (2005 – 2009)
Head Process Development & Manufacturing (since 2009)
Member of the Executive Board (since 2009)
• 2001 - 2003 Gnothis SA, Parc Scientifique EPFL, Lausanne
R & D Scientist and group coordinator (biology division)
Proxy research group leader of the biology division
Group leader of R&D division "enzymes and sample preparation" (2002-2003), Project Manager SNP Analysis (2002-2003)
• 1998 - 2001 Postdoctoral fellow at the Swiss Institute for Experimental Cancer Research (ISREC), Epalinges. Dr. J. Lingner.
Topic: "Regulation and biogenesis of human telomerase"

EDUCATION

• 1994 - 1997 Ph. D. thesis at the Institute of Veterinary Biochemistry, University of Zürich, Prof. U. Hübscher and Prof. P. Sonderegger, Institute of Biochemistry.
Topic: "The reverse transcriptase of the feline immunodeficiency virus"

PUBLICATIONS

Patent publications

• Zurbriggen R, **Amacker M**, Rasi S. Lyophilization of virosomes. WO 2006/069719
• Zurbriggen R, **Amacker M**, Moser C, Rasi S, Kammer AK, Westerfeld N. Virosomes comprising HA derived from viruses produced in cell lines. WO2009/000433.
• Kammer AK, **Amacker M**, Zurbriggen R. Multiepitope vaccine for Her2/neu-associated cancers. EP 2292258.

Recent publications

• Tamborrini M, Stoffel SA, Westerfeld N, **Amacker M**, Theisen M, Zurbriggen R, Pluschke G. Immunogenicity of a virosomally-formulated Plasmodium falciparum GLURP-MSP3 chimeric protein-based malaria vaccine candidate in comparison to adjuvanted formulations. Malar J. 2011;10:359.
• Moser C, Amacker M, Zurbriggen R. Influenza virosomes as a vaccine adjuvant and carrier system. Expert Rev Vaccines. 2011;10(4):437-46.
• Bomsel M, Tudor D, Drillet AS, Alfsen A, Ganor Y, Roger MG, Mouz N, **Amacker M**, Chalifour A, Diomede L, Devillier G, Cong Z, Wei Q, Gao H, Qin C, Yang GB, Zurbriggen R, Lopalco L, Fleury S. Immunization with HIV-1 gp41 subunit virosomes induces mucosal antibodies protecting nonhuman primates against vaginal SHIV challenges. Immunity. 2011;34(2):269-80.
• **Amacker M**, Moese S, Kammer AR, Helenius A, Zurbriggen R. Influenza Virosomes as Delivery Systems for Antigens, in: Delivery Technologies for Biopharmaceuticals, Jorgensen L and Nielsen HN (eds.), 2009, John Wiley & Sons Ltd, London.
• Hofer U, Lehmann AD, Waelti E, **Amacker M**, Gehr P, Rothen-Rutishauser B. Virosomes can enter cells by non-phagocytic mechanisms. J Liposome Res. 2009;19(4):301-9.
• Kopp N, Diaz D, **Amacker M**, Odongo DO, Beier K, Nitsch C, Bishop RP, Daubenberger CA. Identification of a synthetic peptide inducing cross-reactive antibodies binding to Rhipicephalus (Boophilus) decoloratus, Rhipicephalus (Boophilus) microplus, Hyalomma anatolicum anatolicum and Rhipicephalus appendiculatus BM86 homologues. Vaccine. 2009 Dec 10;28(1):261-9.
• Nallet S, **Amacker M**, Westerfeld N, Baldi L, König I, Hacker DL, Zaborosch C, Zurbriggen R, Wurm FM. Respiratory syncytial virus subunit vaccine based on a recombinant fusion protein expressed transiently in mammalian cells. Vaccine. 2009 Oct 30;27(46):6415-9
• Okitsu SL, Mueller MS, **Amacker M**, Vogel D, Westerfeld N, Robinson JA, Zurbriggen R, Pluschke G. Preclinical profiling of the immunogenicity of a two-component subunit malaria vaccine candidate based on virosome technology. Hum Vaccin. 2008 Mar-Apr;4(2):106-14.
• Zurbriggen R, **Amacker M,** Kammer AR. Immunopotentiating Reconstituted Influenza Virosomes. In: Gregoriadis G (ed.) Liposome Technology, Third Edition, Volume 1: Liposome Preparations and Related Techniques. Informa Healthcare, London, 2007.
• Moser C, **Amacker M**, Kammer AR, Rasi S, Westerfeld N, Zurbriggen R. Influenza virosomes as a combined vaccine carrier and adjuvant system for prophylactic and therapeutic immunizations. Expert Rev Vaccines. 2007 Oct;6(5):711-21.
• Kammer AR*, **Amacker M***, Rasi S, Westerfeld N, Gremion C, Neuhaus D, Zurbriggen R. A new and versatile virosomal antigen delivery system to induce cellular and humoral immune responses. Vaccine. 2007 Oct 10;25(41):7065-74. (* first authors).
• Angel J, Chaperot L, Molens JP, Mezin P, **Amacker M**, Zurbriggen R, Grichine A, Plumas J. Virosome-mediated delivery of tumor antigen to plasmacytoid dendritic cells. Vaccine. 2007 May 10;25(19):3913-21.
• Liu X, Siegrist S, **Amacker M**, Zurbriggen R, Pluschke G, Seeberger PH. Enhancement of the immunogenicity of synthetic carbohydrates by conjugation to virosomes: a leishmaniasis vaccine candidate. ACS Chem Biol. 2006 Apr 25;1(3):161-4.
• Schumacher R, **Amacker M**, Neuhaus D, Rosenthal R, Groeper C, Heberer M, Spagnoli GC, Zurbriggen R, Adamina M. Efficient induction of tumoricidal cytotoxic T lymphocytes by HLA-A0201 restricted, melanoma associated, L(27)Melan-A/MART-1(26-35) peptide encapsulated into virosomes in vitro. Vaccine. 2005 Dec 1;23(48-49):5572-82.
• **Amacker M***, Engler O*, Kammer AR*, Vadrucci S, Oberholzer D, Cerny A, Zurbriggen R. Peptide-loaded chimeric influenza virosomes for efficient in vivo induction of cytotoxic T cells. Int Immunol. 2005 Jun;17(6):695-704. (* first authors).

Thomas Andresen

Dr Andresen has since 2007 been an associate professor at the Technical University of Denmark where he established the Colloids & Biological Interfaces (CBIO) group after obtaining experience from industry as Head of R&D in a Danish drug delivery biotech company. His research interest lies at the interface between organic synthesis, biophysical chemistry, and cell biology and he is focused on design of nanocarrier systems for drug delivery and imaging applications where quantification of biomolecular interactions and transport processes in biological milieu is of particular interest. This is both at the systemic, cellular, organelle and membrane/protein level. Cancer is a disease that substantially motivates his research efforts within new drug delivery and treatment principles.

Dr. Andresen publication list covers premier journals in multiple research fields and he has been recognized as a visionary scientific leader by the Technical University of Denmark, where Department of Micro- and Nanotechnology in 2011 appointed him as head of one of four of the department's strategic research areas (Biomedical and Life Science Nanotechnology). The Technical University of Denmark has furthermore appointed him as head of a new cross-department research centre in nanomedicine.

Dr. Andresen is co-founder of Nanovi, a Danish company established by venture capital in 2010 to exploit DTU's proprietary nanoparticle technology for radiation therapy, which was also invented by Dr. Andresen and co-workers. Furthermore, he is co-inventor of DTU's proprietary liposome technology for diagnosing solid tumors, which is planned to form the basis of a new Danish start-up company in 2012. In total, Dr. Andresen has been co-inventor of 6 patent families since starting at DTU in 2007.

Dimitri Aubert

Dimitri Aubert is the Sales Director for Europe, Middle East and Africa at Izon Science Ltd, an innovative company that developed instrumentation for accurate nanoscale particle analysis. He obtained his first degree and MSc in Chemistry from the University of Caen, France, with PhD degree from the University of Nottingham, UK. He has published papers in the field of enantioselective synthetic chemistry.

He worked for a number of scientific companies over the past 8 years, including Ahura Scientific (now Thermo Fisher Scientific) and Cobalt Light Systems Ltd, both in the field of Raman spectroscopy.

Lajos (Lou) P. Balogh

Ph.D., 364 Ocean Ave, #702, Revere, MA 02151 USA Email: baloghl@prodigy.net Phone/Fax: (617) 682-0053, Mobill: (734) 239-3342

CAREER SUMMARY

Conceptualized, launched and directed multidisciplinary drug discovery research program, its facilities and recruited personnel resulting in the publication of 20 original articles per year. Over 200 scientific publications with over 2000 citations (h index = 21), 80 invited lectures, 12 patents in chemistry, drug discovery, drug delivery, biomedical engineering, and nanomedicine. Discovered and pioneered dendrimer nanocomposites, a new organic/inorganic nanoparticle drug delivery platform resulting in several seminal patents. Invented new cancer treatments: nanobrachytherapy and photomechanical therapy. Solved drug delivery problems considerably improving target specificity for cancer drugs. Spearheaded efforts to solve synthetic, analytical and technological problems for drugs, dendrimers, nanoparticles, and polymers.

Developed nanotechnology/nanomedicine nomenclature, terminology, and standards for ASTM, for the American Standard Institute and for ISO, the International Standard Organization.

EXPERTISE

Organic, polymer, and pharmaceutical chemistry, nanotechnology and nanomedicine. Design and characterization of drug delivery platforms. Translating novel technologies into clinical investigations including in vitro and in vivo (toxicity, biodistribution, pharmacokinetic, etc.) preclinical studies, primarily in cancer.

QUALIFICATIONS

Accomplished and creative senior scientist, with broad international and interdisciplinary leadership and consulting experience in chemistry, chemical engineering, nanomedicine, bioengineering, and drug delivery research with a track record of successful independent funding, patenting, mentorship, peer review, and editorship.

EXPERIENCE

- 2011-Pres: Adjunct Professor of Pharmaceutical Sciences, Northeastern University, Boston MA 2009-2013: Editor-in-Chief, Nanomedicine: Nanotechnology, Biology and Medicine (Elsevier).
- Led successful turnaround of Nanomedicine journal by implementing necessary changes in structure, procedures and personnel.
- Received first impact factor of IF=5.44 placing the journal overall 8th in the category of Medicine Research and Experimental, and 11th in the category of Nanoscience and Nanotechnology in the world.
- Increased readership from 49,000 to 113,000, and submissions from 171 to 427 in 2010.
- 2000-Pres.: Scientific Advisor and Principal, AA Nanomedicine and Nanotechnology.
- Intellectual property and technology due diligences. Critically assessed merits of more than 200 medical research proposals, small business proposals, and evaluated originality of venture ideas for private investors and government agencies.
- 2005-2009: Senior Scientist and Research Professor of Oncology, Depts. of Radiation Medicine and of Biophysical Therapies, Roswell Park Cancer Institute, Buffalo, NY and Director of Nanotechnology Research, Radiation Oncology, University of Buffalo, SUNY -Spearheaded nanoparticle and nanodevices drug research including design, synthesis, characterization, in vitro/in vivo toxicology, and physiologically based pharmacokinetics. Developed novel cancer imaging agents and targeted therapy technologies. Supervised and led 14 PhD scientist (chemists, biologists, and materials scientists) producing 20 original publications in two years.
- 2000-2005: Research Associate Professor of Internal Medicine, of Biomedical Engineering and of Macromolecular Engineering, University of Michigan, Ann Arbor, MI, Developed strategies for multifunctional dendrimers selectively targeting breast cancer cells. Invented and pioneered targeted composite nanodevices for medical research. Acquired over $2M independent research funding, recruited and hired scientists to form a productive research group.
- 1998-2000: Assistant Research Scientist, Department of Medicine, Center for Biologic Nanotechnology, University of Michigan, Ann Arbor, MI. Carried out non-conventional research in Biologic Nanotechnology resulting in new medical applications and technologies Equipped chemical research laboratories, according to the synthetic and analytical needs of emerging medical fields.
- 1996-1998: Senior Associate Scientist, Michigan Molecular Institute, ARL Center of Excellence Midland, MI, Developed non-flaming polymer material and new treatment of burnt wounds, Discovered and pioneered a novel class of nanomaterials resulting in seminal patents.
- 1991-1996: Visiting & Adjunct Professor of Chemistry, University of Massachusetts Lowell, MA, Discovered new mechanism of living polymerizations and developed conductive polymers for Libattery applications.
- 1975-1991: Associate Professor of Applied Chemistry, Kossuth University, Debrecen, Hungary, Scaled up and produced fine chemicals on pilot plant scale.

Yechezkel (Chezy) Barenholz

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

Professor 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, and local anesthetic drugs, as well as delivery systems for peptides, proteins, nucleic acids, and vaccines. This is exemplified by Doxil® , which was based on his 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 world by Johnson and Johnson. Doxil 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. NasVax Ltd (now a public company on the Israeli stock market), a vaccine developing company, based on, among others VaxiSomeTM , a Barenholz-invented polycationic sphingolipid adjuvant; 2. Moebius Medical, which develops a liposome-based medical device for treatment of osteoarthritis, now in clinical trials; 3. LipoCure Ltd for 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 special liposomes remote loaded with local anesthetics for prolonging analgesia duration. Two of the liposomal drugs under development in LipoCure are in final preparation for clinical trials.

Professor Barenholz is a coauthor of more than 360 scientific publications having altogether more than 10,000 citations. He is a co-inventor in more than 30 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 4 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 from the Danish Technical University (DTU) in 2012, (Copenhagen, Denmark); and the international Controlled Release Society's (CRS) most prestigious CRS Founders Award for 2012. In 2003 Professor Barenholz founded (from Doxil royalties) the "Barenholz Prizes" for Israeli Ph.D. students to encourage excellence and innovation in applied science.

Professor Barenholz is married to Dr. Hanna Barenholz together they share 4 daughters and 12 grandchildren.

Simon Benita

Simon Benita is a Professor at The Hebrew University of Jerusalem, where he received his Ph.D. in Pharmacy in 1980.

As a result of his research activities, Prof. S. Benita formed and supervises a group of 31 M.Sc., 26 Ph.D. students, and 15 post-doctoral students in pharmaceutical sciences. He has published 144 research articles and 17 book chapters, edited 3 books and been issued 16 patents and 8 patent applications.

Professor Benita has served as a member of the Board of Pharmaceutical Sciences of the International Pharmacy Federation and Governor of the Controlled Release Society. He is currently a Foreign Correspondent of Academie Nationale de Pharmacie, France and Governor of The International Microencapsulation Society. He is a member of the Editorial Boards of Pharmaceutical Development and Technology, Journal of Microencapsulation, Journal of Drug Delivery Science and Technology, AAPS PharmSciTech, Annales Pharmaceutiques Françaises and the European Journal of Pharmaceutics & Biopharmaceutics.

He was selected to be a recipient of The Hebrew University Kaye Innovation Award in 2000 and again in 2005, and is an AAPS Fellow. He is the Founder of the company Novagali Pharma which received in 2009 the "91 d'or" Award from the French Business Confederation and the Siemens "Health Award". Frost & Sullivan recognizes Novagali Pharma for Innovation in Ophthalmic Therapies and grants the 2009 Best Practices Award. Novagali has been listed in the Euronext in 2010.

He is actually the Director of the Institute for Drug Research and Head of the School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem.

François Berger

François Berger, MD-PhD, had a dual scientific and clinical education in the field of neurology, oncology and molecular and cell biology. For the last 4 years he coordinated the Brain Nanomedicine Group in INSERM U 836. He continues to have a dual clinical and research activity has professor of cell biology and neuro-oncology. He develops a translational research activity, trying to validate innovative technologies at the preclinical/clinical level in close collaboration with CEA-LETI micro-nanotechnology centre. At the interface between technology and medicine, he is the director of CLINATEC. Clinatec is a clinical-preclinical research facility devoted to the validation of new implanted micro-nanotechnologies at the human brain interface associating biological and imaging facilities to provide the best environment for the first preclinical and human proof of concept.

Philippe Bergonzo

Dr Philippe Bergonzo Eur.Ing, PhD, HDR, 44, is Research Director at CEA with expertise in the field of diamond research. After a PhD at University College London UK, he Joined CEA in 1994 to focus on novel material development for device fabrication. Since 2003, he took the lead of a team now called the Diamond Sensors Laboratory, that has grown up to over 25 researchers where are currently running 5 French national projects and 5 EU projects. The team developments address novel tools taking advantage on diamond exceptional properties from thermal management to detection properties, via electrochemical applications and bio- and chemical sensor developments. The lab also aims at novel sensors (SAWs and MEMS), to bio-interfaces (cells and retinas), to diamond nanoparticles for drug delivery applications and electrochemical industrial applications. He is author of over 160 contributions in scientific journals and is a member of several diamond conference committees and editorial scientific committees.

Roy Bicknell

Roy Bicknell received his MA (1981) and D. Phil (1984) degrees from Oxford. He then won a NATO postdoctoral fellowship that he held at Harvard Medical School. While at Harvard he first became interested in angiogenesis and angiogenic factors. He returned as a Principal Investigator with the Imperial Cancer Research Fund (now Cancer Research UK) in the new Institute of Molecular Medicine at Oxford in 1984 where he ran the Cancer Research UK angiogenesis group for 16 years. In his time at Oxford he was appointed Professor of Cancer Cell Biology. In 1985 he moved to his current post in Birmingham as Professor of Cancer Studies and Genomics. His principle interests are angiogenesis and vascular targeting on which he has published over 200 articles. A specific interest is the identification of tumour endothelial markers and he first identified Robo4 and CLEC14A.

Gerd Binnig

Gerd Binnig (born July 20, 1947) is a German physicist, and a Nobel laureate.
He was born in Frankfurt am Main and played in the ruins of the city during his childhood. His family lived partly in Frankfurt and partly in Offenbach am Main, and he attended school in both cities. At the age of 10, he decided to become a physicist, but he soon wondered whether he had made the right choice. He concentrated more on music, playing in a band. He also started playing the violin at 15 and played in his school orchestra.
In 1969, he married Lore Wagler, a psychologist, and they have a daughter born in Switzerland and a son born in California. His hobbies are reading, socker and golf.
In 1978, he accepted an offer from IBM to join their Zürich research group. There, he met Heinrich Rohrer, with whom he shared half of the Nobel Prize in Physics in 1986 for their design of the scanning tunneling microscope (STM) (the other half of the Prize was awarded to Ernst Ruska).
The team included Christoph Gerber and Edmund Weibel, and they were soon recognized with a number of prizes: the German Physics Prize, the Otto Klung Prize, the Hewlett Packard Prize, the King Faisal Prize and, ultimately, the Nobel Prize.
In 1994 Professor Gerd Binnig founded Definiens which turned in the year 2000 into a commercial enterprise. Today, companies and institutions around the world use Definiens' technology to maximize the value of images and thereby enabling better decisions. Definiens currently focuses on applications for Life Sciences. Definiens' technology is used to accelerate the drug discovery, development, and diagnostics processes.

Fabio Biscarini

Prof. Fabio Biscarini, Ph. D. FRSC, is Research Director at CNR-ISMN Bologna, where he heads the multidisciplinary group of "Nanotechnology of Multifunctional Materials". He received Laurea cum Laude in Industrial Chemistry from the University of Bologna in 1986 and a Ph.D. in Chemistry in 1993 from the University of Oregon, Eugene, USA. His current research interests include nanotechnology of organic materials and soft matter, organic ultra-thin film field effect transistors and their use as transducers of signals from neural cells. He is author of more than 170 papers in peer-reviewed international journals, with h index = 35 and about 3900 citations, several book chapters. He is inventor in 19 patents and founded two spin-off companies, Scriba Nanotecnologie Srl and Nano4bio Srl. He is the coordinator of the EU NMP Project I-ONE, Grant Agreement n. 280772. He received the EU-Descartes Prize 2007.

Patrick Boisseau

M. Patrick Boisseau joined the French Atomic Energy Commission (CEA) in 1987 to work for 7 years as academic research fellow in plant biology. He then spent 4 years at the Foresight & Strategy Division at the CEA headquarters as expert on strategy in life sciences and environment. From 2001 to 2004, he was committed to the design, organisation and funding of the NanoBio innovation centre in Grenoble. This NanoBio cluster brings together engineers, physicists, chemists, biologists and medical doctors to develop new miniaturised tools for biological applications (130 people involved including SMEs). The NanoBio center is an integral part of the Minatec Innovation Center, the model for France's competitive clusters and #1 European centre for micro- and nanotechnologies. From 2004 till 2008, he was coordinator of the European network of excellence in nanobiotechnology, Nano2Life (www.nano2life.org). This network of excellence integrates 23 full academic partners and 41 associate companies (>400 scientists) in a comprehensive joint programme of activity. Since 2006, he has been a Member of the Executive Board of the European Technology Platforms on Nanomedicine and chairman of the working group on "nanotechnology based diagnostics and imaging." He co-founded the French Technology Platform on Nanomedicine, in 2007. Currently, 15 scientists are devoted there to the preclinical and clinical development of Lipidots®, a lipid nanocarrier platform. He has been part of more than 12 European projects and main coordinator of 5 Framework Projects. Since 2008, he has been Programme Manager at CEA-Leti, on "nanostructures for molecular imaging and therapy." Patrick Boisseau is graduate of the Institut National Agronomique (1983) and of the Ecole Nationale du Génie Rural, des Eaux et des Forêts (1985). He holds a Master's Degree in Human Nutrition (2005).

Donald Bruce

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

Fritz R. Bühler

Fritz R. Bühler, MD is Professor of Pharmaceutical Medicine and Pathophysiology as well as of Internal Medicine and Cardiology at the Faculty of Medicine, University of Basel. He was Director of the Department of Research at the University Hospitals in Basel, Switzerland. Prof Bühler qualified in 1965 at the University of Basel. Between 1970 and 1973 he worked at Columbia University in New York and in 1977 as a visiting professor at Harvard Medical School in Boston. In 1988 he became Professor of Pathophysiology in Basel. Prof. Bühler was Head of world-wide Clinical Research and Development at Hoffmann-La Roche from 1991 to 1995. For twenty years, he was the director of the European Center of Pharmaceutical Medicine (ECPM) at the Medical Faculty and PharmaCenter of the University of Basel. He is on the Board of the Center for Drug Development Science at UCSF in Washington DC and co-founder of the American and Chinese Courses on Drug Development and Regulatory Sciences in 2007/2008 as well as a Chinese version at Peking University. He was on the Executive Committee of the Swiss Academy of Medical Sciences. Fritz R. Bühler co-founded in 1998 the International Biomedicine Management Partners Inc. in Basel, a venture management organization and he was a Managing Partner at Bear Stearns Health Innoventures in New York 2000-2008. He

is also a co-founder and promoter of the trinational BioValley at the Upper Rhine as well as BioValley Basel and Swiss Biotech. In 2002 he received an honorary doctorate of the University Louis Pasteur of Strasbourg, and in 2004 an honorary membership of the Swiss Association of Pharmaceutical Professionals, which he founded in 1995. He now leads a Pharmaceutical Medicine Training Programme, PharmaTrain, part of the Innovative Medicines Initiative in Europe.

Ed Constable

Ed Constable was born in Edinburgh (Scotland), but grew up in Hastings (England) where he went completed his school studies. In 1974 he started his studies in chemistry at St. Catherine's College, University of Oxford, where in 1978 he obtained his BA in Chemistry. He completed his D.Phil at Linacre College, University of Oxford under the supervision of Professor Kenneth Seddon and at that time began a love-affair with ruthenium chemistry and photochemistry that continues to this day. He held a number of research fellowships at the University of Cambridge before being appointed to a University Lectureship and Fellowship of Robinson College in 1984. He remained at Cambridge until 1993 when he was appointed Professor of Inorganic Chemistry at the University of Basel (Switzerland). In 2000 he left Basel to take up a position as Professor of Chemistry at the University of Birmingham (England) where he was shortly appointed Head of School. He returned to the University of Basel as Professor of Chemistry in 2002. He is a Fellow of the Royal Chemical Society and member of the American Chemical Society He is or has been on the Editorial Boards of numerous journals, including Chemical Communications, Chemical Reviews, Chemical Society Reviews, New Journal of Chemistry, Polyhedron and Supramolecular Chemistry. Since 2011 Ed Constable is Vice Principal and Head of Research of the University of Basel.

Vincenzo Costigliola

E-mail: Vincenzo@EMAnet.org

RELEVANT EDUCATION

Dr. Costigliola graduated in Medicine from the University of Naples in 1972 and with distinction, in Anesthesiology and Intensive Care from the University of Pisa in 1978.
He also completed studies in Rheumatology, Dermatology, Procthology, Oncology, Surgery, Drugs Abuse, Emergency Treatment, Disaster Action, Hospital Organization, Medical Teaching Methodology and Computor and Audio-Visual Training for the Medical Profession.

- President of E.M.A. (European Medical Association) http://www.emanet.org
- President of EPMA (European Predictive, Preventive, Personalized Medicine http://www.epmanet.eu
- President of E.D.A. (European Depression Association) http://www.eddas.org
- Member of Board of "The European Biotechnology Association" http://www.ebtna.net

- Member of the International Adisory Board at King Abdulaziz University S.A.
- Member of Editorial Advisory Board Board of EPMA Journal
- Good experience in clinical trials
- Extensive experience in european health care field
- Main contractor and participant in many EU projects of dg 22 and dg 5
- Selected as expert-evaluator in the 5th Framework programme
- Extensive experience in informatics' health programmes
- Teaching experiences
- Nationals and internationals conferences as a speaker
- Publishing experiences

Languages: Italian, French, English, and Spanish

Daxiang Cui

Prof. & Dr. Daxiang Cui, works in Institute of Micro/Nano Science and Technology, National Key Laboratory of Nano/Micro Fabrication Technology, Key Laboratory for Thin Film and Microfabrication of Ministry of Education,Shanghai JiaoTong university, mainly research direction is: synthesis and biosafety evaluation of nanomaterials, and application in biomedical engineering.
Up to date, he has published over 110 papers in international peer-reviewed journals such as Nano letters, Adv. Fun. Mater., Cancer Res., Biosesnors & Bioelectronics, Biomaterials, etc., and his papers were cited over 1800 times, High-Index is 22. So far, he is a chief scientist of nano 973 project.

REPRESENTATIVE PUBLICATIONS

- 1. Zhi, X., Liu, Q., Zhang, X., Zhang, Y., Feng, J., and **Cui, D**. (2012) Quick genotyping detection of HBV by giant magnetoresistive biochip combined with PCR and line probe assay, Lab on a chip 12, 741-745.
- 2. Zhang, F., Braun, G. B., Pallaoro, A., Zhang, Y., Shi, Y., **Cui, D**., Moskovits, M., Zhao, D., and Stucky, G. D. (2012) Mesoporous Multifunctional Upconversion Luminescent and Magnetic „Nanorattle" Materials for Targeted Chemotherapy, Nano Letters 12, 61-67.
- 3. Huang, P., Li, Z., Lin, J., Yang, D., Gao, G., Xu, C., Bao, L., Zhang, C., Wang, K., Song, H., Hu, H., and **Cui, D**. (2011) Photosensitizer-conjugated magnetic nanoparticles for in vivo simultaneous magnetofluorescent imaging and targeting therapy, Biomaterials 32, 3447-3458.
- 4. Huang, P., Bao, L., Zhang, C., Lin, J., Luo, T., Yang, D., He, M., Li, Z., Gao, G., Gao, B., Fu, S., and **Cui, D**. (2011) Folic acid-conjugated Silica-modified gold nanorods for X-ray/CT imaging-guided dual-mode radiation and photo-thermal therapy, Biomaterials 32, 9796-9809.
- 5. He, M., Huang, P., Zhang, C., Hu, H., Bao, C., Gao, G., He, R., and **Cui, D**. (2011) Dual Phase-Controlled Synthesis of Uniform Lanthanide-Doped NaGdF4 Upconversion Nanocrystals Via an OA/Ionic Liquid Two-Phase System for In Vivo Dual-Modality Imaging, Advanced Functional Materials 21, 4470-4477.
- 6. He, M., Huang, P., Zhang, C., Chen, F., Wang, C., Ma, J., He, R., and **Cui, D**. (2011) A general strategy for the synthesis of upconversion rare earth fluoride nanocrystals via a novel OA/ionic liquid two-phase system, Chemical Communications 47, 9510-9512.
- 7. Chen, L., Zheng, J., Zhang, Y., Yang, L., Wang, J., Ni, J., **Cui, D**., Yu, C., and Cai, Z. (2011) Tumor-specific Expression of MicroRNA-26a Suppresses Human Hepatocellular Carcinoma Growth via Cyclin-dependent and -independent Pathways, Molecular Therapy 19, 1521-1528.
- 8. Chen, L., Bao, C.-C., Yang, H., Li, D., Lei, C., Wang, T., Hu, H.-Y., He, M., Zhou, Y., and **Cui, D**.-X. (2011) A prototype of giant magnetoimpedance-based biosensing system for targeted detection of gastric cancer cells, Biosensors and Bioelectronics 26, 3246-3253.
- 9. Song, H., He, R., Wang, K., Ruan, J., Bao, C., Li, N., Ji, J., and **Cui, D**. (2010) Anti-HIF-1 alpha antibody-conjugated pluronic triblock copolymers encapsulated with Paclitaxel for tumor targeting therapy, Biomaterials 31, 2302-2312.
- 10. Kong, Y., Chen, J., Gao, F., Li, W., Xu, X., Pandoli, O., Yang, H., Ji, J., and **Cui, D**. (2010) A Multifunctional Ribonuclease-A-Conjugated CdTe Quantum Dot Cluster Nanosystem for Synchronous Cancer Imaging and Therapy, Small 6, 2367-2373.
- 11. Huang, P., Lin, J., Li, Z., Hu, H., Wang, K., Gao, G., He, R., and **Cui, D**. (2010) A general strategy for metallic nanocrystals synthesis in organic medium, Chemical Communications 46, 4800-4802.
- 12. Li, Z., Huang, P., Zhang, X., Lin, J., Yang, S., Liu, B., Gao, F., Xi, P., Ren, Q., and **Cui, D**. (2009) RGD-Conjugated Dendrimer-Modified Gold Nanorods for in Vivo Tumor Targeting and Photothermal Therapy, Molecular Pharmaceutics 7, 94-104.

• 13. Pan, B., **Cui, D**., Sheng, Y., Ozkan, C., Gao, F., He, R., Li, Q., Xu, P., and Huang, T. (2007) Dendrimer-modified magnetic nanoparticles enhance efficiency of gene delivery system, Cancer Research 67, 8156-8163.
• 14. Ao, L. M., Gao, F., Pan, B. F., He, R., and **Cui, D**. X. (2006) Fluoroimmunoassay for antigen based on fluorescence quenching signal of gold nanoparticles, Analytical Chemistry 78, 1104-1106.

Marco de Boer

Marco de Boer, Head of Research at to-BBB technologies BV.
Marco holds an MSc degree in chemistry/ molecular biology from the University of Amsterdam, and a Ph.D. degree in molecular biology from the Vrije Universiteit Amsterdam.

Before joining to-BBB in 2010, he headed the Department Bioprocessing at the R&D center of Kerry Ingredients & Flavours in Almere. From 2000 to 2005, Marco was Director Biomolecules at Isogen Life Science, and responsible for R&D and the Biomolecules Production Facility. Before that, he has worked as research manager for Unilever Research.

Neil P. Desai

Neil Desai, PhD is currently Vice President of Strategic Platforms at Abraxis Bioscience / Celgene. Prior to its acquisition by Celgene in Oct 2010, he was Sr. Vice President of Global Research and Development at Abraxis Bioscience, in Los Angeles, California, USA, where he was responsible for the company's growing product pipeline and the development of the company's intellectual property portfolio. Dr. Desai is an inventor of ABI's nanotechnology and nanoparticle-albumin bound (nabTM) drug delivery platform, was primarily responsible for the development of its nanotechnology drug, Abraxane® and the discovery of the novel targeted biological pathway utilized by nab®-drugs. This platform has been clinically proven to enhance the efficacy and safety of cytotoxic drugs through a novel targeted biological pathway and is the first protein-based nanotechnology product to be approved globally for the treatment of cancer.

Prior to his positions at Abraxis, Dr. Desai was Senior Director of Biopolymer Research at 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 more than 20 years of experience in the research and development of novel drug delivery systems and biocompatible polymers. He holds over 100 issued patents and peer-reviewed publications. He has served as reviewer for several scientific journals in the area of cancer therapeutics and drug delivery. He also is an active participant in FDA and EU Nanotechnology initiatives. Dr. Desai holds an 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.

Jacques Descotes

Jacques Descotes, MD, PharmD, PhD

Fellow, US Academy of Toxicological Sciences
Eurotox Registered Toxicologist
Professor of Pharmacology, Lyon-Est School of Medicine, Claude Bernard University, Lyon, France

Head, Poison Center and Pharmacovigilance Department, Lyon University Hospitals

Author or co-author of 10 books, 75 chapters, 230 original scientific papers, 74 review papers, in the area of clinical and nonclinical toxicology with special reference to immunotoxicology and regulatory safety evaluation.

Yaniv Dolev

2/2 Shmuel Avitzur St., Tel Aviv, Israel
Mobile: +972 (52) 8911618
yaniv.dolev@gmail.com

EDUCATION

1998 -1999 M.B.A. – Masters Degree in Agribusiness School of Macro-Economic Studies, Buenos Aires, Argentina
(Received a World Bank scholarship for the best admission exam).

1990 –1996 D.V.M. -Veterinary Surgeon, Faculty of Veterinary Science, National University of La Plata, La Plata, Argentina

EXPERIENCE

2008 – present: Moebius Medical Ltd.
Position: CEO
Moebius is a rheumatology/orthopaedics company that is developing a new injectable intra-articular treatment. Taking the company from In-Vitro models stage through the pre clinical activities to the clinical trials stage, including a fund-raising round.
• Initiate, develop and coordinate strategic alliances with leading pharmaceutical companies, seeking for co-development or licensing agreements.
• Responsible for all business development activities of the company. (toxicology, manufacturing, regulatory, site selection, finding key opinion leader to support the project).
• Perform market research, market analysis and define strategic goals for pre-clinical and clinical activities.
• Manage intellectual property activities and strategy.
• Coordinate all research and development activities of research team developing second generation drug.
• Develop project plan and key objectives for pre-clinical research and submission of patent application.
• Design appropriate models for animal experiments, including the use of various laboratory methods and scientific equipment.
• Responsible for budget planning.

Abraham J. Domb

Abraham J. Domb is a Professor for Medicinal Chemistry and Biopolymers at the Faculty of Medicine of the Hebrew University, Jerusalem, Israel. He earned Bachelors degrees in Chemistry, Pharmaceutics and Law studies and PhD degree in Chemistry from The Hebrew University. He did his postdoctoral training at MIT and Harvard Univ. USA and was R&D manager at Nova Pharm. Co. Baltimore US during 1988-1991. Since 1991 he is a faculty member at the Hebrew university with interests in biopolymers, medicinal chemistry and forensic sciences.

Mike Eaton

After a postgraduate training in nucleic acid chemistry Mike Eaton worked in research in the Pharma industry for more than 35 years. At GD Searle he headed the team that synthesised the gene for Urogastrone and was the first to sequence and express human beta fibroblast interferon in E.coli. He was a founding member of Celltech as Head of Chemistry in 1980; later acquired by UCB. He has worked on a number of marketed drugs - Mylotarg in 2000, the first Antibody drug conjugate and Cimzia in 2009; the first PEGylated antibody. Unusually he has worked with both small molecules and large molecules, including DNA at a technical as well as at a strategic level. He built the first automated DNA synthesiser in Europe, which is now owned by the Science Museum in London. This machine was used for the first cloning of pre-prochymosin, a key ingredient in cheese-making. He has worked on low molecular weight drugs including the first non-emetic PDEIV inhibitor and synthetic vectors for gene therapy. He has maintained his interest in nucleic acid based therapeutics and believes this will be an important class once the delivery issues have been solved.

Mike is an active special professor at Nottingham University and has been an executive board member of the European Technology Platform for Nanomedicine, since its inception in 2005.This is a large network of academics and industries, being the European working group chair for Nano-therapeutics. He left UCB in February 2010 and is now a strategic and technical adviser to a number of large and small companies and organisations, including VCs. His particular interest is commercial translation of nanotechnology into Nanomedicines – real medicines to help patients.

Inter alia he is on the scientific advisory board of Future Medicinal Chemistry, Nanomedicine and Nanomedicine J: Nanotechnology, Biology, and Medicine and CLINAM (www.clinam.org) .

Since leaving UCB, as part of the ETP he has tried to improve the design and translation of open innovation. As part of this initiative he has been involved in publishing a white paper for the EC in 2011 http://www.etp-nanomedicine.eu/public/news-events/news/etpn-white-paper-on-improving-translation-of-public-healthcare-nano-research-in-europe. More technical details including milestones are provided in Nanomedicine -NBM 7 (2011) 371–375 including an online supplement, this is now a key, albeit basic source for SMEs.

As a contributor to the prestigious Else Kröner-Fresenius Symposium in Germany he was invited to contribute in 2011 a chapter to Nanomedicine – Basic and Clinical Applications in Diagnostics and Therapy. With the assistance of John Adair this was published in-Else Kröner-Fresenius Symp. Basel, Karger, 2011, vol 2, pp 185–196.

With a background in both small and large molecules he has contributed to Future Medicinal Chemistry both as a reviewer and as an author Future Med. Chem. (2011) 3(15). His early hands-on pioneering knowledge of developing ADCs has been much sought, after this technology has recently been successfully re-evaluated in the clinic.

Lastly he has experience in the courts on litigation relating to IP, having also filed a large number of patents as well as publications over the years.

Rutledge Ellis-Behnke

Professor Rutledge Ellis-Behnke is the Director of the Nanomedicine Translational Think Tank at the Medical Faculty Mannheim of the University of Heidelberg in Germany. In addition, he is Research Affiliate in the Brain and Cognitive Sciences department at the Massachusetts Institute of Technology.

Previously he was Associate Professor in the Faculty of Medicine at the University of Hong Kong, as well as Associate Director of the Technology Transfer Office.

Ellis-Behnke's research is focused on reconnecting the disconnected parts of the brain—with the goal of being able to provide a prescription to restore quality of life after brain or spinal cord trauma, or stroke. In animals he was the first to repair the brain showing reversal of blindness; to stop bleeding in less than 15 seconds without clotting; to preserve stem cells; and to immobilize prostate cancer stem cells.

He has more than 100 patent applications and his "Nano Neuro Knitting" and "Immediate Hemostasis" technologies have each been licensed to companies for translation to humans. Technology Review named his "Nanohealing" discoveries one of the "Top 10 Emerging Technologies."

Ellis-Behnke received a PhD from MIT in Neuroscience; a Bachelor of Science from Rutgers University and graduated from Harvard Business School's Advanced Manager's Program (AMP).

Ellis-Behnke is an Associate Editor for the journal Nanomedicine and is on the Editorial Board of Nanomedicine & Biotherapeutic Discovery. He is on both the Executive and Scientific Advisory Boards of the Glaucoma Foundation; and is on the Executive Board of the Asia Foundation for Cancer Research.

Noam Emanuel

Dr. Emanuel holds a Ph.D. degree from the Faculty of Medicine at the Hebrew University of Jerusalem, Israel. His main experience is in the development of drug delivery systems and immunology. Dr. Emanuel is widely experienced in different biotechnological industrial projects in various areas, including immunotherapy, vaccines, immunodiagnostics, systemic and local drug-delivery and medical devices. Dr. Emanuel is the co-founder and the first CEO of PolyPid Ltd., founded in 2008. Dr. Emanuel is currently serving as the CTO of PolyPid Ltd.

Sandra Erb

Sandra Erb is a graduate of Wittenburg University (B.A., Chemistry) and has completed the course work for an M.B.A. at New York University. Prior to joining Technology Catalysts in 1989, she held positions in Research at a major healthcare company and positions in Technology & Business Assessment, Mergers & Acquisitions, and Marketing at a major chemical company.

Ms. Erb has over 20 years experience consulting in the drug delivery, pharmaceutical (Rx, OTC, biopharmaceutical), specialty and fine chemical, and food and consumer products industries. Ms. Erb has overall program management responsibility for TCI's consulting activities for pharmaceutical, drug delivery, and consumer care clients worldwide. In addition, she participates as a team member in selected special projects on behalf of clients.

RECENT PRESENTATIONS AND PUBLICATIONS INCLUDE:

• Entry Strategies for Specialty Pharmaceutical Companies in BRICKTM Countries (Drug Delivery Partnerships 2012, Las Vegas, NV USA)
• Future of Drug-Device Combinations (BIT's 1st Annual Symposium on Drug Delivery 2011, Shenzhen China)
• Innovative Delivery of Actives (CPhI 2011, Frankfurt Germany)
• Nanotechnology in drug delivery Japan Society of Drug Delivery Systems Vol.24, January 2009

Christoph Eymann

Born in Basel, 1951

AFTER HIGH SCHOOL

1970 studies in Medicine
1973 changing to the Faculty of Jurisprudence, University of Basel
1978 diploma in Jurisprudence
1980 LLD, Doctor of Laws

1980 – 1984 jurist, Federation of employers, Basel.
1984 – 2001 director of the Basel Union of small and medium sized enterprises.

POLITICAL ACTIVITIES

1981 member of the municipal Council.
1984 to 1995 member of the Cantonal Council.
1991 to 2001 member of the National Parliament (National Council).
1999 to 2001 member of the Congress of Constitution, Canton of Basel-Stadt.
October 2000: elected into the Governing Council of the Canton of Basel-Stadt.
Married to Corinne Eymann-Baier, one daughter. Two children (daughter and son) with Patricia von Falkenstein.

Bengt Fadeel

Bengt Fadeel holds M.D. and Ph.D. degrees from Karolinska Institutet in Stockholm. He is Full Professor of Medical Inflammation Research and Head of the Division of Molecular Toxicology at the Institute of Environmental Medicine, Karolinska Institutet and Adjunct Professor of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA, USA. He also serves as Vice Chairman of the Institute of Environmental Medicine. Fadeel has participated as coordinator or partner in several nanosafety projects funded through the Seventh Framework Programme of the European Commission including FP7-NANOMMUNE and FP7-MARINA. He was the main organizer of the 1st (2006) and 2nd (2010) Nobel Forum Mini-Symposium on Nanotoxicology, Karolinska Institutet, and the 6th Key Symposium on Nanomedicine, Stockholm (2009), and he is a co-organizer of the annual Autumn School on Nanosafety in Venice, Italy. He is editor of the recent publication (2012) "Adverse Effects of Engineered Nanomaterials: Exposure, Toxicology, and Impact on Human Health" (Elsevier). Fadeel was awarded the national Environmental Medicine Prize (2011) for bringing attention to the potential risks and medical opportunities of nanotechnology.

Elias Fattal

Elias Fattal is a full professor in Drug Delivery Science at the University of Paris-Sud 11 in Châtenay-Malabry, France and has been President of APGI from 2003 to 2010. He received his Pharmacy Degree (1983) and Ph.D. (1990) from the University of Paris-Sud 11 and followed an internship in Pharmacy at the University of Lille (1984-1986). After visiting the Department of Pharmaceutical Chemistry at the University of California, San Francisco for a post-doctoral position (1990-1991), he became associate Professor (1992) and full Professor at the University of Paris-Sud 11 (2000). Elias Fattal is the director of the UMR CNRS 8612, a research unit dealing with the design of nanomedicines where he is also leading one of the research group "Drug targeting and delivery of poorly stable drugs". His research activity deals with the design of nano and microtechnologies for the delivery of peptides/proteins, nucleic acids to the eye and lungs, for the formulation of imaging contrast agents for theranostics. Recent studies are related to nanotoxicology to the lungs. He has received the Pharmaceutical Sciences World Congress (PSWC) Research Achievement Award in 2007. He has also been elected Honour member of APV (Arbeitsgemeinschaft Pharmazeutische Verfahrenstechnik) in 2005. He is an elected member of the French National Academy of Pharmacy. He serves in the editorial board of several Pharmaceutical Sciences Journals (Journal of Pharmaceutical Sciences, European Journal of Pharmaceutical Sciences, Journal of Drug Delivery science and Technology and Expert Opinion on Drug Delivery) and the International Journal of Nanomedecine. He has been regularly appointed as an expert at the European Union as member of the working group on Nanotechnology "Emerging and Newly Identified Health Risks (SCENIHR)") or the working group on Nanosubstances in Cosmetics du Scientific Committee on Consumer Products (SCCP).

Xavier Fernàndez Busquets

Date and place of birth: July 13, 1963, Barcelona, Spain. Present positions and affiliations: (1) Associate Researcher, Biomolecular Interactions Team, Nanobioengineering Group, Institute for Bioengineering of Catalonia (IBEC), Barcelona Science Park, Baldiri Reixac 10-12, E-08028 Barcelona, Spain. (2) Assistant Research Professor, Nanomalaria Group, Barcelona Centre for International Health Research (CRESIB), Rosselló 132, E-08036 Barcelona, Spain. Work phone: +34 93 403 7180 Work fax: +34 93 403 7181, e-mail: xfernandez_busquets@ub.edu

CURRENT RESEARCH: NANOBIOMEDICINE

1. Single-molecule studies of proteoglycan and glycosaminoglycan interactions.
2. Application of nanotechnology to the study of functional amyloids.
3. 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: 54, Coference Contributions: 102

Marianna Foldvari

Dr. Marianna Foldvari is the Canada Research Chair in Bionanotechnology and Nanomedicine. She is also a Professor of Pharmaceutical Sciences and served as the Associate Director, Research and Graduate Studies in the past 4 years, at the University of Waterloo's School of Pharmacy. Dr. Foldvari's expertise is in pharmaceutics, dosage form and drug delivery system design, nanotechnology, non-viral delivery methods, vaccine development, and computational modeling. She has over 20 years of experience as an academic researcher and in research and development in the pharmaceutical industry through technology transfer activities. Her research program is focusing on non-invasive drug delivery, gene therapy and pharmaceutical development of nano-enabled products. Dr Foldvari's research is supported by grants from Canadian Institutes of Health Research (CIHR), Canada Foundation for Innovation (CFI) and Natural Sciences and Engineering Research Council of Canada (NSERC). Dr Foldvari is a member of the American Association for the Advancement of Science, American Association of Pharmaceutical Scientists, American Society for Gene and Cell Therapy and the Controlled Release Society. She currently serves as Associate Editor of Nanomedicine: Nanotechnology, Biology and Medicine and perform editorial activity for the Journal of Nanomedicine and Biotherapeutic Discovery, Current Patents in Nanomedicine, Journal of Bionanoscience, Current Drug Delivery, and International Journal of Pharmaceutical Compounding. Dr Foldvari served as a grant reviewer on CIHR, NSERC, CFI and NIH panels and the Bill and Melinda Gates Foundation Global Health Initiatives review board. Dr Foldvari is one of the Founding Directors of the American Society for Nanomedicine (ASNM) and the International Society of Nanomedicine (ISNM). She served as Board Member for Genome Prairie and was a Member of the Advisory Committee to the Prime Minister on Science and Technology and a Founder of the Canadian Society of Pharmaceutical Sciences (CSPS). She has received the YWCA Women of Distinction Award and the Sabex Award of Innovation. She is a frequently invited speaker at national and international conferences on gene and protein delivery systems and nanomedicine development and applications. Dr. Foldvari has authored more than 120 papers and 100 conference presentations and is the inventor on 18 patents. She founded two spin-off companies, PharmaDerm Laboratories Ltd. and DDS Research Inc., which focus on nanomedicine product development to commercialize technologies that she and her research team have developed.

Alberto Gabizon

Alberto Gabizon received his M.D. at the School of Medicine in Granada, Spain, and his doctorate (Ph.D.) in Cell Biology from the Weizmann Institute of Science in Rehovot, Israel. He completed his medical residency and obtained the Israeli board certification in Radiation and Medical Oncology in 1985 at Hadassah Medical Center in Jerusalem. Between 1985-1988, he was a research associate fellow at the Cancer Research Institute of the University of California in San Francisco, U.S.A., where he helped to develop a new generation of long-circulating liposomes known as Stealth liposomes which have greatly improved stability and selective accumulation in tumors. In 1988, Dr. Gabizon returned to Israel, and continued his research and clinical activity at the Hadassah Medical Center until 2001. In 2002, he was appointed Chairman of the Oncology Institute at Shaare Zedek Medical Center, and Professor of Oncology at the Hebrew University-Faculty of Medicine in Jerusalem, his current title.
Dr. Gabizon is active in the medical oncology field, and in preclinical pharmacology research with special emphasis on applications of liposomes in drug delivery, targeting, and experimental cancer therapy, and has published around 135 original articles and specialized book chapters.
Dr. Gabizon's research contribution placed a central role in the development of pegylated liposomal doxorubicin (known as Doxil or Caelyx), a unique anticancer formulation extensively used in the clinic with important advantages over conventional chemotherapy. He recently founded LipoMedix Pharmaceuticals Inc., a start-up company aimed at developing further his inventions in the field of cancer nanomedicine.

Rogério Gaspar

Born Lisboa (Portugal), 29th August 1961

Professional address: Faculdade de Farmácia da Universidade de Lisboa
Av. Prof Gama Pinto, 1649-003 Lisboa, Portugal Tel +351-217946400 (ext.14210) Mobile +351-918269217 Fax: +351-217937703 E-mail: rgaspar@ff.ul.pt

• Full Professor, Faculty of Pharmacy at the University of Lisboa (FFUL), Portugal
• Head of "Nanomedicine & Drug Delivery Systems" research group at iMed.UL (Research Institute for Medicines and Pharmaceutical Sciences at the University of Lisboa)
• Head of Departament of Pharmaceutical Technology at FFUL (2011-2014) Member (elected) at the General Council from the University of Lisboa (2008-2012) Member (elected) at General Assembly and also of the Scientific Council both at FFUL (2009-2013)
• Member of the Executive Committee of EUFEPS (European Federation of Pharmaceutical Sciences, 2009-2011) and currently its Vice-President (2011-2013)
• Member of Scientific Advisory Board of CIBBER-BBN (Publicly funded Research Network in Biomaterials, Bioengineering and Nanomedicine, Spain) Member of the External Advisory Board of EuroNanoMed (ERA-NET Nanomedicine, FP7)

ACADEMIC QUALIFICATIONS

• 1985 PharmD in Pharmaceutical Sciences (Industrial Pharmacy), University of Coimbra, Portugal
• 1991 "Docteur ès Sciences Pharmaceutiques" (La plus grande distinction avec les félicitations du júri), Université Catholique de Louvain, Brussels, Belgium
• 1999 Habilitation ("Agregação") in Pharmacy, University of Coimbra, Portugal

CARREER

Professional/Academic
• 1984 - 1985 Monitor, Faculty of Pharmacy University of Coimbra (FFUC)
• 1985 - 1991 Training-assistant and Assistant, FFUC
• 1991 - 1998 Assistant Professor, FFUC
• 1998 - 2006 Associate Professor, FFUC
• 2006 - 2007 Associate Professor, Faculty of Pharmacy at the University of Lisboa (FFUL)
• since 2007 Full Professor, FFUL

Professional/Regulatory Affairs
Portugal
• 1995-1996 Member of National Medicines Committee, CTM (INFARMED)
• 1996-1999 Member of CTM, new nomination (INFARMED)
Note: self-suspended in 1999 to act as diplomatic counsellor at the REPER (Permanet Representation of Portugal at the European Union,

Brussels), active up to 2008 due to exercise of political or private mandates (2000-2002: Vice-President of INFARMED national regulatory agency; 2002-2008 . Consultant at the Pharmaceutical Industry).
• 1996-1999 Vice-President of CTM (INFARMED)
• 1998-2002 Member of Committee for Research Activities (CRAF) at INFARMED
• 2000-2002 Vice-President of Management Board of INFARMED
• 2000-2002 Member of Management Board of LEMES (Laboratoty for Studies & Metrology in Health, Portuguese, Notified Body to the European Union for Medical Devices Certification (CE mark), representing INFARMED)
• 2001-2002. Coordinator for strategic plan of implementation of Quality Management System at INFARMED, ISO 9001:2000 certification obtained in 2002 for INFARMED (Licensing and Inspection Department).
• 2008-2011 Member of National Medicines Committee (CAM) at INFARMED, self-suspended in June 2011 to initiate a consultancy business.

European Medicines Agency (EMEA, now EMA) & European Union
• 1995-2002 Expert of EMEA (European Medicines Evaluation Agency)
• 1997-1999 Member of QWP (Quality Working Party - EMEA) • 1998-1999 Member of CPMP (Committee for Proprietary Medicinal Products, CPMP-EMEA)
• 1999-2002 Member of Working Group of Medicines at the European Union Council, acted has its President during the Portuguese rotating presidency of the EU in the first semester of 2000 – in that period was the responsible coordinator for the political agreement at the Council of Ministers of the EU concerning the Clinical Trials Directive (approved at the European Parliament and published in 2001)
• 2000-2002 Member of Management Board at EMEA
• 2000-2002 Member and participant of various committees related with the activities of INFARMED within the European Union regulatory network and EU institutions (total of 12 working groups)
• 2009-2011 Member of the adhoc expert group in Nanomedicines at EMA (European Medicines Agency, ex-EMEA)

Global
• 2002 Member of the EU delegation that started the implementation of Mutual Recognition Agreement (MRA) between Japan and the European Union (Tokyo, March 2002)
• 2002 Member of expert group that elaborated a project for Cape Vert regulatory agency (contract with EU and World Bank)
• 2005 Consultant for regulatory authorities for medicinal products at ASEAN in GMPs and Quality Management Systems.
• 2006 Consultant for ASEAN and Republic of Indonesia for GMPs and Quality Management Systems.

Professional/Coordination functions at national or international level
• 1993-1997 Coordinator of an European Network Erasmus/PIC-P3017/12 (Galenos) for mobility of students and teachers, as well as intensive programs in drug delivery systems (18 Universities of 9 countries of EU12).
• 1999-2000 Member of the Board at Portuguese Society of Pharmaceutical Sciences (SPCF, member of EUFEPS)
• 2002-5 President of the Spanish-Portuguese Local Chapter of the Controlled Release Society (SPLC/CRS)
• 2004-5 Expert of the European Science Foundation (ESF) Look Forward Initiative in Nanomedicine.
• Since 2005 Member of the Board at Portuguese Society of Pharmaceutical Sciences (SPCF, member of EUFEPS) and since 2011 its Vice-President.
• 2006 Co-chairman da 1st ESF European Research Conference in Nanomedicine, Barcelona, Spain
• 2007 Co-chairman da 1st ESF European Summer School in Nanomedicine, Cardiff, UK
• 2008 Chairman da 2nd ESF European Research Conference in Nanomedicine, Barcelona, Spain
• 2009 Chairman da 2nd ESF European Summer School in Nanomedicine, Quinta da Marinha (Cascais), Lisboa, Portugal
• 2011 Co-chair of 3rd ESF European Summer School in Nanomedicine, Halle-Wurtemberg, Germany
• Since 2009 Member of the Executive Committee of the European Federation of Pharmaceutical Sciences (EUFEPS) and since 2011 its Vice-President

Professional/other
• 2002-8 Consultant Tecnimede, pharmaceutical company (for R&D)
• 2008-11 Consultant ANF – Associação Nacional das Farmácias (business area not related to medicines)
• Since 2011 Owner of a consultancy business "ROGERIO SA GASPAR, Pharma Consulting Unipessoal Lda"
Member of the Editorial Board of national and international publications in Health and Nanomedicine

RESEARCH INTERESTS AND PUBLICATIONS

All along more than 27 years his research interests focused in drug delivery systems and nanomedicine. In the University of Coimbra started the first group in this field (second ever in Portugal) in 1993. His work developed in the areas of polymeric nanoparticles and liposomes (e.g. Leishmaniasis and Cancer) as well as non-viral vectors for delivery of nucleic acids ("cytosolic delivery"). Research interests included also imaging (NMR) and ocular delivery. Basic research was associated to celular interactions of coloidal systems and currently more focused in its relevance for oncology using targeted combinatorial systems.

SELECTED PUBLISHED WORK

• M Videira and **R Gaspar** - Patentability and Intellectual Property Issues Related to Chitosan-Based Biopharmaceutical Products – Chapter 25 in Sarmento, Bruno & das Neves, Jose (editors) "Chitosan-based Systems for Biopharmaceuticals: Delivery, Targeting and Polymer Therapeutics" John Wiley and Sons Ltd. ISBN: 978-0-47097-832-0 (Hardback, 584 pages), Publication date: March 2, 2012
• R Duncan and **R Gaspar** - Nanomedicine(s) under the microscope, Molecular Pharmaceutics (2011) 8(6): 2101-2141
• **R Gaspar** – Medicamento: a regulação entre a inovação & as políticas de Saúde, in "Cadernos Saúde & Sociedade" (nº2 - O Medicamento e o Sistema de Saúde), pp 141-147, Edição "Diário de Bordo", ISBN 978-989-97087-3-0
• **R Gaspar** – Therapeutic products: regulating drugs and medical devices. Chapter 14 in "International Handbook on Regulating Nanotechnologies", editors Graeme Hodge, Diana Bowman and Andrew Maynard, Edward Elgar Publishing, 2010, pp 291-320, ISBN 978-1-84844-673-1.
• **R Gaspar**, R. Duncan – Polymeric carriers: Preclinical safety and the regulatory implications for design and development of polymer therapeutics. Advanced Drug Delivery Reviews (2009), 61: 1220-1231
• **R Gaspar** - The regulatory landscape: implications for design and development of nanomedicines. Journal of Pharmacy and Pharmacology 61 (Suppl. 1): A148-A149 (2009)
• **R. Gaspar** - Inovação Farmacêutica, equidade no acesso e sustentabilidade financeira, in "Financiamento: inovação e sustentabilidade". edição APDH, Coordenação Ana Escoval, pags. 374-383, ISBN 978-989-95247-1-2 (2008)
• **R. Gaspar** – Regulatory issues surrounding nanomedicines: setting the scene for the next generation of nanopharmaceuticals. Nanomedicine 2, 143-147 (2007)
• J.N. Moreira, **R. Gaspar** – Antagonist G-mediated targeting and cytotoxicity of liposomal doxorubicin in NCI-H82 variant small cell lung cancer. Brazilian Journal of Medical and Biological Research 37, 11851192 (2004)
• J.N. Moreira, **R. Gaspar**, T.M. Allen — In vivo targeting of stealth liposomes against human small cell lung cancer. Journal of Liposome Research 13, 76-77 (2003)
• C. Fonseca, S. Simões, **R. Gaspar** — Paclitaxel-loaded PLGA nanoparticles: preparation, physicochemical characterization and in vitro anti-tumoral activity. Journal of Controlled Release 83, 273-286 (2002)
• J.N. Moreira, T.Ishida, **R. Gaspar**, T.M. Allen — Use of the post-insertion technique to insert peptide ligands into pre-formed stealth liposomes with retention of binding activity and cytotoxicity. Pharmaceutical Research 19, 265- 269 (2002)
• J.N. Moreira, **R. Gaspar,** T.M. Allen — Targeting Stealth liposomes in a murine model of human cell lung cancer. Biochimica-Biophysica Acta 1515, 167-176 (2001)
• J.N. Moreira, C.B. Hansen, **R. Gaspar**, T.M. Allen — A growth factor antagonist as a targeting agent for sterically stabilized liposomes in human small cell lung cancer Biochimica Biophysica Acta 1514, 303-317 (2001)

• S. Simões, V. Slepushkin, **R. Gaspar**, M.C. Pedroso de Lima and N. Düzgünes — Human serum albumin enhances DNA transfection by lipoplexes and confers resistance to inhibition by serum. Biochimica Biophysica Acta, 1463, 459-469 (2000)
• **R Gaspar** – European drugs: Europe faces the future. Journal de Pharmacie de Belgique 55(3): 69-73 (2000)
• P. Pires, S. Simões, S. Nir, **R. Gaspar**, N. Düzgünes and M.C. Pedroso de Lima — Interaction of cationic liposomes and their DNA complexes with monocytic leukemia cells. Biochimica Biophysica Acta, 1418, 71-84 (1999)
• S. Simões, V. Slepushkin, E. Pretzer, **R. Gaspar**, M.C. Pedroso de Lima and N. Düzgünes — Transfection of human macrophages by lipoplexes via the combined use of transferring and pH-sensitive peptides. Journal of Leukocyte Biology, 65, 270-279 (1999)
• M. C. Pedroso de Lima, S. Simões, P. Pires, **R. Gaspar**, V. Slepushkin and N. Düzgünes — Gene delivery mediated by cationic liposomes: from biophysical aspects to enhancement of transfection. Molecular Membrane Biology, 16, 103-109 (1999)
• S. Simões, V. Slepushkin, P. Pires, R. **Gaspar,** M.C. Pedroso de Lima and N. Düzgünes — Mechanisms of gene transfer mediated by lipoplexes associated with targeting ligands or pH-sensitive peptides. Gene Therapy, 6, 1798- 1807 (1999)
• S. Simões, V. Slepushkin, **R. Gaspar**, M.C. Pedroso de Lima and N. Düzgünes —Successful transfection of lymphocytes by ternary lipoplexes. Bioscience Reports, 19, 601-609 (1999)
• S.Simões, V.Slepushkin, **R.Gaspar,** M.C.Pedroso de Lima and N.Düzgünes — "Gene delivery by negatively charged ternary complexes of DNA, cationic liposomes and transferrin or fusogenic peptides". Gene Therapy, 5: 955-964 (1998)
• M.B.F. Martins, A.Supico, S.I.D. Simões, **R. Gaspar**, M.E.M. Cruz — "An analytical methodology to quantify the incorporation of enzymes in polyalkylcyanoacrylate nanoparticles based on size exclusion chromatography — Journal of Pharmaceutical and Biomedical Analysis 15: 811-818 (1997)
• S.Simões, V.Slepushkin, E.Pretzer, **R.Gaspar**, M.C. Pedroso de Lima and N.Düzgünes — "Transfection of human blood monocyte-derived macrophages by transferrin-cationic lipid-DNA complexes", FASEB J. 11:9, Abst.1090 (1997)
• P.Pires, S.Simões, **R.Gaspar**, S. Nir, N.Düzgünes and M.C.Pedroso de Lima — "Interaction of cationic liposomes with cells: effect of several factors on liposome-cell fusion", FASEB J. 11:9, Abst.1266 (1997)
• T. Cruz, **R. Gaspar**, A. Donato, C. Lopes, "Interaction between polyalkylcyanoacrylate nanoparticles and peritoneal macrophages: MTT metabolism, NBT reduction and NO production" — Pharmaceutical Research 14 (1): 73-79 (1997)
• M.B.F.Martins, S.I.D.Simões, A.Supico, M.E.M.Cruz, **R. Gaspar,** "Enzyme-loaded PIBCA nanoparticles (SOD and L-ASNase): optimization and characterization. International Journal of Pharmaceutics 142: 7584 (1996)
• M.B.F. Martins, S.I.D.Simões, M.E.M. Cruz, **R. Gaspar** — "Development of enzyme loaded nanoparticles: effect of pH." Journal Materials Science - Materials in Medicine 7: 413-414 (1996)
• C. Lourenço, M. Teixeira, S. Simões, **R. Gaspar** — Steric stabilization of nanoparticles: size and surface properties. International Journal of Pharmaceutics 138: 1-12 (1996)
• **R. Gaspar**, V. Préat, F. Opperdoes, M. Roland — "Macrophage activation by polymeric nanoparticles of polyalkylcyanoacrylates: activity against intracellular Leishmania donovani is associated with hydrogen peroxide production" Pharmaceutical Research 9 (6): 782-787 (1992)
• **R. Gaspar**, F. Opperdoes, V. Préat, M. Roland — "Drug targeting with polyalkylcyanoacrylate nanoparticles: in vitro activity of primaquine-loaded nanoparticles against intracellular Leishmania donovani " Annals of Tropical Medicine and Parasitology 86(1): 41-49 (1992)
• **R. Gaspar** — "Nanoparticulas de poli-alquilcianoacrilato e vectorização de medicamentos", Revista Portuguesa de Farmácia XLI (3): 36-45 (1991)
• **R. Gaspar,** V. Préat, M. Roland — "Nanoparticles of polyisohexylcyanoacrylate (P.I.H.C.A.) as carrier of primaquine:
formulation, physico-chemical characterization and acute toxicity" International Journal of Pharmaceutics 68: 111-119 (1991)
• **R. Gaspar**, V. Préat, F. Opperdoes, M. Roland — "Drug targeting in visceral leishmaniasis with polymeric nanoparticles: pharmaceutical development and in vitro anti-leishmanial activity" Journal de Pharmacie Belgique 45(1):73 (1990).

Ehud Gazit

Ehud Gazit is a Professor at the Department of Molecular Microbiology and Biotechnology, Tel Aviv University and the incumbent of the Chair for Nano-Biology. From 2008-2012 Gazit served as Tel Aviv University Vice President for Research and Development and the Chairman of the board of directors of Ramot Ltd, the technology transfer company of Tel Aviv University. Prior to his appointment as Vice President, Gazit served in different academic and administrative positions at Tel Aviv University, including the Head of The Chemistry-Biology double major track, a member of the University Committee for Appointments and Promotions, the Head of the Academic Committee of the Ilona Rich Institute for Nano-Biology and Nano-Biotechnology, and a member of the managing board of the Center for Nanoscience and Nanotechnology.
Gazit received his B.Sc. (summa cum laude) after completing his studies at the Special Program for Outstanding Students of Tel Aviv University (Currently the Adi Lautman program), and his Ph.D. (with highest distinction) as a Clore Fellow at the Department of Membrane Research and Biophysics, Weizmann Institute of Science in 1997. For his Ph.D. work, he received the John F. Kennedy Award in 1996. He has been a faculty member at Tel Aviv University since 2000, after completing his postdoctoral studies as an European Molecular Biology Organization (EMBO) and Human Frontiers Science Program (HFSP) fellow at Massachusetts Institute of Technology (MIT) where he also held a visiting appointment (2002-2011).

Christoph Gerber

Christoph Gerber is the Director for Scientific Communication of the NCCR (National Center of Competence in Research Nanoscale Science) at the Department of Physics, University of Basel, Switzerland, and a founding member of the NCCR. He was formerly a Research Staff Member in Nanoscale Science at the IBM Research Laboratory in Rueschlikon, Switzerland, and has served as a project leader in various programs of the Swiss National Science Foundation and in the European Framework 6.
For the past 30 years, his research has been focused on Nanoscale Science. He is a pioneer in Scanning Probe Microscopy, and he made major contributions to the invention of the Scanning Tunneling Microscope and the Atomic Force Microscope (AFM), he is also a co-inventor of Biochemical sensors based on AFM Technology.
He is the author and co-author of more than 160 scientific papers that have appeared in peerreviewed journals and has been cited more than 23'000 times in cross-disciplinary fields. He belongs to the one hundred worldwide most cited researchers in Physical Sciences. He has given numerous plenary and invited talks at international conferences. His work has been recognized with multiple honorary degrees and various awards and appeared in numerous articles in daily press and TV coverage. He is a Fellow of the American Physical Society, a Fellow of the World Technology Network and a Fellow of the IOP Institute of physics UK. He serves in the advisory board of several nano institutes and has chaired and co-chaired various international conferences. His IP portfolio contains 37 patents and patent publications. His private interest range from literature (scientific and a good novel) to art and sports (he is a passionate skier and plays an acceptable round of golf).

HIS CURRENT INTERESTS INCLUDE

• Biochemical sensors based on AFM Technology
• Chemical surface identification on the nanometer scale with AFM
• Nanomechanics, nanorobotics, and molecular devices at the ultimate limits of measurement and fabrication
• Atomic Force microscopy research on insulators
• Single Spin Magnetic Resonance Force Microscopy (MRFM)
• Self-organization and self-assembly at the nanometer scale

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

Nahum Goldberg

S. Nahum Goldberg, M.D., FSIR
Professor of Radiology
Harvard Medical School / Hebrew University-Hadassah Medical Center

POSITIONS AND EMPLOYMENT

• 1992-1993 Internal Medicine Internship - Hospital of St. Raphael, New Haven, CT
• 1993-1997 Diagnostic Radiology Residency, Massachusetts General Hospital, Boston, MA
• 1997-1998 Abdominal Imaging and Intervention Clinical Fellowship and RSNA Research Fellowship, Massachusetts General Hospital, Harvard Medical School, Boston, MA
• 1998-1999 Instructor in Radiology, Harvard Medical School, Boston,
• 1998-present Staff Radiologist, Abdominal Imaging / Intervention, Beth Israel Deaconess, Boston, MA
• 1999-2001 Assistant Professor in Radiology, Harvard Medical School, Boston, MA
• 1999-2009 Director of CT Intervention and Tumor Ablation Therapy Program, BIDMC, Boston, MA
• 2000-present Director, Minimally Invasive Tumor Therapies Laboratory, BIDMC, MA
• 2001-2007 Associate Professor in Radiology, Harvard Medical School, Boston, MA
• 2006-2009 Director, BIDMC Radiology Residency Scholars Track, Boston MA
• 2006-present Director, Applied Radiology Laboratory, Hadassah Hebrew University, Jerusalem, Israel
• 2007-present Fellow, Society of Interventional Radiology
• 2007-present Professor of Radiology, Harvard Medical School, Boston, MA
• 2008-2009 Visiting Fulbright Scholar, Hadassah Hebrew Univ. Medical Center, Jerusalem, Israel
• 2009-present Section Chief – Image-guided therapy and Interventional Oncology, Dept. of Radiology, Hadassah Hebrew University, Jerusalem, Israel
• 2010-present Professor of Radiology, Hebrew University, Jerusalem, Israel

HONORS

1989	Yale School of Medicine Research Scholarship
1991	American College of Rheumatology Medical Student Award
1995	Society of Thoracic Radiology; Best resident physician manuscript
1996	Radiologic Society of North America Resident Research Award
1997-1998	Caesar Gianturco RSNA Research Fellowship, Harvard Medical School
2001-2003	Radiology – Reviewer with Special Distinction
2007	Society of Interventional Radiology (SIR) Service Award
2010	BIDMC, Harvard Medical School – Excellence in Academic Mentoring Award

SELECTED RECENT PUBLICATIONS

(10 most relevant to the proposal from over 130)

• 1. Goldberg SN, Girnan GD, Lukyanov AN, et al. Percutaneous tumor ablation: Increased necrosis with combined RF and IV liposomal doxorubicin in a rat breast tumor model. Radiol. 2002; 222:797-804
• 2. Monsky WL, et al. Radiofrequency Ablation Increases Intratumoral Liposomal Doxorubicin Accumulation in an Animal Breast Tumor Model. Radiology 2002; 224:823-829
• 3. Goldberg SN, Kamel IR, Kruskal JB, et al. Radiofrequency ablation of liver tumors: Increased tumor destruction with adjuvant liposomal doxorubicin therapy. AJR 2002 179:93-101
• 4. D'Ippollito G, Ahmed M, et al. Increased survival from combined RF ablation and liposomal doxorubicin in an animal model. Radiol. 2003 228:112-18.
• 5. Ahmed M, et al. RF thermal ablation sharply increases intratumoral liposomal doxorubicin accumulation and tumor coagulation. Cancer Res. 2003; 63(19):6327-33.
• 6. Ahmed M, Goldberg SN. Combination RF thermal ablation and adjuvant IV liposomal doxorubicin increases tissue coagulation and tumoral drug accumulation. IJH 2004; 20:781-802
• 7. Solazzo S, et al. RF ablation with adjuvant therapy: Comparison of external beam radiation and liposomal doxorubicin on ablation efficacy in an animal tumor model. Int J Hyperth. 2008 29:1-8.
• 8. Solazzo SA, et al. Liposomal doxorubicin increases RF ablation-induced tumor destruction by increased cellular oxidative/nitrative stress & accelerated apoptotic pathways. Radiol 2010; 255:62-74
• 9. Yang W, Ahmed M, et al. Do liposomal apoptotic enhancers increase tumor coagulation and end-point survival in percutaneous RF ablation of tumors in a rat tumor model? Radiol. 2010;257:685-96.
• 10. Ahmed et. al. RF Ablation Combined with Liposomal Quercetin to Increase Tumor Destruction by Modulation of Heat Shock Protein Production in a Small Animal Model. IJH 2011 [In press]

RECENT GRANT SUPPORT (MAJOR PI GRANTS ONLY)

NCI 1U54CA151881-01, Dates: 1/9/10-31/8/15 NIH

"Combined Cancer Therapy with RF Ablation and Drug-Loaded Nanopreparations" Role: PI

This research project is part of the NEU/BIDMC Center for Cancer Nanaotechnology Excellence. The study goal is to improve tumor destruction by rationally combining image-guided, minimally invasive image-guided radiofrequency (RF) thermal ablation with adjunctive nanotherapies. This includes nanopreparations containing powerful proapoptotic agents such as paclitaxel, cell stress inducers such as GLA and BSO, and quercitin a known down-regulator of HSP.

Israel Science Foundation **#989/11**, Dates 1/10/11-30/9/15

"Combined Cancer Therapy with RF Tumor Ablation and Drug-Loaded Nanopreparations"

Our goal is to improve tumor destruction by rationally combining image-guided, minimally invasive image-guided thermal ablation with adjunctive nanotherapies, including extension into the field of theranostics.

1R01CA112533-01, Dates 08/01/04-07/31/09 NIH

"Enhanced RF Tumor Ablation with Liposomal Chemotherapy" (Goldberg)

This research project developed and optimized combination therapy of RF ablation, hyperthermia, and liposomal doxorubicin in order to increase the completeness and extent of tumor destruction. Key findings including definition of potential mechanisms such as cell stress, apoptosis and heat shock production that can be potentially further modulated to improve therapy.

LICENSING OF RESEARCH IP

All current 8 patents are licensed to Harvard University.

Nicolas Gouze

Nicolas Gouze has an engineer's degree in optronics from the University Paris XI and studied Innovation Management at the University of Valenciennes (France). Since 2004 he is working with the Department Future Technologies and Europe of VDI/VDE-IT. From 2004-2008 he was involved in technology transfer and innovation issues within the Innovation Relay Centre (IRC). He became the project manager after the IRC's transformation in 2008 into the Enterprise Europe Network. Further to SME support and cross-border cooperation he is working on following topics: technology transfer and consultancy on European R&D programmes, innovation and technology policy issues and commercialisation of R&D results. He is also experienced in innovation management teaching.
Nicolas is involved in the management of the ETP Nanomedicine by offering support to the members and being responsible for the platform's events.

Piotr Grodzinski

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 is materials scientist by training, but like many others found bio- and nanotechnology fascinating. In mid-nineties, he left the world of semiconductor research and 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 15 patents and published 52 peer-reviewed papers, 7 book chapters, and delivered over 100 invited conference presentations. Dr. Grodzinski has been an invited speaker and served on the committees of numerous bio- and nano-MEMS conferences in the past years.

Hans-Joachim Güntherodt

Professor of Experimental Physics (Ordinarius), Department of Physics, University of Basel, Klingelbergstrasse 82, 4056 Basel, Switzerland, Hans-Joachim.Guentherodt@unibas.ch.

RESEARCH PROJECTS:

STM, AFM, MFM, FAMARS. Hans-Joachim Güntherodt, born 1939, received a diploma in physics in 1963 and a PhD in 1967 from ETH Zürich. In 1974 he became full professor of physics at the Institute of Physics, University of Basel. Sabbatical leaves at the IBM Thomas J. Watson Research Center, Yorktown Heights, USA, and Tokyo Institute of Technology, Japan. He was Dean of the Faculty of Science, then Rector of the University of Basel. Prof. Güntherodt also was the director of the major research programs MINAST and TOPNANO 21.

- 2001 Director of NCCR Nanoscale Science
- 2004 member of the Executive Board of IUVISTA; head of Nanometer Structure division
- 2005 Doctor honoris causa of University of Neuchâtel
- 2006 Honorary President of NCCR Nanoscale Science and Swiss Nanoscience Institute (SNI)

RESEARCH SUMMARY

During the last 40 years the research interests shifted from condensed matter physics (liquid metals, liquid semiconductors, metallic glasses, rapidly quenched quasi-and nanocrystalline alloys, graphite intercalation compounds, electron spectroscopy) to nanoscale science (scanningprobe microscopy, application to new materials, such as high-temperature superconductors, fullerenes, carbon-nanotubes, self-assembly, molecular storage, molecular recognition, nanomechanics, cantilever arrays, in particular thermal properties, and nanomaterials).

SELECTED PUBLICATIONS

1. two book series “Glassy Metals”, and “Scanning Tunneling Microscopy”, Springer. 2. over 550 publications, including numerous articles in Physical Review Letters, Nature, Science, and PNAS

Michael Hehenberger

As Life Sciences Business Development Executive, Michael Hehenberger is focused on the creation of partnerships between IBM Research and global Life Sciences organizations, including bio-pharmaceutical, diagnostics and food industry, academic medical research centers, and government sponsored research. The partnerships are based on the joint desire to improve the productivity of life sciences and medical research. IBM Research has built expertise and innovative capabilities in fields as diverse as computational chemistry, computational biology and genomics, „deep“ unstructured and structured data analytics, and biomedical applications of nanotechnology. Dr. Hehenberger has published over 40 scientific papers and has presented at and organized conferences in high performance computing, computational chemistry and biology, cheminformatics, text analytics, clinical genomics, biobanking and (imaging) biomarker informatics. He has also been leading the creation of a significant IBM Research partnership in the area of DNA Sequencing.

Michael Hehenberger holds advanced degrees in physics (Dipl.Ing., TU Vienna, Austria) and quantum chemistry (Ph.D. and Dr.Sc., Uppsala University, Sweden).

Göran Hermerén

Göran Hermeren, Ph. D., earlier professor of theory of science and philosophy, since 1991 professor of medical ethics at Lund University, Sweden, in the faculty of medicine, currently senior professor at this university. He has done research at Trinity College, Ireland and several universities in the US, among others Princeton and University of Michigan.

He has been a member of the National Council on Medical Ethics in Sweden since its start, president of the European Group on Ethics 2002-2011, and is chair of the advisory board of the German Reference Center for Ethics in the Life Sciences (DRZE) in Bonn and of the ethics committee of the Swedish Research Council. He has also served on hospital ethics committees.

In addition to writings on aesthetics and theory of art he has written on the ethics of priority setting in health care and ethical problems raised by science and new technologies, such as stem cell research and nanomedicine. Recent publications include a book on the goals of medicine (together with Kurt Fleischhauer), contributions to books on research ethics, papers on fraud in science and on challenges in the ethical evaluation of nanoscale research, as well as on ethical issues raised by transplantation and on the principle of proportionality.

Hermeren has edited, and contributed to, a large number of anthologies in different areas. The most recent one is Hug, K and Hermeren, G (eds), Translational Stem Cell Research. Issues beyond the Debate on the Moral Status of the Human Embryo, Springer/Humana Press, New York, 2011.

Further details, including a bibliography, can be found at the website www.hermeren.nu

Inge Herrmann

Inge Herrmann was born in 1985. She studied Chemical and Bioengineering at the ETH Zurich with a stay at the TU Delft in the Reaction and Catalysis Engineering Laboratory (Moulijn/Kapteijn) in 2007. During her PhD studies in the group of Professor Wendelin Stark at the ETH Zurich, she developed a nanomagnet-based blood purification technology in collaboration with the University Hospital Zurich. Since 2011, Inge Herrmann is a postdoctoral research fellow in the group of Professor Beatrice Beck-Schimmer at the Center of Clinical Research at the University Hospital in Zurich. Her research focuses on therapeutic applications of magnetic nanoparticles and on organ protection with small molecule drugs for patients suffering from inflammatory diseases (e.g. sepsis) or ischemia reperfusion injuries (myocardial or cerebral infarction).

Jeffrey Hubbell

Jeffrey Hubbell is Professor in the Institute of Bioengineering and the Institute of Chemical Sciences and Technology of the Ecole Polytechnique Fédérale de Lausanne (EPFL; in English the Swiss Federal Institute of Technology, Lausanne), where he is also Director of the Institute of Bioengineering, a department of the School of Engineering and the School of Life Sciences. Trained as a chemical engineer, he uses biomaterials and protein engineering approaches to investigate topics in regenerative medicine and immunotherapeutics. Previous to moving to Lausanne, he taught at the Swiss Federal Institute of Technology Zurich, at the California Institute of Technology, and at the University of Texas in Austin. He holds a BS from Kansas State University and a PhD from Rice University.

Patrick Hunziker

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

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

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

Nadia Jessel

Dr. Nadia Benkirane-Jessel is the Leader of the "Active Biomaterials and Tissue Engineering" team at INSERM U977, Strasbourg. She received her Ph.D. from University Louis Pasteur, ULP, Strasbourg, France for the work on Development of pseudopeptides as synthetic vaccines. Dr. Jessel (Benkirane) then held a postdoctoral position in collaboration with the Institut Pasteur, Paris, France, working on Immunotherapy HIV, and another postdoctoral position on the application of modified peptides as vaccines against FMDV (Plum Island Animal Disease Center, ARS, USDA, Greenport, NY 11944-0848, USA). She joined the INSERM U595 in 2002 as a post-doc, and received the diploma to direct the research (HDR) in 2004. Dr. Jessel got the permanent position (CR1) in the INSERM 595 laboratory in 2004 and currently Research Director (DR2) in the INSERM 977 and heads the team. Dr. Jessel possesses expertise in diverse fields of molecular and cellular biology, immunochemistry, tissue engineering and biomedical engineering. In the last 5 years, she focused her research on the bio-functionalization of multilayered polyelectrolyte architectures with emphasis on the use of these architectures to induce specific cellular responses and gain control over cell proliferation and differentiation. Dr. Benkirane-Jessel is a co-author of 60 peer-reviewed publications in high impact factor journals (Proc. Nat. Acad. Sci. USA; Adv. Mater.; Adv. Funct. Mater.; Small; Nanoletters, Biomaterials, ACS Nano), 5 chapters reviews and 4 international patents, she is a regular referee for a number of scientific journals (Nature nanotechnology, Nature Materials, Biomaterials, Nanoletters…). She is under the contract (Interface INSERM vers l'hôpital 2008-2013) and she got also (Prime d'Excellence Scientifique from the INSERM for 2010-2014).

SCIENTIFIC SUMMARY

Recently, tissue engineering has merged with stem cell technology with interest to develop new sources of transplantable nanostructured and "living" material for injury or disease treatment. Eminently interesting, are bone and joint injuries disorders because of the low self-regenerating capacity of the matrix secreting cells.

In recent years, considerable effort has been devoted to the design and controlled fabrication of nanostructured materials with functional properties. The nanstructured films from oppositely charged polymers offer new opportunities for the preparation of functionalized biomaterial coatings. This technique allows the preparation of supramolecular nano-architectures exhibiting specific properties in terms of control of cell activation and may also play a role in the development of local drug/gene delivery systems. Peptides, proteins, drugs or DNA, chemically bound to polypeptides or Cyclodextrins (CDs), adsorbed or embedded in nanostructured films, have been shown to retain their biological activities. Recently, we have demonstrated the sequential induction of nuclear and /or cytoplasmic expression products, mediated by β-cyclodextrin embedded in a nanostructured films7. We have also reported that embedded BMP-2 and TGFβ1 in a nanostrutured film can drive stem cells to bone or cartilage differentiation depending on supplementary co-factors. Our results demonstrate clearly that we are able to induce osteogenesis in embryonic stem cells mediated by growth factors embedded into the nanostructured films on a planar surface or as a nanostructured capsules for bone induction in vivo. We have also reported that we are able to induce dental pulp regeneration by using an active nanostructured gel based on alpha-melanocortin (alpha-MSH) active peptide.

Scientific topics: Material Science, Nanomedicine, Regenerative Medicine, Tissue Engineering

Keywords: Active living Biomaterials, Osteo-Articular Systems, Bone-tooth unit regeneration

Web:
http://cvscience.aviesan.fr/cv/862/nadia-benkirane-jessel
http://www.linkedin.com/pub/nadia-benkirane-jessel/24/39b/345

Andreas Jordan

Founder & Chief Scientific Officer. Dr. Andreas Jordan founded MagForce Nanotechnologies AG and serves on its management board as chief scientific officer, with responsibility for all research and development activities. He began his career with studies in biology at the Free University of Berlin, followed by further studies in biochemistry at the Technical University of Berlin. His highly praised doctoral dissertation in 1993 addressed the production of nanoparticles and their application for cancer therapy. This pioneering work was based on research which began in 1987, long before the subject of nanotechnology had achieved any international significance. He subsequently managed scientific projects for the Berlin's Virchow Clinic (now Charité) as well as for the Institute for Diagnostic Research, a subsidiary of Schering. Dr. Jordan has already delivered more than 500 scientific lectures about Nano-Cancer® therapy. He has authored more than 45 articles for peer-reviewed scientific journals and has cleared the way for twelve families of international patents, some of which have been licensed. His contacts to NASA, the National Cancer Institute (NCI), the Institute of Nanotechnology (IoN), the U.S. Food and Drug Administration (FDA), and such renowned U.S. hospitals as the University of California, San Francisco (UCSF), the Cleveland Clinic Foundation (CCF) and Duke University, as well as throughout Asia, continue to provide an essential foundation for his professional activities through the world.

Michael Juhnke

Dr.-Ing. Michael Juhnke
Novartis Pharma AG, Technical R&D, Basel, Switzerland
Michael is process engineer graduated 1997 from Cologne University of Applied Sciences (BSc) and 2000 from Clausthal University of Technology (MSc). He continued his scientific education with a doctorate degree (Dr.-Ing.) in particle technology graduated 2006 from Clausthal University of Technology. His industrial career started 2006 at Novartis Pharma AG in the Technical R&D department, focusing on the process development of engineered drug particles for oral, parenteral and respiratory applications from pre-clinical to production scale. He is steering committee member of the comminution and classification working parties in the VDI-GVC/Dechema ProcessNet association and in the European Federation of Chemical

Varvara Karagkiozaki

Dr. Med. Karagkiozaki Varvara, MSc
Specialist Cardiologist, MSc in Nanosciences & Nanotechnologies, PhD in Nanomedicine

Nanomedicine Group, Lab for "Thin Films -Nanosystems & Nanometrology", Department of Physics, Aristotle University of Thessaloniki, Greece; AHEPA University Hospital, AUTh, Greece
She is a specialist Cardiologist, receiving her diploma from Aristotle University of Thessaloniki (AUTH). She holds a BSc in Medicine (Medical School, AUTH, 1998). She received an MSc diploma with distinction of excellence, (2007), after attendance of two years' interdisciplinary Postgraduate Program "Nanosciences & Nanotechnologies" of AUTH, focusing on Nanobiotechnology & Nanomedicine field. Formerly she had been working as an Honoured Clinical Fellow in Congenital Cardiac Unit at University Hospital of Southampton in United Kingdom, to become a specialist in 3d heart echo in adult and congenital cardiac diseases. She received her nanomedical thesis from the Medical School of AUTH, with distinction of excellence (2009). The thesis is on the advances of Nanotechnology that can bring to Stent coating technology aiming at manufacturing stent nanocoatings that avoid the late stent thrombosis which is considered to be the major pitfall of drug eluting stents. She is a specialist in the implementation of nanoscale imaging techniques such as Atomic Force Microscopy (AFM) and SNOM for the in depth analysis of bio and non-bio interactions and in the development of thrombo-protective nanomaterials to meet the demands for cardiovascular implants. Her latest nanomedical activities involve: biofunctionalization of nanomaterials for tissue regeneration; development of drug delivery nanocoatings for implants and studies on drug release kinetics; bioelectronics for diagnosis and treatment of atherosclerosis within ROleMak Project of the European Commission under the 7th Framework Program, FP7-REGPOT-2011-1.
She is a Member of Nanomedicine – Nanobiotechnology team of LTFN, member of Lab for Cardiovascular Engineering & Atherosclerosis, Ahepa Hospital, Medical School AUTH, and member of European Society of Clinical Nanomedicine, a founding member of International Society of Nanomedicine, member of American Society of Nanomedicine, and member of Greek Pediatric Cardiology Association.
She is a member of the scientific board of North Greek Society of Atherosclerosis. She had many oral and poster presentations in European, International and Greek at Nanomedical, Nanotechnology, Cardiology Conferences and numerous attendances at Greek, European and International congresses. Especially, these research presentations have been focused on many aspects of cardiology, such as atherosclerosis, hypertension, acute coronary syndromes, innovations in diagnosis and treatment of coronary artery disease, etc as well as on nanotechnology, nanomedicine, biocompatibility issues, biomaterials and stent technology. She received a scholarship from the North Greek Society of Atherosclerosis and research awards from Greek Cardiology and Atherosclerosis Societies.
She published several academic research papers in peer reviewed journals, chapters in Nanomedicine books and has been active in Nanomedicine field with papers on Nanocardiology, Nanomedicine for cardiovascular disease and thrombosis. She gave lectures at Nanomedicine Summer Schools (ISSON9, ISSON10, and ISSON11) and at N&N Postgraduate Program of Auth, during the period of 2010-2012. She had established her private cardiology clinic and she is a member of LTFN, working for EU research projects, promoting the research in Nanomedicine in Greece and Europe. She was a chair of the Organizing Committee of the Nanomedicine Workshop during the 8th International Conference on Nanosciences & Nanotechnologies NN11. She is a chair of the Organizing Committee of the Nanomedicine Workshop during the upcoming 9th International Conference on Nanosciences & Nanotechnologies (NN12), 3-6 July 2012 at Thessaloniki, Greece. She is also a member of the Organizing Committee of the Bioelectronics Workshop during NN12 and of the upcoming 6th International Summer School "N&N: Organic Electronics & Nanomedicine" (ISSON12), 30 June -7 July, at Thessaloniki, Greece. More information can be found at the website: http://nanotexnology.com

ALASTAIR KENT

Alastair Kent is the Director of the Genetic Interest Group (GIG) - the UK alliance of charities and support groups for people affected by genetic disorders. GIG's mission is to promote the development of the scientific understanding of genetics and the part that genetic factors play in health and disease, and to see the speedy transfer of this new knowledge into improved services and support for the treatment of c urrently i ncurable c onditions.
He is also President of EGAN (the European Genetic Alliances Network) a coalition of national umbrella groups for genetic patient support groups that campaigns for improved therapies and support for all affected individuals and families throughout the EU.
Prior to joining GIG Alastair worked for a number of voluntary organisations on issues concerning policy, service development and disabled people.

Alexander N. Kharlamov

M.D., [Ph.D.], Ural Institute of Cardiology, 78A, 8th March street, Yekaterinburg 620144, Russia. E-mail: drskharlamov@gmail.com, phones: +79638547663, +31627849118.

Dr. Kharlamov born 21st of May 1981 in Yekaterinburg, Russia, received his M.D. cum laude in 2005 from Ural State Medical University (Yekaterinburg, Russia). After finishing his internship in therapeutics and general cardiology in 2008 at the Department of Internal Medicine in Ural Institute of Cardiology (supervisor – Prof. Jan Gabinsky, Yekaterinburg, Russia) he started as a physician and translational researcher at the Department of Interventional Cardiology, Acute Care Unit, and founded a Department of Science in the Ural Institute of Cardiology working on the field of novel nanobiotechnologies in cardiology. The main research direction of the group now is a RTD of the new multifunctional nanoparticles for plasmonic photothermic angioplasty and imaging of coronary arteries. The Biotechnology Lab of the Institute is also involved in the growing of the bioengineered on-artery patch structures for the management of atherogenesis. Since 2007 he is working as a scientific assistant to C.E.O. Ural Institute of Cardiology and chief-cardiologist of the Ural Federal District (Russia) Prof. Jan Gabinsky in the field of international collaboration and innovative development of bio- and nanotechnologies. He has received his Ph.D. in Russia from Ural State Medical Academy in 2011. Since 2009 he has been working as a research fellow in some institutes in the Netherlands, including collaboration with Prof. Patrick W. Serruys (Erasmus MC, Rotterdam, The Netherlands). He is an author of some grant proposals (NANOPLASTY, REVOLUTION projects) for the European Commission and FP7/CORDIS, and has received for his research work some national and international awards.

Andreas Kjær

Andreas Kjær, MD, PhD, DMSc is professor and chief physician at Dept. of Clinical Physiology, Nuclear Medicine & PET, Rigshospitalet, The National University Hospital of Denmark and head of Cluster for Molecular Imaging at University of Copenhagen. His research focus is on molecular imaging with PET for tissue characterization, tailored therapy and drug testing in cancer. Professor Kjær has received several prizes for his research in molecular imaging and published more than 200 scientific peer-review articles. Currently he is president of Scandinavian Society of Clinical Physiology and Nuclear Medicine. National Director of the European Advanced Translational Research Infrastructure in Medicine (EATRIS). Member of the Research Council of the Danish Cancer Society. Editor-in-chief of the Open Neuroendocrinology Journal and MDPI Diagnostics.

George Kordas

Present Address: "Demokritos", NCSR D, IMS, 153 10 Ag. Paraskevi Attikis Athens, Greece;

EDUCATION:

1979 Ph.D. in Engineering (Doktor Ingenieur), WWIII, Germany.

1974 M.S. in Physics (Diplom Physiker), University of Erlangen, Germany.

CURRENT PROFESSIONAL EXPERIENCE:

1990- Group Leader, NCSR, "Demokritos," Athens, Greece.

SCHOLASTIC ACHIEVEMENTS & AWARDS (selected):

• 2012 "Award for Scientific Excellence", National Center for Scientific Research, Thursday 23 February 2012

• 2009 ERC-IDEAS Grant - 232959, NANOTHERAPY A Novel Nano-container drug carrier for targeted treatment of prostate cancer (http://erc.europa.eu/index.cfm?fuseaction=page.display&topicID=492)

• 2002 Weston Visiting Professor, Department of Physical Chemistry, Weizmann Institute of Technology, Rehovot, Israel

• 2001 DAAD Invitation, Max Plank Institute for Polymer Science, Mainz, Germany

SELECTED ARTICLES IN JOURNALS

Novel PEGylated pH-sensitive polymeric hollow microspheres, Bilalis P.; Boukos N.; Kordas G., MATERIALS LETTERS, 67, 1,180-183 DOI: 10.1016/j.matlet.2011.09.062, JAN 15 2012

Synthesis and characterization of SiO2-CaO-P2O5 hollow nanospheres for biomedical applications, Pappas G. S.; Bilalis P.; Kordas G., MATERIALS LETTERS, 67, 1, 273-276 DOI: 10.1016/j.matlet.2011.09.089 Published: JAN 15 2012,

New approach in synthesis, characterization and release study of pH-sensitive polymeric micelles, based on PLA-Lys-b-PEGm, conjugated with doxorubicin, Efthimiadou E. K.; Tapeinos C.; Bilalis P.; et al., JOURNAL OF NANOPARTICLE RESEARCH, 13, 12, 6725-6736 DOI: 10.1007/s11051-011-0579-5, DEC 2011

CURRENT RESEARCH

We are interested in the synthesis and characterization of nanocontainers for medical, energy and corrosion applications.

CURRENT GROUP

Dr. G. Mitrikas, Dr. J. Danilidis, J. Kartsonakis, A. Balaskas, E. Mekeridis, A. Angelopoulou, C. Tapeinos, G. Pappas, L. Chiotinis, A. Kikidis, A. Palaiologos

Silke Krol

Silke Krol was born in Gronau (Germany) and received her degree in Chemistry and Biochemistry at the University of Muenster, Germany. Her aim of study for the Ph.D. was the "Biophysical characterization of the surfactant proteins B and C in phospholipid monolayers". Then she studied at the University of Genoa (Italy) as fellow in a EU project in the 5th framework "Nanocapsules with functionalized surfaces". Here she studied the encapsulation of living cells (yeast, fungi etc.) and their possible use as biosensor. In a second EU project "Development of a Bioartificial Pancreas for Type I Diabetes Therapy" she focused her interest on encapsulation of pancreatic islets in order to improve long-term survival and stability. Then she worked for three years in CBM (Cluster in Biomedicine), working as head of the NanoBioMed lab @ LANA3DA (laboratory for Nanodiagnostics, drug delivery and Analysis). Her major research interests there was the development and characterization of polyelectrolytes either as nanoparticles for selective drug delivery or as drugs. Actually she is now with European Center for Nanomedicine in Fondazione IRCCS Neurologic Institute "Carlo Besta" in Milan as Principal Investigator and assistant to the director. The focus of her work is the nanoparticle biodistribution and delivery to the brain and their passage of the blood brain barrier. She continues also her works about multilayer nanocoated pancreatic islets as immune protected transplants for diabetes therapy.

Twan Lammers

Twan Lammers studied Pharmacy at the University of Utrecht. He obtained a D.Sc. degree in Radiation Oncology from Heidelberg University in 2008 ('Role of PP2Ca in growth regulation, in cellular stress signaling and in tumorigenesis'), and a Ph.D. degree in Pharmaceutics from Utrecht University in 2009 ('Drug targeting to tumors using HPMA copolymers'). From 2007-2010, he worked as a postdoctoral fellow at the Department of Pharmaceutics at Utrecht University, focusing primarily on the MediTrans project (FP6: 'Targeted delivery of nanomedicines'). Since 2009, he has been appointed as a group leader at the Department of Experimental Molecular Imaging at RWTH - Aachen University and at the Helmholtz Center for Biomedical Engineering. He furthermore works as an assistant professor at the Department of Targeted Therapeutics at the University of Twente, and still holds an adjunct position at the Department of Innovative Cancer Diagnosis and Therapy at the German Cancer Research Center in Heidelberg. He has published approximately 50 research articles and reviews, mostly dealing with polymeric drug delivery systems, combination therapies, functional and molecular imaging, and nanotheranostics. He is on the editorial boards of Theranostics, the Journal of Controlled Release, and the American Journal of Nuclear Medicine and Molecular Imaging. In 2010, he edited a theme issue on HPMA copolymers for Advanced Drug Delivery Reviews, and in 2012 a theme issue on Drug Delivery Research in Europe for the Journal of Controlled Release. His primary research interests include drug targeting to tumors, image-guided drug delivery and tumor-targeted combination therapy.

Sebastian Lange

Dr. Sebastian Lange studied physics at Heidelberg University. During the course of his studies he completed an academic year abroad at the Univeristy of Massachusetts, Amherst. After his studies he joined LifeBits AG in Tübingen as an assistant lecturer and finished his doctor's degree in physics at the University of Tübingen in close cooperation with the EMBL Heidelberg. After being awarded his PhD Sebastian Lange worked as a Postdoctoral Researcher at EMBL. In 2004 he took the function of management consultant in the consultancy company Droege&Comp (Arideon). Since 2006 Sebastian Lange has been senior consultant by VDI/VDE-IT in the department Innovation Europe. Among others projects he was Deputy Head of Office of the European Technology Platform (ETP) EPoSS. Moreover, he is Head of the ETP Nanomedicine Office and thus member of the Executive Board of the industry initiative. Since 2009 he is the coordinator of the European research project „Internet of Things Architecture" (IoT-A, 20 Mio. €). In 2010 Sebastian Lange became a member of the IoT Expert Group established by the European Commission.

Claus-Michael Lehr

Claus-Michael Lehr is Professor at Saarland University, and also cofounder and head of the department "Drug Delivery" at the recently established Helmholtz Institute for Pharmaceutical Research Saarland (HIPS). HIPS is the first permanently installed institution explicitly dedicated to Pharmaceutical Research; it belongs to the Helmholtz Centre for Infection Research (HZI), Braunschweig.

The main focus of research of Prof. Lehr's team has been on the one hand exploring the biological barriers, in particular the gastro-intestinal tract, the skin and the lungs, and on the other hand developing the appropriate carriers capable of crossing these epithelial barriers and deliver the active molecule to the target. The expansion of this approach by developing new in vitro models of higher sophistication and relevance, based on the epithelial cells and tissues concerned, is in advanced progress. In parallel, the nanotechnology approach is improving and has broadened in terms of formulating multifunctional nanocarriers that allow in vivo tracking of the system, targeting to the site of action, releasing the payload in a controllable manner and last but not least being safely biodegraded and excreted from the body.

Laurent Levy

PhD, has a deep understanding and practical knowledge of the technical, scientific, intellectual property, development and marketing issues associated with nanotechnologies, because he has been working-and achieving- in these areas for more than 15 years. His pioneering research at the frontier of molecular biology and physics has empowered him to develop a number of practical applications, not the least of which is nanoXray, the technology foundation of Nanobiotix, which is focused on making possible a whole new era in cancer medicine. Laurent Levy has worked for many years as a consultant in business development and in the implementation of nanotechnologies with major companies, including Sanofi Aventis, Guerbet, and Rhodia, as well as start-up biotechs.
He is the president of the French Technology Platform of Nanomedicine (FTPN) and is involved with many international groups working in the field. The author of 35 international publications and communications, Dr. Levy holds several patents and completed postdoctoral work at the Institute for Laser Photonics and Biophotonics, State University of New York (SUNY), Buffalo. Dr. Levy holds a PhD in Physical Chemistry specializing in Nanomaterials from Pierre et Marie Curie University-CEA, and a DEA (first doctoral diploma) in Condensed Matter from UPVI-ESPCI (Paris).

David Lewis

David Lewis is Vice President of Biology at Arrowhead Research Corporation and Site Head of Arrowhead's research and development facility in Madison WI, USA. Dr. Lewis was a pioneer in the use of RNAi in animals and was the first to show that siRNAs could be used to inhibit gene expression in multiple tissues of adult mammals. Dr. Lewis is also co-inventor of Dynamic PolyConjugate (DPC) nanotechnology for targeted delivery of siRNA, which is currently in clinical development. Prior to his role at Arrowhead, Dr. Lewis was Site Head and Director of Research at Roche-Madison.
Dr. Lewis received his B.S. degree in Biochemistry and Molecular Biology from the University of Wisconsin-Madison and his Ph.D. degree in Biochemistry from Michigan State University. He received Michigan State University's "Outstanding Graduate Student" award in recognition of his Ph.D. thesis research. His post-doctoral research was performed at the Howard Hughes Medical Institute at the University of Wisconsin-Madison under Dr. Sean Carroll. While there, he developed viral and non-viral methods to modulate gene expression in animals, and discovered novel gene regulatory mechanisms involved in body patterning.
Dr. Lewis has authored 25 scientific papers and book chapters, has more than 20 patents or patent applications, and has been awarded several government-sponsored research grants. He has given numerous invited speaking presentations at both scientific conferences and research institutions. He has served on several NIH review panels and since 2008, has been a lecturer for the Masters in Biotechnology Program at the University of Wisconsin-Madison.

Roderick Y. H. Lim

Born in Singapore in 1974, Roderick Y. H. Lim studied physics as an undergraduate at the University of North Carolina at Chapel Hill. He went on to use the atomic force microscope to investigate the fundamental properties of confined molecular liquids at the Institute of Materials Research and Engineering in Singapore, where he obtained his PhD in 2003. Following this, Rod joined the group of Prof. Ueli Aebi at the Maurice E. Müller Institute for Structural Biology, Biozentrum, University of Basel as a postdoc where he worked within the framework of the NCCR-Nano from 2004 to 2008. There he pioneered the use of nanotechnological fabrication, imaging and measurement methods to reproduce in vitro the molecular functionality of cellular nanomachines in a stepwise manner. He has published in journals such as Science, Nature Nanotechnology, PNAS and Physical Review Letters, and was awarded the Pierre-Gilles de Gennes Prize: "From Solid State to Biophysics" in 2008. In 2009, Rod joined the faculty of the Biozentrum and the Swiss Nanoscience Institute as the Argovia Professor for Nanobiology at the University of Basel where he innovates across disciplines so as to obtain "bottom-up" insight into the inner workings of key biological problems.

Julianna Lisziewicz

PhD, President, Chief Executive Officer, Genetic Immunity. Dr. Julianna Lisziewicz co-founded Genetic Immunity in 1998 and has served as the President and Chief Executive Officer of Genetic Immunity since its founding. In 1994, Dr. Lisziewicz co-founded the non-profit Research Institute for Genetic and Human Therapy (RIGHT) and directed its research and business affairs in the USA. RIGHT was focusing on the treatment of HIV/AIDS from multiple perspectives: virology, molecular biology, immunology and medicine. From 1990 to 1995, she was Head of the Antiviral Unit in the Laboratory of Tumor Cell Biology at the National Cancer Institute of the NIH in Bethesda, Maryland. While at NIH, she discovered and developed antisense oligonucleotide therapy and gene therapy for HIV/AIDS treatment. In 2005, she was appointed as the Marie Curie Chair at the Semmelweis University Budapest. She received her Ph.D in molecular biology from the Max-Planck Institute (Goettingen, Germany) and two Masters of Science in Chemistry and Biochemistry from the Technical University (Budapest, Hungary). She has co-authored over 100 peer reviewed scientific publications.

Beat Löffler

studied Communications Science, Philosophy and Political Science. He received his MA at Freie Universität Berlin. In 1983 he started his first company for concepts and new media. 6 years later he became Director of the International Hightech-Forum of Messe Basel. After working further 6 years in the new technology sector as developer and conference organiser, creating concepts for emerging technology events he created in 1994 his present Company "L&A Concept Engineering" and specialised in the fields of the development of innovation concepts and the development of science and knowledge promotion initiatives as well as to leadership-training and interdisciplinary bridging events.
Fields of work are • Computational Fluid Dynamics • Materials Science, • Energy Technology and • Life Sciences.

Beat Löffler had numerous mandates for projects developed by his company and was mandated for 4 years as leader of the life sciences business development EMEA for the Japanese company NEC High Performance Computing. 2005 he concepted and realised the European Summit for New Materials in Energy and Mobility in Essen, Germany for "Initiativkreis Ruhrgebiet". He co-concepted and realised the first world Summit for New Materials in Energy Technology in Lisbon.
In October 2006 he started the development of a concept for a conference for applied Nanomedicine. He founded together with Patrick Hunziker, MD in 2007 the European Foundation for Clinical Nanomedicine and started up the European Society for Nanomedicine. The company focuses since then besides on novel materials with mandates of the European Materials Forum and the European Materials Research Society predominantly on Nanotechnology. Since 2007 he is CEO of the CLINAM Foundation and is Secretary General of the European Society for Nanomedicine. He signs responsible for the European Conference for Clinical Nanomedicine.

Stergios Logothetidis

Department of Physics, Aristotle University of Thessaloniki, Greece
Tel: +30 2310 998174, Fax: +30 2310 998390, e-mail: logot@auth.gr
Prof. S. Logothetidis, is the Director and Founder of the Lab of Thin Films - Nanosystems & Nanometrology (LTFN) (http://ltfn.physics.auth.gr).

He received his degree in Physics from the Aristotle University of Thessaloniki (AUTh) at 1977, his Master in Electronics in 1980 and his PhD in 1983 from the Physics Dpt. of AUTh. He then worked as a postdoctoral researcher in Max-Planck Institute - MPI in Stuttgart 1983-1985 and Research Associated at MPI and at the Synchrotron Radiation Laboratory at BESSY, Berlin at 1985 and 1988-1989.

In 1985 he became a Lecturer and in 1991 he founded the Lab for Thin Films-Nanosystems and Nanometrology – LTFN (http://ltfn.physics.auth.gr), in the Physics Department of AUTh, with equipment and infrastructure by European and National R&D projects. LTFN is internationally acknowledged as a powerful center for research and innovation, with hundreds of students, postgraduate and postdoctoral researchers being trained. In 1999 he was elected Full Professor of Physics, while during 2005-2009 he served as Chairman of the Physics Department of AUTh.

The research interests of Prof. S. Logothetidis include the following:
• Development of organic semiconductors, transparent electrodes and barrier layers for flexible organic electronic devices
• Fabrication of Organic Electronic Devices that include Organic Photovoltaic cells (OPVs), Organic Light Emitting Diodes (OLEDs), Organic Thin Film Transistors (OTFTs), Sensors & Biosensors
• Fabrication of Thin Films and Nanomaterials by vacuum, wet and printing techniques in batch and roll-to-roll (r2r) configuration
• Development of optical sensing techniques for in-situ & real time monitoring, and probing materials properties and processes
• NanoBiology, NanoMedicine
• Development of functionalized polymers and biocompatible materials and investigation of the adsorption of biological agents onto their surface by non-destructive optical techniques.
• Optical Spectroscopies, Electronic and Mechanical Properties of Thin Films and Nanomaterials
• Computational modeling of electronic properties, growth mechanisms of thin films and materials

His research activity is complemented by over 650 papers and review articles in International Journals & Conferences, and his over 5500 citations by other researchers. He has given more than 130 Invited talks. He is a referee in more than 25 International Scientific Journals and in the board of several Journals.

He is the Coordinator and Principal Investigator in more than 60 R&D Projects funded by European Commission (EC) in FP4 to FP7 (ICT, NMP, GROWTH, BRITE-EURAM, BRITE, EPET, CRAFT, etc.), many of which have been distinguished and honored by the European Commission EC as Outstanding, and by the General Secretariat for Research and Technology Greece (STRIDE, EPET, Bilateral, PENED, PAVE, CO-OPERATION, etc.).

He has developed long standing partnerships with more than 70 Research Institutes / Centres as well as more than 60 Greek and European Research and Manufacturing Organizations. He is a reviewer and knowledge-expert in dozens of Research Programs (National, EC, NSF, etc.). The period 2008-09 he was a member of the NSRF Committee for subjects in Research and Technology in the areas of Nanotechnology, Materials & Infrastructure and Analytical Techniques.

He is the Director and Founder of the **Interdisciplinary Post-Graduate Program "Nanosciences & Nanotechnologies" (N&N)** (http://nn.physics.auth.gr), of AUTh with participation of Depts. of Physics, Chemistry, Biology and Polytechnic School at 2002, and other Depts or Institutes from Greece and abroad.
In 2003 he has founded the Thematic Research Network "NANONET" (www.nano-net.gr), which has more than 285 Research Labs from Greece, Europe and USA.

He has organized several National and International Conferences. Since 2003 he organizes the "International Conference on Nanosciences & Nanotechnologies (NN)", whereas for 4 continuous years he is co-organizing the "Global Plastic Electronics Conference", the "International Symposium on Flexible Organic Electronics (ISFOE)" and the "International Summer Schools on Nanosciences & Nanotechnologies (ISSON)". During the last 2 years he also organizes the NANOTEXNOLOGY Exhibition, Conferences & Summer School on Organic Electronics, Nanotechnologies and Nanomedicine (http://www.nanotexnology.com/).

He is Editor of several books such as "Nanomedicine and Nanobiotechnology" Springer 2012, "Nanostructured Materials and their applications" 2011, several of his books and monographs, such as " Nanometrology, in Handbook NANOPHYSICS", 2009, "Introduction to Advanced Materials Growth" , "Thin Films and Vacuum Technology", "Optical properties of Solids" and "Technology - Materials and Social - economic enviroment" are distributed to under- and post- graduates at Physics Department.

Prof. S. Logothetidis has supervised more than 25 Ph.D. Theses carried out in the LTFN, more than 50 MSc. theses and more than 28 Post-Doctoral researchers. He has trained numerous researchers and scientific collaborators at the LTFN, Max Planck Institute Stuttgart and at the Synchrotron Radiation Lab BESSY, Berlin in subjects that are related to his scientific activities.

His administrative activities include the organization and coordination of more than 80 research and industrial groups, the organization of cooperations at educational, scientific and research level between AUTh and Universities, Research Institutes / Centres of Greece and abroad (e.g. H. Max-Planck, Ecole Polytechnique, BESSY, CERN, etc.).

Prof. S. Logothetidis is a member of the Materials Research Society (MRS), European MRS, Optical Society of America (OSA), European Physical Society (EPS), European Synchrotron Radiation Society (ESRS), Plastic Electronics, the Hellenic Physical Society (HPS), Hellenic Society of Condensed Matter Technology (HSCMT), Hellenic Society of Ceramics (HSC), and Clinical Nanomedicine (Clinam).
He received research Stiftungs from the Alexander von Humbolt-Stiftung Institute and the Max-Planck Institute in Germany. Also he received a research fellowship from the Onassis Foundation for the completion of his doctoral dissertation, a Scholarship Award from OTE for his excellent academic performance during his postgraduate studies (first in rank), and a State Scholarship Foundation for the whole duration of his undergraduate study (first in rank). He won the Award of Honor and Offer in the ceremony for the Pioneers of Spirit, Art, Science and Social Value of the "Association of the Greek Literary Men".

Bertrand Loubaton

MBA, Chairman ETP Nanomedicine
GEHC Director, Pharmaceutical & Academic Research Collaboration.

He has more than 20 years experience in Drugs & Medical Technologies in both R&D and marketing.
Since 2009 he is Elected Chairman of the ETP Nanomedicine (European Technology Platform Nanomedicine). His current responsibility in GE Healthcare involves collaboration between academia and industry, for new technologies & biomarkers development. He's jointed GE Healthcare in 2005 as European Director Sales/Marketing & Business Development of IMANET a PET research Network of Academic Sites.

PREVIOUSLY

He was Business Development & Licensing Director at SMV (Gamma Camera), Director of Operations/France Executive Director and Co-founder of the First European clinical sites network (SMO), Business Director of IBAH (Clinical Research Organization; & Product Manager at 3M Pharma & Roussel-Uclaf group.

Philip S. Low

Dr. Philip S. Low is the Ralph C. Corley Distinguished Professor of Chemistry at Purdue University where he has been on the faculty since 1976. While Dr. Low has conducted research on protein thermodynamics, plant cell signal transduction, and erythrocyte membrane structure, he has spent the last ~20 years developing ligand-targeted therapeutic and imaging agents for various cancers and inflammatory diseases. In the course of this research, he has published over 300 scientific articles and received ~35 US patents/patent pending. Dr. Low has received several national and international awards including an NIH MERIT Award and Purdue University's award for outstanding research. He has also guided the discovery and development of 7 drugs that are currently in human clinical trials for imaging and therapy of various cancers and has founded two companies (Endocyte Inc. and On Target Laboratories) focused on commercialization of these and other targeted drugs. Dr. Low received his Ph.D. in Biochemistry from the University of California, San Diego in 1975.

Lina H. Machtoub

Priv. Doz. Dr. Lina H. Machtoub
UniversitätsKlinik für Radiologie
Medizinische Universität Innsbruck
Anichstr.35 A-6020 Innsbruck
Tel.office: (43) 512-504-28963
E-mail: lina.machtoub@i-med.ac.at

ACADEMIC ACHIEVEMENTS

My field of study encompass the development of novel therapeutic approaches and high sensitivity diagnostic targeted imaging probes for noninvasive characterization of molecular events associated with the inflammatory process in the vascular system and motor neurodegenerative disorders. I received my Ph.D. in 2002 from the University of Tokyo in Japan, where I was awarded honourable Japanese research fellowship for 5 years. In 2003, I was granted a fellowship award from Alexander von Humboldt foundation and started my research work at Max Planck Institute in Germany, where I worked on the development of combined ultra high magnetic field and optical imaging. Since 2007, my work was focused on the development

of clinical multimodal imaging techniques based on nanobiotechnology. I joined Universitätsklinik für Radiologie in Innsbruck, in 2009, where I was appointed as a senior scientist, leading clinical studies on microscopic nanobiophotonic imaging. During this time, one of my main projects was the development of an emerging innovative diagnostic imaging modality based on surface-enhanced coherent anti-Stokes Raman scattering (SECARS) microscopy using nanoscale MR contrast agents. The major advantage of this novel imaging modality is the drastic signal enhanced in the vicinity of nanoparticles that can increase the detection sensitivity reaching the single-molecule level, which make it a very promising high resolution imaging tool for clinical applications. In 2010, intensive studies were focused on the molecular mechanisms of neurodegenerative disorders established experimental models of amyotrophic lateral sclerosis (ALS) using MR imaging with the first novel application of SECARS microscopy (highlighted in publications). In addition to conducting clinical research projects, I have been appointed as a senior lecturer in the Image-Guided Diagnosis and Therapy (IGDT) program of the Medical University core curriculum for the Nanotechnology. My current projects implement the combination of SECARS and MR imaging in the characterization of the inflammatory process in CNS disorders and atherosclerotic models which can lead promising approaches for molecular imaging and future therapeutic intervention.

FUNDING & HONORS

- 2011 Deutsche Forschungsgemeinscaft Sonderforschungsbereich
- 2009-2010 National grant -143054 MSTD-RS
- 2007-.2008 Research grant -Ministry of Education, Japan
- 2004-2006 Research fellowship- Max Planck Institute, Stuttgart, Germany
- 2003-2004 Research fellowship -Alexander von.Humboldt Foundation, Germany
- 1996-2002 Research fellowship, Japanese government-Ministry of Education, Japan

HIGHLIGHTED RECENT SELECTED PUBLICATIONS

- Machtoub L, Bataveljić D, Andjus PR. Molecular imaging of brain lipid environment of lymphocytes in amyotrophic lateral sclerosis using Magnetic resonance imaging and SECARS microscopy. Physiol Res. 2011; 60 Suppl 1:S121-7
- Machtoub L, Monitoring SERS-based contrast agents in atherosclerosis-experimental models. Proc. of SPIE 2011. Vol. 7890; Artikel# 789003
- Machtoub L., Michalska M., Herold V., Bauer E., Hildenbrand MF, Jakob P, Bauer WR, Characterisation of The Infalammatory Process in Atherosclerotic Models by MRI and SECARS Microscopy. Proc. of 16th World Congress on Heart Disease.2011.
- Machtoub, LH. Investigating neurodegenerative disorder systems using USPIO-nanoparticles with (SECARS) microscopy, Journal of Neurology 2010; 257: P. S65
- Machtoub L, Pfeiffer R, Backovic A, Frischauf S, Wick MC. Molecular Imaging Cellular SPIO Uptake with Nonlinear Optical Microscopy, Journal of Medical Imaging and Radiation Sciences. 2010 Sep; 41(3):159-164.
- Machtoub L, Pfeifer R, Bataveljić D, Andjus PR, Monitoring lipids in Neurodegerative disorders by SECARS microscopy, EJSSNT 2010: vol 8; 362-366.
- Machtoub, LH., Imaging USPIO Nanoparticles Uptakes in Biological Systems with Vibrational Microspectroscopy AIP Conference Proc.: 2010; 1267: P. 471

HIGHLIGHTED CONFERENCES

- **Alpach, Tirol, Austria**, Organiser of the International Meeting of Advanced Imaging Nanobiotechnology, AINBT2012.
- **Los Anglos, USA** "Characterisation of the early and advanced vascular inflammatory processes in the atherosclerotic mouse model by USPIO-VCAM-1 particles" World Molecular Imaging Congress, WMIC 2011.
- **Vancouver, Canada** "Characterisation of The Inflammatory Process in Atherosclerotic models by MRI and SECARS Microscopy ", 16th World Congress on Heart Disease,2011.
- **Strasbourg, France** "Monitoring cellular nanoparticles uptake with stimulated Raman & SECARS Microscopy". Hybrid Materials 2011.
- **San Francisco, USA** "Monitoring SERS-based contrast agents in atherosclerosis", Advanced Biomedical & clinical diagnostic systems, SPIE 2011.
- **Berlin, Germany** "Monitoring cellular metabolic interactions of nanoparticles in biological systems with Stimulated Raman and SECARS Microscopy." 7th International Conference on Biomedical Applications of Nanotechnology, Nanomed2010.
- **Boston, USA** "Imaging USPIO Nanoparticles Uptakes in Biological Systems with Vibrational Microspectroscopy", International Conferences on Raman Spectroscopy, ICORS2010.
- **Kyoto, Japan**, "Non-invasive Molecular Imaging of USPIO Uptakes in Atherosclerotic Aorta using Nonlinear Raman Microscopy," 2010 World Molecular Imaging Congress: WMIC2010
- **Berlin, Germany** "Investigating neurodegenerative disorder systems using USPIO-nanoparticles," Twentieth Meeting of the European Neurological Society, ENS2010.
- **Paris, France** "Investigating neurodegenerative disorder systems using USPIO-nanoparticles with (SECARS) and stimulated Raman scattering microscopy", Third European Workshop on Lipid Mediators. Pasteur Institute,2010.
- **Houston, USA**, First Global Congress on NanoEngineering for Medicine, ASME 2010.
- **Venice, Italy**, Molecular Mechanisms of Neurodegeneration, 2010
- **Boston, USA**, CRS International meeting, Harvard University, 2009

Helmut Maecke

Prof. Helmut Maecke, PhD

TRAINING

Undergraduate and graduate work at the University Basel, Switzerland
Thesis 1971 under Prof. S. Fallab in bioinorganic chemistry: "Synthetic Oxygen Carriers"

1971-1977	Teaching and research assistant, reader, Chemistry department, University Basel
1977-1980	Research fellow (postdoc), University of Southern California, Los Angeles: Prof. A. Adamson: work in Inorganic Photochemistry/ photophysics
1984	„Habilitation" in Inorganic Chemistry, Photochemistry and photophysics, University Basel
1984	Head of Radiopharmacy, University Hospital Basel
1988	Tenured position
1992	Professor of Radiopharmacy (AP), University Hospital Basel
1996-2009	Head of Division of Radiological Chemistry, University Hospital Basel
2009-	Guest professor (emeritus) at the university hospital Freiburg, Germany

HONORS

- Co-Awardee Prize of the Swiss Society of Gastroenterology
- Several prizes for best scientific and most cited papers of the European Aassociation of Nuclear Medicine (Springer prizes)
- Marie Curie Award Lecture of the European Association of Nuclear Medicine (EANM) 2008
- Becquerel medal of the Royal Society of Chemistry for life time achievemnet (2011)
- Pioneer Award at the first world congress on Ga-68 PET and Peptide Receptor Radionuclide Therapy, 2011, Bad Berka.
- Distinguished Pioneer`s Award , 2012, Austrian Society of Nuclear Medicine

Over 230 peer reviewed publications
6 patents in the field of diagnostic and therapeutic radiopharmaceuticals

INTERESTS

- Radiopharmacy
- Radiopharmacology
- Targeted MRI Contrast Agents
- Bioinorganic Chemistry
- Medical Application of Metal Based Drugs

MAIN AREA OF RESEARCH

- Design, synthesis, in vitro and in vivo characterization of radiolabeled peptides and antibodies for imaging and internal radiotherapy; patient studies. The bench-to-bed approach. About 2000 patients were studied and treated with radiopeptides and antibodies developed in my lab.
- $^{68}Ga/^{64}Cu$-based PET radiopharmacy.
- Chelator chemistry, bifunctional chelators for radiometals
- Gd-based MRI contrast agents.

PROFESSIONAL ACTIVITIES

- Member of the American, European, German and Swiss Societies of Nuclear Medicine
- Member of the American, European, German and Swiss Chemical Societies
- Member of the German, Austrian and Swiss Society of Radiation Safety
- Member of the Society of Molecular Imaging
- Member of the Academy of Molecular Imaging
- Founding Member of the European Society of Molecular Imaging (ESMI)
- Member of the Swiss Society of Radiopharmacy and Radiopharmaceutical Chemistry (Chairman 2001-2005)
- Member of the Swiss Society of Pharmaceutical Sciences
- Member of the International Research group in Immuno-Scintigraphy and Therapy (IRIST)
- Chairman of the working group "Radiolabelled Peptides and Oligonucleotides" within an action of the European Cooperation in the field of Scientific and Technical Research (COST)
- Member of the Editorial Board of the European Journal of Nuclear Medicine and Molecular Imaging
- Member of the Editorial Board of Bioconjugate Chemistry
- Advisory Board Member of the journal Contrast Media and Molecular Imaging
- Member of the Editorial Board of the Journal of Nuclear Medicine
- Member of the Scientific Board of Nexatio AG, Basel
- Member of the Scientific Board in the field of immunospecific tumor targeting of radiopharmaceuticals of INSERM U601, Nantes
- Chairman of the working group "Targeting Probes" within an action of the European Cooperation in the field of Scientific and Technical Research (COST D38)
- Member of the Therapy Committee of the European Association of Nuclear Medicine (EANM)
- Co-chairman of the COST action BM 0607 (Targeted radionuclide therapy)
- Founding member of 3B Parmaceuticals

Mira Marcus-Kalish

miram@post.tau.ac.il

Dr. Mira Marcus-Kalish is currently a Senior Research Fellow at ICTAF – Interdisciplinary Center for Technological Analysis and Forecasting, and the director for international research affairs at Tel Aviv University. Her main areas of interest are mathematical modelling, data Analysis, converging technologies and data mining (mainly a targeted rule discovery tool for Bio-Medicine).
Recent projects focused on personalized skin treatments, rehabilitation of the discrete sensory motor learning function, cerebellar motor learning, protein- protein interactions, drug toxicity analysis, learning machine systems, smart sensors for tackling oil spill, multi-level multisource data mining applied to neurology, etc.
Mira Marcus-Kalish holds a Ph.D in operations research from the Technion, Haifa, where she developed a computerized E.C.G. diagnosis system.
She did her post doctorate training at Harvard University, at the MBCRR laboratory (Molecular Biology Computer Research and Resource) and the Dana Farber Cancer Institute.
Her B.Sc. is in Statistics and Biology from the Hebrew University in Jerusalem.
Coming back to Israel, she joined the Tel-Aviv University Business School, focusing on Medical informatics and management, than moved to the Weizmann Institute working with Prof. Ephraim Katzir mainly on protein interactions.
She was involved at the private business enterprise and served as the scientific advisor and later as the head of the Enterprise Marketing Department in IBM Israel.
Dr. Kalish is involved in many EU framework projects: ReNaChip, SkinTreat, EpoCan, HBR, etc and was the joint research work package leader in the Nano2Life NoE (Network of Excellence) yielding 43 funded projects.

Stephan Marsch

Stephan Marsch is Professor of Intensive Care Medicine and Chairman of the Medical Intensive Care Unit at the University of Basel, Switzerland. He graduated in Zürich and acquired a MD in Zürich and a DPhil in Oxford, UK. He is board certified in Internal Medicine, Anaesthesiology, and Intensive Care. He is currently Study Dean of the Medical Faculty of the University of Basel. Since the late 1990s he is involved in the simulator-based training of interdisciplinary teams. Under his leadership a high-fidelity patient simulator was build within the Medical Intensive Care Unit of Basel in 2000 where workshops are performed for health-care workers of the Intensive Care Unit, general practitioners and hospital physicians from all over Switzerland, and medical students. The focus of his current academic work is on Human Factors relevant for the settings of acute medicine and improvements in medical education of individuals and teams.

Scott McNeil

Dr. Scott McNeil serves as the Director of the Nanotechnology Characterization Laboratory (NCL) for SAIC-Frederick and the National Cancer Institute at Frederick (NCI-Frederick), 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 more than 250 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 also directs SAIC-Frederick's Imaging and Nanotechnology Group (ING), and is a Vice President of SAIC-Frederick.
Prior to establishing the NCL, he served as a Senior Scientist in the Nanotech Initiatives Division at SAIC where he transitioned basic nanotechnology research to government and commercial markets. He advises Industry and 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.

Phillip C. Miller

Phillip C. Miller, B.A., Ph.D.

Dr. Phil Miller is the Senior Vice President of Technology & Applied Research at Ventana Medical Systems, Inc. (Ventana). With over 25 years of experience in the healthcare industry, his primary responsibilities include research, development, and commercialization of new technology-based products.

In 1988, Phil was involved in the early development of Ventana and was a key participant in raising venture capital. During the venture start-up, he led the research and development phase and successful market launch of the first Ventana IHC system, including an instrument and a broad menu of reagent kits.

Prior to Ventana, Phil was the Vice President of Research and Development at Nichols Institute Diagnostics in California where he was responsible for numerous business development activities. Phil also served as a member of Quest Diagnostics' Biotechnology Research Innovation team with responsibility for technology assessment, acquisition and development, and he played a key role in the development of novel assays with large pharmaceutical companies and clinical research sites.

Phil has extensive experience in intellectual property, patents and licensing. He has worked with university inventors, patent counsel and marketing consultants to assess new technology for patentability and commercialization of new technology from academic institutions and research foundations.

Phil received his Bachelor of Arts in Chemistry from Indiana University in 1971 and his honorary Doctor of Science from the University of Arizona in 2011. He holds 14 U.S. patents and has authored numerous publications, papers, abstracts and scientific sessions.

Moein Moghimi

BSc (Man.), PhD (Lond.), FIoN
Department of Pharmaceutics and Analytical Chemistry
University of Copenhagen
Universitetsparken 2
DK-2100 Copenhagen, Denmark
Tel: +45 35 33 65 28
E-mail: momo@farma.ku.dk
http://www.farma.ku.dk/index.php/Nanomedicine/8313/0/

Moein Moghimi (British National) is Professor of Nanomedicine and Head of the Nanomedicine Group at the Department of Pharmaceutics and Analytical Chemistry (Faculty of Medicine, University of Copenhagen, Denmark). He further serves as the Director of the Centre for Pharmaceutical Nanotechnology and Nanotoxiocology (CPNN) and Group Leader in Pharmaceutical Nanotechnology at the NanoScience Center (Faculty of Science, University of Copenhagen). He is also Guest Professor of Nanomedicine at the Multidisciplinary Research Center, Shantou University (China) and the elected Fellow of the Institute of Nanotechnology (FIoN) in UK. Previously he was Senior Lecturer in Biopharmacy and Molecular Pharmaceutics at the School of Pharmacy, University of Brighton (UK), and The University Research Fellow in Advanced Drug Delivery at the Department of Pharmaceutical Sciences, University of Nottingham (UK). His research activities are focused on experimental nanomedicine, nanotoxicology and pharmaceutical nanotechnology. He has pioneered research in design and surface engineering of nanoparticles and functional nanosystems for parenteral site-specific targeting and imaging modalities (e.g., splenotropic entities, lymphotropic agents, 'phagocyte-resistant' nanoparticles and cancer nanomedicines) as well as the molecular basis of nanomaterial cytotoxicity (single cell studies) and adverse immunological reactions (complement activation mechanisms). Professor Moghimi has been the recipient of numerous awards and most recently was honoured with the Pharmaceutical Sciences Research Achievement Award (Copenhagen University). His contributions to peer-reviewed high impact international journals include over 100 original full research papers and invited critical reviews (with over 4500 citations and h-index of 32) and more than 40 book chapters, business reports, editorials, and patents. Since 2009, Professor Moghimi has secured over 7 million Euros in competitive research funds as principal investigator in nanomedicine and bionanotechnology and partnering European Commission FP-7 programmes.

Professor Moghimi has previously served as invited Theme Editor for four Theme Issues of the prestigious Advanced Drug Delivery Reviews (Elsevier, The Netherlands) and currently act as Associate Editor for both Nanomedicine: Nanotechnology, Biology and Medicine (Elsevier) and Journal of Biomedical Nanotechnology (American Scientific Publishers, USA). He is a member of editorial/advisory board of 17 international journals to include Advanced Drug Delivery Reviews, Nanomedicine-UK (Future Medicine), Journal of Liposome Research (Informa Healthcare), Drug Delivery (Informa Healthcare), Recent Patents in Drug Delivery and Formulation (Bentham) and Current Drug Discovery Technologies (Bentham). He further practices in the capacity of consultant to numerous pharmaceutical, biotechnology, health, and food industries as well as investment banks, management consultancy firms and other entrepreneurial enterprises world-wide and was an invited evaluator for Nanotechnology/Nanomedicine Centres of Excellence in Germany and Austria. Other responsibilities include being a regular invited assessor and expert in drug delivery systems and nanomedicine for various research councils and organizations world-wide (over 50 establishments in 20 countries). To date, Professor Moghimi has been an appointed reviewer to over 1000 manuscripts for more than 100 international journals and has delivered over 200 invited presentations and keynote lectures in more than 25 countries as well as being regular conference chair and organizer.

In 1985, he graduated with Honors in Biochemistry from The University of Manchester (UK) and in 1989 completed a PhD in Biochemistry (liposome immunobiology) at the Charing Cross Hospital Medical School (Imperial College, University of London, UK).

Professor Moghimi is listed in Marquis Who's Who in the World, USA, Marquis Who's Who in Science and Engineering, USA, and Marquis Who's Who in Medicine and Healthcare, USA (by invitation).

Jan Mollenhauer

Jan Mollenhauer, born in Kiel, Germany, in 1968, studied biology from 1989 till 1994 at the University of Cologne and received his PhD in 1998 from the University of Heidelberg, Germany. He worked in the field of genomics and functional genomics at the Division of Molecular Genome Analysis with Prof. Annemarie Poustka at the German Cancer Research Center (DKFZ) Heidelberg from 1994 till In 2008 and received his habilitation in Molecular Medicine from the University Heidelberg, which was mentored by the Nobel laureate in Medicine, Prof. Harald zur Hausen. Since 2008 he works as Professor for Molecular Oncology at the University of Southern Denmark, Odense. Jan Mollenhauer received the Future Award in Health Sciences 2005 and was listed in the 2007 edition of the Who Is Who of Emerging Leaders. In 2009, he received the Leo og Ingeborg Dannins Fonden award and was included in the Portrait Collection of the Danish Royal Library. His work was further awarded with the Fyens Stiftstidende R esearcher Award 2010. Since 2010, he is director of the Lundbeckfonden Center of Excellence in Nanomedicine NanoCAN and he will lead the Danish-German High Technology Platform starting from 2011. Research focuses on pattern recognition molecules, synthetic biology, functional genomics, drug target discovery, and nanomedicine.

Ýrr Mørch

Ýrr Mørch, Ph.D.
Post doc at SINTEF Materials and Chemistry/Dept. of Physics, Norwegian University of Science and Technology, Sem Sælandsvei 2, Kjemihallen 3rd floor, N-7491, Trondheim, Norway.
Phone: +47 40234744, e-mail: yrr.morch@ntnu.no / yrr.morch@sintef.no

EDUCATION

Ph.D. degree in biotechnology at Dept. of Biotechnology, Norwegian University of Science and Technology (NTNU), "Novel Alginate Microcapsules for Cell Therapy" (2008).
Master degree in chemical engineering, NTNU (1999).

PROFESSIONAL EXPERIENCE

2011 –	present: Post.doc at Dept. of Physics, NTNU and SINTEF Materials and Technology
2010	Research Scientist at Dept. of Physics, NTNU
2007 – 2009	Research Scientist at Dept. of Biotechnology, NTNU, funded by The Chicago Diabetes Project, University of Illinois at Chicago, USA.
2002 – 2007	Ph.D. student at Dept. of Biotechnology, NTNU
2000 – 2002	Research assistant/teacher, Dept. of Biotechnology, NTNU.

PROFESSIONAL SKILLS

Microencapsulation, nanoencapsulation, cell encapsulation (cell therapy), miniemulsion method, polymerization, surface modification and targeting of nanoparticles, biopolymer gelling techniques (internal and external gelling, capsules, beads, films, foams, cylinders), surface modifications and chemical modification of biopolymers, enzymatic modification of biopolymers, cell handling and culturing (including staining and imaging and various cell function techniques), rheological measurements (reduced capillary viscosity, uniaxial compression measurements, oscillation measurements), permeability measurements, optical microscopy techniques (including fluorescence microscopy and CLSM), image analysis (including high-speed camera imaging), Circular Dichroism Spectroscopy, lithography, S(T)EM, Zetasizer, Coulter Counter, microfluidics.

AWARDS

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

PUBLICATIONS

16 peer reviewed publications (times cited: 367 ICI Web of Science, March 2012), 4 public science publications, 8 (first author) presentations at international scientific conferences. numerous publications, papers, abstracts and scientific sessions.

Maria Jose Morilla

Maria Jose Morilla is currently Adjunct Professor of chemistry at Universidad Nacional de Quilmes and Adjunct Researcher of the National Science Research Council (CONICET). She received a Biotechnology degree of the Universidad Nacional de Quilmes in 1999 and a Ph.D. in Natural and Exact Science in 2003. Her Ph.D. research focused on the design of anti-chagasic liposomes. She is member of the Nanomedicine Research Program where she supervises projects on development of dendrimers and megamers for oral and mucose nano-delivery systems. She has supervised (2011) and co-supervised (2009, 2008) three PhD thesis in nanomedicine. Her main contributions in the last five years have been published as corresponding author, in the following journals:, Journal of Controlled Release, Expert Opinion in Drug Delivery, Advanced Drug Delivery Reviews, BMC Biotechnology, International Journal of Pharmaceutics. She is Founding Member of the Argentinean Association for Nanomedicines (Nanomed-ar).

Christian Moser

The virosome technology is the focus of Christian Moser's work ever since he joined the vaccine industry ten years ago. From 2001 to 2005, he held the position as head virus research at Berna Biotech where he was involved in the preclinical and clinical vaccine development as well as in life cycle management of commercial vaccines. In 2005 he joined Pevion as principal scientist and was appointed as head research in 2009.
Christian Moser graduated as a veterinary surgeon at the University of Bern in 1990. After two years in practice, he obtained a doctoral degree and a PhD in veterinary medicine from the University of Bern (Switzerland) and held a post-doc position at the Institute for Human Gene Therapy at the University of Pennsylvania (Philadelphia, USA) from 1999 to 2001.

Bert Müller

Bert Müller received a diploma in mechanical engineering (1982), followed by the M.Sc. degree from the Dresden University of Technology and the Ph.D. from the University of Hannover, Germany in 1989 and 1994. From 1994 to 2001, he worked as a researcher at the Paderborn University, Germany, EPF Lausanne, ETH Zurich. 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 Hospital Basel in September 2006, he founded the Biomaterials Science Center. He also teaches physics and materials science at the ETH Zurich and the Universities of Basel and Bern.

Fabrice Nesslany

PhD In Toxicology
Head of the Genetic Toxicology Laboratory of Institut PASTEUR de LILLE, FRANCE.
Date of Birth: 18.11.1965
Nationality: French
Professional Address:
1, rue du Pr CALMETTE – BP245
59019 LILLE Cedex - FRANCE

Member of the Expert Committees for Plant protection products: chemical substances and preparations at the French Agency for Food, Environmental, and Occupational Health & Safety (ANSES)

Chairman of the Expert Committees for Food contact materials at the French Agency for Food, Environmental, and Occupational Health & Safety (ANSES)

Chairman of the working group nano3 (2009-2010) at the French Agency for Environmental and Occupational Health Safety (AFSSET).

Member of the Working Group Nano & Food at the French Agency for Food, Environmental, and Occupational Health & Safety (ANSES)

Member of the Working Groups on Non-clinical innovation and on tattoo products at the French Agency of French Agency of Human Health Products (AFSSAPS)

Expert in OMNT (Observatory for Micro & NanoTechnologies),

Involved in many expertise and research activities particularly in the field of nanogenotoxicology

- NANOGENOTOX (2010-2013) a Joint Action on « the Safety of Nanomaterials which aims at establishing a robust methodology to screen potentially genotoxic manufactured nanomaterials using standard in vitro/in vivo assays completed with specific tests,
- Research project IRENI (Funding from European, Feder) which aims at developing a model enabling the study of the in vitro genotoxicity (comet assay & μnucleus test) of Nanoparticles on respiratory cell line (16HBEo-)

INTERNATIONAL PUBLICATIONSFOR THE LAST 5 YEARS

- Platel A, **Nesslany F**, Gervais V, Claude N, Marzin D. Study of oxidative DNA damage in TK6 human lymphoblastoid cells by use of the thymidine kinase gene mutation assay and the in vitro modified comet assay: Determination of No-Observed-Genotoxic-Effect-Levels. Mutat Res. 2011 Dec 24;726(2):151-9.
- Stephen D. Dertinger, Souk Phonethepswath, Pamela Weller, John Nicolette, Joel Murray, Paul Sonders, Hans-Werner Vohr, Jing Shi, Ljubica Krsmanovic, Carol Gleason, Laura Custer, Andrew Henwood, Kevin Sweder, Leon F. Stankowski Jr., Daniel J. Roberts, Amanda Giddings, Julia Kenny, Anthony M. Lynch, Céline Defrain, **Fabrice Nesslany**, Bas-jan M. van der Leede, Terry Van Doninck, Ann Schuermans, Kentaro Tanaka, Yoshie Hiwata, Osamu Tajima, Eleanor Wilde, Azeddine Elhajouji, William C. Gunther, Catherine J. Thiffeault, Thomas J. Shutsky, Ronald D. Fiedler, Takafumi Kimoto, Javed A. Bhalli, Robert H. Heflich and James T. MacGregor. International Pig-a gene mutation assay trial: Evaluation of transferability across 14 laboratories. Environmental and Molecular Mutagenesis 2011 Sep 11.
- Merhi M, Dombu CY, Brient A, Chang J, Platel A, Le Curieux F, Marzin D, **Nesslany F** and Betbeder D. Study of serum interaction with a cationic nanoparticle: Implications for in vitro endocytosis, cytotoxicity and genotoxicity. Int J Pharm. 2011 Jul 27.
- Arnich N, Canivenc-Lavier MC, Kolf-Clauw M, Coffigny H, Cravedi JP, Grob K, Macherey AC, Masset D, Maximilien R, Narbonne JF, **Nesslany F**, Stadler J, Tulliez J. Conclusions of the French Food Safety Agency on the toxicity of bisphenol A. Int J Hyg Environ Health. 2011 Jan 7.
- Sultan A, **Nesslany F**, Violet M, Bégard S, Loyens A, Talahari S, Mansuroglu Z, Marzin D, Sergeant N, Humez S, Colin M, Bonnefoy E, Buée L, Galas MC. Nuclear tau, a key player in neuronal DNA protection. J Biol Chem. 2011 Feb 11;286(6):4566-75.
- Platel A, Gervais V, Sajot N, **Nesslany F**, Marzin D, Claude N. Study of gene expression profiles in TK6 human cells exposed to DNA-oxidizing agents. Mutat Res. 2010 Jul 7;689(1-2):21-49.
- **Nesslany F**, Marzin D. Cytosine arabinoside, vinblastine, diethylstilboestrol and 2-aminoanthracene tested in the in vitro human TK6 cell line micronucleus test (MNvit) at Institut Pasteur de Lille in support of OECD draft test guideline 487. Mutat Res. 2010 Oct 29;702(2):212-8.
- **Nesslany F**, Parent-Massin D, Marzin D. Risk assessment of consumption of methylchavicol and tarragon: The genotoxic potential in vivo and in vitro. Mutat Res. 2010 Feb 1;696(1):1-9.
- Khandoudi N, Porte P, Chtourou S, **Nesslany F**, Marzin D, Le Curieux F. The presence of arginine may be a source of false positive results in the Ames test. Mutat Res. 2009 Sep-Oct;679(1-2):65-71.
- **Nesslany F**, Simar-Meintières S, Ficheux H, Marzin D. Aloe-emodin-induced DNA fragmentation in the mouse in vivo comet assay. Mutat Res. 2009 Aug;678(1):13-9.
- Platel A, **Nesslany F**, Gervais V, Marzin D. Study of oxidative DNA damage in TK6 human lymphoblastoid cells by use of the in vitro micronucleus test: Determination of No-Observed-Effect Levels. Mutat Res. 2009 Aug;678(1):30-7.
- Gaudin J, Le Hégarat L, **Nesslany F**, Marzin D and Fessard V. "In vivo genotoxic potential of microcystin-LR, a cyanobacterial toxin, investigated both by the unscheduled DNA synthesis (UDS) and the comet assays after intravenous administration". Environ Toxicol. 2008 Jun 17.
- **Nesslany F**, Simar-Meintieres S, Watzinger M, Talahari I, Marzin D. "Characterisation of the genotoxicity of nitrilotriacetic acid". Environ Mol Mutagen. 2008 Jul;49(6):439-52.
- **Nesslany F**, Zennouche N, Simar-Meintieres S, Talahari I, Nkili-Mboui EN, Marzin D. "In vivo Comet assay on isolated kidney cells to distinguish genotoxic carcinogens from epigenetic carcinogens or cytotoxic compounds.". Mutat Res. 2007 Jun 15;630(1-2):28-41.

Maj-Inger Nilsson

Maj-Inger Nilsson is a pharmacist by training, Ph.D. and Associate Professor in Biopharmaceutics and Pharmacokinetics, all from Faculty of Pharmacy, Uppsala University, Uppsala, Sweden. She has an extensive experience of preclinical and clinical research from the pharmaceutical industry, both in Sweden and internationally. She has also worked with public health organisations, regulatory agencies and research councils.

Maj-Inger Nilsson has published about 60 papers in international journals and at international meetings. In addition, she has written more than 150 scientific reports related to preclinical and clinical study programmes, project summaries and expert reports in the development of new drug compounds. She is a member of a several scientific associations.

Nataliya V. Nukolova

Date of Birth: June 10, 1982
Place of Birth: Moscow, Russian Federation
Present Position: Senior Research Scientist
Office Address: Department of Fundamental and Applied Neurobiology, Serbsky National Research Centre for Social and Forensic Psychiatry, Kropotkinsky per.23, 119991, Moscow, Russia
Telephone: +7 926 820-3665 E-mail: nnukolova@gmail.com

EDUCATION

07/1999-06/2004 Master of Science in Organic Chemistry, Department of Chemistry, Lomonosov Moscow State University, Russia
10/2004-9/2009 Graduate Student at Polymer Science Division, Department of Chemistry Lomonosov Moscow State University, Russia

POSITIONS AND EMPLOYMENT

2005-2006	Junior Research Assistant, School of Chemistry, Moscow State University, Department of Chemistry, Russia
2006-2010	Research Assistant, College of Pharmacy, University of Nebraska Medical Center, Omaha, Nebraska, USA
2011-pres.	Senior Research Scientist, Department of Fundamental and Applied Neurobiology, Serbsky National Research Centre for Social and Forensic Psychiatry, Russia
2012-pres.	Senior Research Scientist, Department of Medical Nanobiotechnology, Russian State Medical University, Russia

HONORS

2003 "Chembridge" corporation stipendium for students;
2012-2014 Special Grant of the President of Russia for Young Scientists

SELECTED PEER-REVIEWED PUBLICATIONS

- 1. Kudryavtsev K.V., **Nukolova N.V.**, Kokoreva O.V., Smolin E.S.; Stereoselective Synthesis of Functional Derivatives of 2-(2-Carboxyethyl)pyrroliddine-2-carboxylic Acid. Russian Journal of Organic Chemistry, 2006, 42 (3) 412-422. (stereoselective synthesis of precursor of kaitocephalin)
- 2. Kim J.O., **Nukolova N.V.**, Oberoi H.S., Kabanov A.V., Bronich T.K.; Block ionomer complex micelles with cross-linked cores for drug delivery. Polymer Science Series A, 2009, 51(6), 708-718. (relations between structure of micelles and loaded drugs)
- 3. Nukolova N.V., Zigang Y., Kim J.O., Kabanov A.V., Bronich

T.K.; Polyelectrolyte nanogels decorated with monoclonal antibody for targeted drug delivery. Reactive & Functional Polymers 2011, 71, 315–323. (new approach for modification of nanogels)

- 4. **Nukolova N.V.**, Oberoi H.S., Cohen S.M., Kabanov A.V., Bronich T.K.; Folate-decorated nanogels for targeted therapy of ovarian cancer. Biomaterials, 2011, 32, 5417-5426. (first paper on folate-modified nanogels for delivery cisplatin in vivo)

Marisa Papaluca

MD, Specialist in Internal Medicine

Graduated in Medicine in Rome in June 1978 Marisa engaged in the emerging Clinical immunology research area and worked in clinical practice as specialist in internal medicine (1978-1988).
Medical director at the Italian Ministry of Health (1984 – 1994) she actively contributed to Pharmacovigilance, Safety and Efficacy of pharmaceuticals as Rapporteur for a number of products and member of National and International Committees and expert groups such as the CPMP and the ICH1. In 1991 she established the first national Operational Centre for Community Procedures, leading innovative activities in collaboration with EU and international regulatory partners2 and since contributed to the drafting of Regulation 2309/93 establishing the European Medicines Evaluation Agency.
From 1994 to date at the EMA, she took scientific and managerial leadership 3 maintaining special focus on innovation in pharmaceuticals4 and in regulatory science and processes5.
Being an engaging communicator Marisa has been active in global platforms since 1991 bringing into the stakeholders' debate clear and independent information from Regulators. Landmark contributions include the workshops on Pharmacogenomics, on Biosimilars, on the Qualification Process for Biomarkers and the recent track on Personalised Medicine and Nanotechnology conferences. Marisa also contributed to 14 books and published more than 40 articles in scientific and regulatory Journals.

[1] National Committee for Medicines (CUF), CPMP (Committee for Proprietary Medicinal Products), international and European Working Parties (WHO, CIOMS, CPMP Pharmacovigilance and Efficacy), ICH expert groups. S6, E5, Gene Therapy Discussion Group and pharmacogenomics guidelines (E14 and 15).
[2] E.g. scientific advice on clinical trials design in conjunction with other National Competent Authorities, overview of safety profile of r-h-DNA products in the EU market, focus expert group on safety of hypnotics, computer assisted New drugs applications (CANDA)
[3] Previously deputy Head of Sector and currently Section Head of Scientific Support and Projects in the Unit for Human Medicines Development and Evaluation.
[4] Gene therapy, cell therapy, pharmacogenomics, nanomedicines, combined and borderline health products
[5] Start-up of the centralised procedure, Innovation Task Force early dialogue platform, Advanced therapies classification, Business Pipeline Project, Biosimilars concept development, Biomarkers Qualification, modular EU referrals management, transversal scientific support to regulatory scientific opinions.

Carlos Peña

Carlos Peña, Ph.D., M.S.

Dr. Carlos Peña is Director of Emerging Technology Programs in the Office of the Chief Scientist, Office of the Commissioner, at the U.S. Food and Drug Administration (FDA). He currently serves a lead role in the development of the agency's position and current thinking on science, regulatory research, policy, and communication needs for emerging technology areas, with an emphasis on nanotechnology. His position includes service on the FDA Nanotechnology Task Force composed of key officials across the agency and establishing and enhancing partnerships with national and international regulatory agencies as well as others stakeholders focused on nanotechnology. He also serves as Chair of the FDA Standards Committee, a committee dedicated to ensuring effective participation by FDA in the development of both domestic and international standards relevant to emerging technologies and other product areas.

Before joining FDA, Dr. Peña served at the National Institute of Neurological Disorders and Stroke, National Institute of Health. He completed his neurosciences doctoral training at Case Western Reserve University in Cleveland, Ohio. Prior to graduate school, he attended the University of Connecticut for the Masters in Comparative Physiology, and the City College of New York, City University of New York, where he received a Bachelors specializing in Developmental Biology.

Jean-Yves Pierga

Prof. Jean-Yves Pierga is Professor of Medicine and Medical Oncology at Paris Descartes University since 2005. He is a full time medical oncologist at the Institut Curie, Paris Cancer Center. His main research interests are breast cancer treatments, early clinical trials and translational research.
Pr Pierga trained as a medical oncologist and obtained a Silver medal from Paris Medical School in 1994. His PhD thesis focused on disseminated tumor cells detection and characterization in breast cancer patients (2003, Paris University XI; Thesis Director: Dr Jean-Paul Thiery).
After a post-doctoral fellowship in 2004 at the Royal Marsden London in the Breast Cancer Unit of Pr IE Smith and the Academic Biochemistry Department of Pr M. Dowsett, Pr. Pierga became head of the Day-clinic at the Institut Curie until 2007. He is currently Head of the research program on disseminated and circulating tumor cells at the Institut Curie since 2010.
He has contributed to over 120 peer-reviewed publications. He is member of Société Française de Cancérologie (SFC), European Society for Medical Oncology (ESMO), American Society of Clinical Oncology (ASCO), Breast Cancer Group of the EORTC.

Adriele Prina-Mello

Dr. Adriele Prina-Mello is a CRANN Investigator, a Senior Research Fellow of the School of Medicine and a part-time lecture at Trinity College Dublin (Ireland), a Nanosafety Cluster member and the vice-chair of the Nanodiagnostic working group of the European Technology Platform of Nanomedicine.
Dr Prina-Mello has extensively published his work in biomedicine, nanotechnology, nanotoxicology and nanomedicine research area. Dr Prina-Mello main research interest is on the development of future applications of nanoparticles and nanomaterials in the Biomedical field.Dr Prina-Mello is involved in developing and advancing several multidisciplinary research projects between University, Research Hospital and Industry partners for future applications in medicine and nanotechnology industry. Currently involved in several EU FP7 funded projects: NAMDIATREAM (NMP) MULTIFUN (NMP), and Celtic Alliance in Nanomedicine (INTERREG).

Michael Reinert

1986 – 1992 Medical School in Basel Switzerland
1993 – 1995 Internship in Internal Medicine and Surgery
1996 – 2002 Neurosurgical Training
2007 Habilitation
2011 Associate Professor

Prof. Dr. med. Michael Reinert is secretary oft he Swiss Society of Neurosurgeons and Member of the American Association of Neurological Surgeons. The main research topics are cerebral metabolism of severe head injury and hemorrhage and the development of minimal invasive surgical techniques.

Prof. Reinert is collaborator in three peer reviewed grants:

- 1. KTI Projekt Nr. 11478.1 PFLS-LS: Tissue soldering with nanoparticle scaffold
- 2. SNF 32003B_133083: Sutureless endoluminal and transluminal microvscular laser anastomosis
- 3. NRP 64: 406440_131297: Transport of nanoparticles after release from a biodegradable implant

Bernd Riebesehl

Dr. Bernd Riebesehl joined Novartis as Principal Fellow, Technical Research & Development, Novartis Pharma AG, Basel in 2007, acting as Formulation Expert and Technical Project Leader. The scope of his responsibilities includes the development of drug substances and parenteral drug formulations from early development to regulatory submissions of new drug applications to health authorities. He is pursuing the integration of nanomedicine formulations into the parenteral technology platform.
Bernd Riebesehl is Pharmacist and completed a thesis on solubilization in the field of Pharmaceutical Technology at the Technical University Braunschweig, Germany. He joined Beiersdorf-Lilly, the later Lilly Forschung GmbH in 1992 focusing on drug product development and drug delivery in the Department Pharmaceutical Research & Development. In 2007 he joined Speedel Pharma AG, Basel as Director Pharmaceutical Research & Development. He is active member of the APV Focus Group Drug Delivery.

Christoph Rochlitz

23.09.1957 Born in Karlsruhe, Germany

- 5/76 High-school graduation at the Bismarck-Gymnasium in Karlsruhe, Germany
- 10/76-9/79 Medical school at the Freie Universität in Berlin, Germany
- 10/79-9/83 Medical school at the Ruprecht-Karls University in Heidelberg, Germany
- 10/80-9/81 Research fellowship in Montpellier, France
- 10/82-9/83 Internship in Gynecology and Internal Medicine in Heidelberg, and Surgery at the University of Cambridge, England
- 10/83 Approbation in Heidelberg, Germany (= MD licence)
- 2/84-1/86 Research assistant at the University Hospital in Heidelberg, Germany
- 2/85 American medical graduate examination (FMGEM = Foreign Medical Graduate Examination in the Medical Scienees)
- 1/86-12/87 Postdoctoral research fellowship at the University of California in San Francisco, USA
- 3/88-11/92 Research assistant at the University of Berlin, Germany, Department of Hematology and Oncology
- 5/92 Approbation for Internal Medicine in Berlin, Germany
- 5/93 Docentship (Privatdozent)
- 12/92-6/93 Postdoctoral research fellowship at the Institute Pasteur in Paris, France
- since 7/93 Attending in Oncology, Department of Internal Medicine and Research Group Leader at the Department of Research of the University Hospital in CH-4031 Basel, Switzerland
- since 8/97 Deputy Head of the Department of Oncology, University Hospital in Basel, Switzerland
- since 8/99 Associate Professor (Titularprofessor) University of Basel, Switzerland
- since 2006 Head of the Brustzentrum of the University of Basel
- since 7/2011 Full Professor of Oncology, Head of the Department of Oncology, University Hospital in Basel, Switzerland

Eder Lilia Romero

Eder L. Romero was educated at University of La Plata, Argentina where she obtained her M.A. Biochemistry and PhD in Exact Sciences (1996). Following a post-doctoral research in Groningen University, The Netherlands under the supervision of Prof. Gerrit Sherphof (1997-1998), she returned to Argentina where currently is an Independent Researcher at the National Council of Scientific and Technological Research (CONICET) (2006) and Associate Professor of Chemistry (2008), at the Department of Science and Technology, National University of Quilmes, Buenos Aires, Argentina.
From 2007 she is leading the Program of Nanomedicine Research (PNM), being under her supervision four finished PhD thesis in nanomedicine (2003, 2008, 2009, 2010) and other four ongoing doctoral research subjects on different nanomedical therapeutic strategies. Her research interest deals with a) development of targeted nanomedicines (photodynamic therapy) and nanocosmetics across the skin b) across the oral (delivery of macromolecules) and olfactory mucosa (bypassing the blood brain barrier), c) treatment of infectious parasitic diseases. Additionally the PNM is developing vaccination strategies employing biodegradable nano vesicles prepared with total polar archaeolipids extracted from extreme halophile archaeas, to be applied by parenteral/topical/ mucosal routes, as cattle and human adjuvants. Her main contributions in the last five years as corresponding author have been published in: International Journal of Pharmaceutics, Journal of Controlled Release, Expert Opinion in Drug Delivery, BMC Biotechnology, Advanced Drug Delivery Reviews. She is president of the Advisory Committee of the Argentinean Foundation for Nanotechnology (FAN), president and founding member of the Argentinean Association for Nanomedicines (Nanomed-ar) since 2010. Romero has been responsible for the first and second Nanomedicine School in Latinoamerica (2008, 2010). Currently she is a scientific advisor for regional pharmaceutical companies interested in developing therapeutic nanomedicines. Since 2010 she is Member of the Editorial Board of Nanomedicine: Nanotechnology, Biology and Medicine (Elsevier).

Barbara Rothen-Rutishauser

Prof. 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 lung cell culture models and various micros-

copy techniques such as laser scanning and transmission electron microscopy. In addition, she has overlooked the establishment of a wide-variety of commonly used and specialist cell biology assay methods within her research group. Since 2011 she is an independent group leader at the Respiratory Medicine, Department of Clinical Research and Bern University Hospital, Switzerland, and since 1st of July 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.

Abraham Rubinstein

Abraham Rubinstein, Ph.D.
Abraham (Avri) Rubinstein is a Full Professor of Pharmaceutical Sciences at The Hebrew University of Jerusalem, Faculty of Medicine, School of Pharmacy Institute for Drug research. After graduating with a degree in Pharmacy and completing his M.Sc. degree (physical pharmacy) he joined the R&D division of Teva Pharmaceutical Industries in 1973. In 1978 he was promoted to head the Pharmacy R&D laboratory of the company. In 1981 he returned to the Hebrew University to complete his Ph.D. studies (physical pharmacy and pharmacokinetics), after which he spent two years as a postdoctoral fellow in the University of Wisconsin-Madison (GI physiology and oral delivery). In 1987 Prof. Rubinstein joined the faculty staff of the School of pharmacy. He is the author of 97 research articles, reviews and book chapters, as well as and 5 patents. Three of the technologies he developed were purchased by start-up companies. His research interests are focused on site-specific therapy and real-time diagnostics of malignant processes in the GI tract, which includes: colon-specific drug delivery, site specific therapy of IBD, mRNA hybridization for diagnostic purposes and mechanistic understanding of affinity processes between sugar-containing polymers and the intestinal epithelium. Prof. Rubinstein was the President of the Israeli Chapter of the Controlled release Society of Bioactive Materials and serves as a consultant in the area of drug delivery and industrialization of orally administered formulations. He is the Head of the Pharmaceutical Sciences Teaching Program of The School of Pharmacy of Jerusalem.

Ralph Schiess

Dr. Ralph Schiess graduated with a master of science from the University Zurich. After training at the Institute for Systems Biology in Seattle, US, he earned his Ph.D. in science from the ETH Zurich where he developed a novel biomarker discovery strategy and worked on prostate cancer biomarkers. In 2010, Ralph Schiess co-founded ProteoMediX, a diagnostic company with the mission to enable personalized medicine by developing more accureate non-invasive diagnostics.

Raymond Schiffelers

Raymond Schiffelers was born on January 21st, 1971 in The Hague (The Netherlands). He studied Bio-Pharmaceutical Sciences at Leiden University and worked shortly for the Dept of Vascular Biology at SmithKline&Beecham Pharmaceuticals in Welwyn (UK).
His PhD studies were performed at the dept of Medical Microbiology & Infectious Diseases at Erasmus University Medical Center in Rotterdam. His thesis entitled 'Liposomal targeting of antimicrobial agents to bacterial infections'was successfully defended in 2001.
During his post-doc on a project by the Dutch Cancer Society at Utrecht University on targeting angiogenic tumor vasculature, he spend one year in a small biotech company Intradigm Co. working on the delivery of siRNA.
After his post-doc, he stayed at Utrecht University to become assistant (2004) and subsequently associate professor (2008). In 2011, he transferred to University Medical Center Utrecht to the Laboratory of Clinical Chemistry & Haematology where he is heading a research team working on exosomes and microvesicles in the framework of a European Research Council Starting Grant.

SUMMARY

- >100 research papers, >2500 citations
- > 5 M€ research grants acquired in last 5 years, most notably ERC-Starting Researchers grant (2010)
- ERC- Proof of Concept grant (2011)
- Netherlands Organization for Scientific Research Vidi grant (2007)
- TIPharma grant (2009)
- Galenus Research Prize 2009

Ruth B. Schmid

Ruth B. Schmid (59), at present Chief Business Developer at SINTEF Materials and Chemistry, is a Swiss citizen living in Norway since 1979. She gained her Diploma (1975) and PhD (1979) in Natural Sciences at ETH Zürich, Switzerland. She is a member of ACS, CRS, the European Technology Platform in Nanomedicine, two COST Actions and the External Advisory Board of the ERA-Net EuroNanoMed, the Austrian Nano Initiative and the Austrian Nano EHS program. Her present research activities include the preparation and characterisation of micro- and nanoparticles, as well as the surface modification of polymers and polymer particles. Lately, focus has been on the encapsulation and immobilisation of liquids and solids and on coating of biomaterials by self-assembling methods and covalent attachment with biocompatible, biomimetic and functional coatings. Fields of special interest are the emerging fields of nanomedicine and application of encapsulation technologies and controlled release in various industrial segments. She has worked with interdisciplinary research the last 30 years and is at present the project manager of SINTEFs strategic research project in medical technology (MedicalACTION). She is author/co-author of 53 publications/patents, 48 oral/poster presentations, 11 mass media and popular science articles and 14 confidential SINTEF reports.

Simó Schwartz

Dr. Simó Schwartz Jr. began his career as a postdoctoral researcher at the Burnham Institute for Biomedical Research, where, under the guidance of Dr. Manuel Perucho, he worked intensively on the molecular pathways involved in the development of colorectal cancer. In 2000, he was appointed head of the Molecular Oncology and Aging laboratory at the Molecular Biology and Biochemistry Research Center for Nanomedicine (CIBBIM Nanomedicine) of the Vall d' Hebron University Hospital in Barcelona, where he is now Coordinator and member of the board. Furthermore, he is a member of the Science Advisory Board of the Vall d' Hebron Research Institute (VHIR).
Dr. Schwartz also leads the group on Drug Delivery and Targeting at the CIBBIM Nanomedicine. In this context, he is coordinator and collaborator of numerous research projects directly related to the obtention of therapeutic drug delivery systems, such as the CENIT Oncnosis project, two ERANET projects and an international project at the Iberian Nanotechnology Institute (OncoNanoTarget). He is also a member of the Spanish Platform on Nanomedicine (NanomedSpain) and of the European Platform for Nanomedicine.

Dr. Schwartz acts as the Nanomedicine Coordinador at the national level for CIBER-BBN, where he also leads two intramural projects and collaborates on seven other coordinated projects.
In 2004, Dr. Schwartz began a number of collaborations with biotech companies in the field of diagnostic and prognostic biomarkers and new therapeutic targets in colorectal cancer. He holds 10 patents, which have been transferred to leading companies in the biotech and pharma sectors.

Giacinto Scoles

Place of birth: Torino (Italy)

Citizenships: USA, Canadian and Italian

Languages: Speaks and writes fluently Italian, English, Spanish, French and Dutch

Email: gscoles@princeton.edu

http://www.princeton.edu/~chemdept/Scoles/SCOLES%20NEW/Scolessite/

PRESENT POSITIONS

• 1) ERC Advanced grant holder and adjoint professor at the University of Udine (Italy), Dept. of Medical and Biological Sciences
• 2) Donner Professor of Science, Emeritus Princeton University, Princeton, NJ, USA
• 3) Distinguished Adjunct Professor of Physics, Temple University, Philadelphia, PA, USA

RESEARCH INTERESTS

Scoles has studied for 50 years intermolecular forces gradually increasing the complexity of the systems studied from isolated noble gas atoms, via solid noble gases and physically adsorbed systems, through polyatomic molecules and atomic and molecular clusters to reach finally the complexity of biochemical systems that he is studying at present. Scoles hopes to live long enough to study (always with the same quantitative spirit and with the use of comparisons with theory and computations) living cells to understand cell cycles and the nature of cell evolution from stem cells to fully differentiated cells, both sick and healthy cells, so to be able to find the mechanisms (and therefore the cure) for brain degenerative diseases and cancer.

Patrick W. Serruys

Patrick W. Serruys is Professor of Interventional Cardiology at the Interuniversity Cardiological Institute of the Netherlands (1988-1998), and the Erasmus University with respectful h-index - 109. Since 1980 he has been Director of the Clinical Research Program of the Catheterization Laboratory, Thoraxcenter, Erasmus University, Rotterdam, The Netherlands and since 1997 the Head of the Interventional Department, Heart Center Rotterdam. He is a Fellow of the American College of Cardiology and a Fellow of the European Society of Cardiology and scientific council of the International College of Angiology. He is the author or coauthor of over 1600 papers and editor or coeditor of 37 books, and a member of 20 Editorial Boards of Scientific Journals. Dr. Serruys received the M.D. degree (1972) from the Catholic University of Louvain, Louvain, Belgium and his PhD degree (1986) from the Erasmus University, Rotterdam, The Netherlands. He has been associate editor of Circulation for Europe for five years and he co-edited the Textbook of Cardiology of the European Society of Cardiology. In 1996 he received the TCT Career Achievement Award and in 1997 he was awarded the Wenkebach Prize of the Dutch Heart Foundation. In 2000 he was awarded the Gruentzig Award of the European Society of Cardiology. In 2001 he held the Paul Dudley White Lecture at the American Heart Association in the USA. In 2004 he received the Andreas Gruentzig Award of the Swiss Society of Cardiology. In 2005 he held the 4th International Lecture at the AHA and Mikamo Lecture at the Japanese heart Association. In 2006 he received the highest award of the Clinical Council of the American Heart Association: the James Herrick Award. In 2007 he received the Arrigo Recordati International Prize (Italy) and the ICI Achievement Award (bestowed by the President of Israel – Shimon Perez). In 2008 he received the Einthoven Penning (Leiden). In 2009 he became Doctor Honoris Causa from the University of Athens. In 2011 he received the Lifetime Achievement Award, bestowed by the American College of Cardiology, in recognition of many years of service and invaluable contributions to the ACC. At the end of 2011 Dr.Serruys received the Ray C. Fish Award, bestowed by the Texas Heart Institute, for outstanding achievement and contribution to cardiovascular medicine.

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 and from 1991 to 1998 she was acting in different managerial roles within the Medical Devices Bureau. In 1998 she joined the Office of Science within the Therapeutic Products Directorate (TPD), at the Health Products and Food Branch (HPFB) as an Associate Manager. She is currently a Senior Science Advisor in the Office of Science. Her duties include management of the activities of Expert Advisory Committees and Appeal Panels. Mrs. Shahbazian coordinates the Nanotechnology file for the TPD and is one of the key members working on the development and implementation of nanotechnology related activities in Health Canada. She currently serves as a chair of the HPFB Working Group on Nanotechnology. 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.

Louis Shenkman

Professor of Medicine, Tel Aviv University

EDUCATION

AB, New York University; MD, New York University

RECENT RESEARCH

• Partner, NISAN Project, Aministered Tests with Network Diagnostics via a Non-invasive Sensor, FP5: IST-2001-38052.
• Coordinator, P. CÉZANNE Project, FP-6: Development of an Implanted Biosensor for Continuous Care and Monitoring System of Diabetic Patients, Contract No. 031867, 6/2006 – 4/2011.
• Coordinator, SaveMe Project, FP7: A novel nano-technological platform for pancreatic cancer diagnosis and therapy, Contract No. 263307

OTHER ACTIVITIES

• Dean, New York State/American Program, Sackler School of Medicine, Tel Aviv University, 1994-2010.
• Consultant in Endocrinology and Internal Medicine,Herzilia Medical Center, Israel, 2009-present.
• Medical Director, Elfi-Tech LTD, Rehovot, Israel

Robert Sinden

Bob Sinden D.Sc., F.Med.Sci.

EDUCATION

1986 Doctor of Science, University of London
1966-1969 Doctor of Philosophy, University of Edinburgh

RECENT APPOINTMENTS HELD

Division of Cellular and Molecular Biology, Faculty of Life Sciences, Imperial College London

2007-2008 Senior Dean

2005-2008 Dean of Life Sciences
1992-2002 Head of Infection and Immunity
1987-present Professor of Parasite Cell Biology

HONOURS

2004 Elected Fellow of the Academy of Medical Sciences
2007 Elected Honorary Fellow of the American Society of Tropical Medicine and Hygiene

BRIEF RESUME

Sinden developed techniques to study malaria in a wide range of hosts and vectors. He was amongst the first to bring to bear electron microscopic, and cell-biological techniques to observe protozoal infections in natural, and laboratory hosts. Perhaps his most seminal contributions were on the cellular events and regulation of sexual development including his discovery of the meiotic divisions of the parasite.

He pioneered numerous techniques for the culture of Plasmodium spp, and finally in 2002 his laboratory became the first to culture all stages of the life cycle. These methods are now regarded as part of the standard repertoire used by labs throughout the world. The ability to study all stages of the life cycle of a convenient laboratory model (P. berghei) in vitro and in vivo permitted penetrating analysis of the cell and molecular strategies of the parasite (especially when combined with genetic modification of mouse, mosquito and parasite).

Fundamental work on the structure, control of protein expression, function and immunogenicity of the gametes and ookinete in the rodent malarias has underpinned the development of novel transmission-blocking strategies including vaccines that kill the parasite in the mosquito midgut. Ongoing lab screens examine the transmission-blocking potential of extant and new antimalarial drugs.

He served as a member of the steering committee of the BMGF-MalERA review of malaria research.

SELECTED PEER-REVIEWED PUBLICATIONS

275 papers published; 6 most cited:

• FLORENS, L., et al. "A proteomic view of the Plasmodium falciparum life cycle." Nature 419, 2002, 520-526

• SCHNEIDER, J., et al. "Enhanced immunogenicity for CD8+ T cell induction and complete protective efficacy of malaria DNA vaccination by boosting with modified vaccinia virus Anka." Nature Medicine, 4, 1998, 397-402.

• CARLTON, J.M., et al. "Whole genome shotgun sequencing of a model rodent malaria parasite and comparative analysis with Plasmodium falciparum." Nature 419, 2002, 512-519.

• MCCONKEY SJ et al. "Enhanced T-cell immunogenicity of plasmid DNA vaccines boosted by recombinant modified vaccinia virus Ankara in humans." Nature Medicine. 2003 Jun; 9 (6) :729-35

• HALL, ,N. et al. A global analysis of the molecular life-strategies of malaria parasites by integrated genomic, transcriptomic and proteomic analyses of both vertebrate and mosquito stages. Science 2005; 307. 82-86.

• BILLKER, O., et al.. "Identification of xanthurenic acid as the putative inducer of malaria development in the mosquito." Nature, 392, 1998, 289-292.

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 for 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). He is the first or last author of publications related to all these 10 products.

Skotland is now 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 endocytosis and intracellular transport of protein toxins and nanoparticles. He is co-author of approximately 80 publications and is used as referee for many journals in the field of bioanalysis, metabolism, biochemistry, nanomedicine and contrast agents for medical imaging.

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 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.

Wendelin Stark

Wendelin Stark (1976) is an Associate Professor at the Institute for Chemical and Bioengineering of the ETH Zurich and heads the chair in Functional Materials Engineering. He studied Chemistry at the ETH with a stay at the UC Berkeley in 1999 and pursued a Ph.D. in Mechanical and Process Engineering at ETH. He has written over 140 papers and 17 patents and cofounded 4 spin-off companies (3 running, one stopped).

The bioactive glasses and amorphous calcium phosphates today provide a key tool for biomaterials development where the corresponding mineral nanoparticles convey bioactivity (tissue bonding) to virtually all medically useful polymers.

Metal nanomagnets with a well-defined, covalently attached chemical surface now enable the use of "magnetic chemical reagents" as a powerful tool for accelerating organic synthesis. In medicine, such metal nanomagnets enable in vivo extraction of toxic intermediates or compounds out of living blood.

Most recently developed "living materials" include a microorganism as part of a material composition and can tackle very complex functions, such as "eating".

Prof. Stark has developed method and risk evaluation concepts for safe nanoparticles since 2003 and identified key concepts in nanotox, such as the role of particle agglomeration, diffusion and sedimentation, solubility, and catalytic activity. His group experimentally pioneered investigations on nanoparticles in wastewater treatment plants and quantitative translocation studies in plants. He has assisted Swiss Government agencies in developing regulatory evaluation tools for nanoparticle containing products and served as the chairman of the 2nd International Conference in Nanotoxicology. His work has led to fundamental understanding on the behavior of nanoparticles in biological systems andthe recognition that persistent nanoparticles should not be used in consumer goods.

Fabrizio Stocchi

Date of Birth: 22.09.1958
Place of Birth: Montereale (AQ)

Address VIA DEI MONTECATINI, 5
00186 ROMA
Phone +39.335.8412976
Fax +39.06.6789158
E-mail Fabrizio.stocchi@sanraffaele.it

Fabrizio Stocchi, MD, PhD, is Professor of Neurology, Consultant in Neurology and Director of the Parkinson's disease and Movement disorders research centre at the Institute for Research and Medical Care IRCCS San Raffaele Rome and University "La Sapienza" Rome. He is also Scientific advisor of the Institute for Parkinson's Disease Research in Vicenza. Professor Stocchi was awarded his MD from the University of L'Aquila and his PhD from the University of Catania.
Professor Stocchi's research activities have centred on neuropharmacology in the field of movement disorders and neurodegenerative diseases. He has published many books and papers on the genetics, clinical diagnosis, characterisation and treatment of Parkinson's disease, as well as in preclinical research into the disease. He is an active member of 11 societies, including the Movement Disorders Society, the WFN society where is member of the extrapiramidal committee, the European Clinical Neuropharmacology Society and the European Federation Neurological Society.

Gert Storm

contact: g.storm@uu.nl

Professor Gert Storm studied biology at the Utrecht University, The Netherlands. He graduated in 1983. He obtained his Ph.D. degree in 1987 at the Dept. of Pharmaceutics of the same university. His research interests are in the fields of biopharmaceutics and drug targeting. In 1988-1989 he was a visiting scientist at Liposome Technology Inc. in Menlo Park, USA, and visiting assistant professor at the School of Pharmacy, UCSF, San Francisco. In 1990-1991 he was senior research scientist at Pharma Bio-Research Consultancy B.V. in Zuidlaren, The Netherlands. During this period he contributed to the design, co-ordination and evaluation of clinical pharmacological studies. In September 1991 he took up his present position. In 1999, he was appointed adjunct professor at the Royal School of Pharmacy, Copenhagen. From July 2009 on, he is Honorary Professor in Biomacromolecular Drug Delivery at the University of Copenhagen. In 2000, he was appointed as professor (Targeted Drug Delivery) at Utrecht University. Form 2012 on, he is also professor (Targeted Therapeutics) at the MIRA institute of the University of Twente. He is author/co-author of around 400 original articles, reviews and book chapters, in the field of advanced drug delivery/drug targeting, and theme (co-)editor of Advanced Drug Delivery Reviews and the book 'Long Circulating Liposomes. Old Drug, New Therapeutics'. He is co-ordinator of an Integrated Project (FP6) on targeted nanomedicines (MediTrans) based on the collaboration of 30 european partners and funded by the EC and industry. He is program director of the program Drug Delivery embedded within the recently approved New Nano Initiative (NanoNextNL) strongly sponsored by the dutch government and industry. He is also principal investigator of a national industry-academia partnership (HIFU-CHEM) studying the clinical application of MRI-guided high-intensity focused ultrasound (HIFU) to improve cancer chemotherapy with temperature-sensitive targeted nanomedicines. He is course director of the GUIDE/UIPS/LACDR Course on Advanced Drug Delivery & Drug Targeting, co-sponsored and accredited by EUFEPS and the GALENOS Network, and held in The Netherlands. He is involved in organizing conferences in the field of advanced drug delivery, e.g. chairman of the recent ESF-UB Conference "Nanomedicine: Reality Now and Soon", held 23-28 October in San Feliu de Guixols, Spain. He is member of the editorial (advisory) board of a variety of scientific journals. He is on the board of the Dutch Society for Gene Therapy. He was involved in the foundation and is currently on the board of the European Society for Nanomedicine (ESNAM/CLINAM) and The Netherlands Platform for Targeted Nanomedicine (NPTN).

Rudolf W. Strohmeier

Deputy Director-General, EC - DG Research. Mr. Rudolf W. Strohmeier studied laws and economics in Wurzburg and Bonn. After working as assistant teacher at Wurzburg University he joined in 1980 the Bavarian Liason Office to the Federal Gouvernment in Bonn from which he was seconded as national expert to the EU-Commission for nearly 2 years. In 1987 he established the Bavarian Information Office in Bruxelles and became its first director. In the same year he moved back into the EU Commission as member of German Commissioner Peter Schmidhuber's first Cabinet. Having served also in Commissioner Schmidhuber's 2nd and 3rd Cabinet he became Deputy Head of Cabinet of the first Austrian Commissioner, Dr. Franz Fischler. Having worked in DG Agriculture and as Adviser in the Commission President's Cabinet, Italian Prof. Romano Prodi, he became Head of the 2nd Cabinet of Luxembourger Commissioner Viviane Reding. In February 2010 he was nominated as Deputy Director-General of DG Research.

Janos Szebeni

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

Thomas Teuscher

Dr Thomas Teuscher, Executive Director a.i. Roll Back Malaria Partnership

Thomas Teuscher is the currently the Executive Director ad interim of the RBM Partnership.

Thomas Teuscher is a physician and public health specialist with extensive professional experience in developing countries and in public private partnerships.

Thomas worked in remote rural areas in Africa for over 20 years. First as a pediatrician in Gabon, then as a public health practitioner in Togo, later as Director of the Ifakara Health Research and Development Centre in Tanzania, and finally as General Manager of the ECOWAS Health Research Consortium in Côte d'Ivoire.Under Thomas' direction, the Ifakara Research and Development Centre provided the first testing ground in Africa for a disease-modulating malaria vaccine and also tested the efficacy of deploying intermittent preventive treatment through the Expanded Immunization Programme to protect infants from the consequences of malaria infections. Thomas is a founding member of the Mapping Malaria Risk in Africa, or MARA, a collaboration that generated the first evidence-based malaria risk map for Africa.

Dr Thomas Teuscher joined the Roll Back Malaria Cabinet Project as a Senior Adviser in 2000. During his tenure, Thomas helped to facilitate RBM's transition from a partnership 'built on the strength of loose ties' to a well-structured, accountable, and internationally recognized body for the coordination of global and regional malaria control efforts.

Thomas has played a key role in strengthening RBM's governance and decision-making processes, contributing to the establishment of formal RBM Partnership mechanisms such as the RBM Board, Constitution and By-Laws. Thomas also helped to set increasingly ambitious objectives and priorities for the Partnership, particularly by facilitating the development of the Global Strategic Plan (GSP) and Global Malaria Action Plan, which strengthened the Partnership's shift away from an exclusive focus on vulnerable populations to the adoptoin of the wider goal of universal coverage for all populations at risk. More recently, Thomas worked on expanding the Partnership beyond Africa by forging strategic alliances with malaria control coalitions in Asia and the Americas. He has also served as the research focal point within the RBM Secretariat.

Thomas holds a Doctor in Medicine (MD), and Master's of Science degrees in Clinical and Tropical Medicine and in Human Nutrition from the London School of Hygiene and Tropical Medicine. Thomas has published over 40 articles in international peer-reviewed scientific journals focussing on generating evidence that informs public health policy, planning and programme implementation. Thomas is a former commercial pilot and keen mountaineer.

Donald A. Tomalia

Dr. Tomalia is the CEO/Founder of NanoSynthons, Mt. Pleasant, Michigan. He serves as Associate Editor, Nanomedicine (Elsevier); Editorial Advisory Board, Bioconjugate Chemistry; Faculty Member, Faculty 1000 Biology; Director of The National Dendrimer & Nanotechnology Center; Distinguished Visiting Professor, Columbia University, Affiliate Professor (Department of Physics), Virginia Commonwealth University, Richmond, VA and External Faculty, University of Wisconsin-Madison (School of Pharmacy). He received his B.A. in chemistry from the University of Michigan and while at The Dow Chemical Company completed his Ph.D. in physical-organic chemistry from Michigan State University under the mentorship of Professor Harold Hart. He progressed from research chemist to research manager/scientist at The Dow Chemical Company. He is recognized as the pioneering scientist/inventor associated with the discovery of dendrimers/dendritic polymers and poly(oxazolines). His discovery of the cationic polymerization of 2-oxazolines led to two international industrial research awards (R&D-100) for creative research in 1978 and 1986. His discovery of dendrimers (dendritic polymer architecture) in 1979 led to a third R&D-100 Award in 1991 and the Leonardo da Vinci Award (Paris, France) in 1996. He received the Society of Polymer Science Japan (SPSJ) Award for Outstanding Achievement in Polymer Science (Yokohama, Japan) in 2003 for his discovery of the fourth major macromolecular architectural class, namely; dendritic polymers. Tomalia is the recipient of the 35th Carothers Award (2012) presented by the Delaware Section of the American Chemical Society for his outstanding contributions and advances in industrial applications.

Tomalia was recently inducted into the Thomas Reuters (2011) –"Hall of Citation Laureates in Chemistry" (i.e., 40 most highly cited scientists in the field of chemistry). Tomalia is the recipient of the Louis W. Busse Lecturership, School of Pharmacy, University of Wisconsin, Madison, WI (2011); ACS Eminent Scientist Lecture Award, 240th ACS National Meeting, Boston, MA (2010); Linus Pauling Memorial Lecturer, Portland, OR (2010); Chevron Lectureship, Texas A&M University, College Station, TX (2009).

He is listed as the inventor of over 128 U.S. patents and is author/co-author of more than 245 peer reviewed publications. Over 175 papers are focused on the dendrimer/dendritic polymer field. His forthcoming book entitled: Dendrimers, Dendrons and Dendritic Polymers: Discovery, Applications and the Future coauthored with J.B. Christensen and U. Boas will be available in Spring, 2012 (Cambridge University Press). His research interests include: dendrimer-based nanomedicine, dendrimer structural design for drug delivery, imaging and nanopharmaceuticals. More recently, he has been focused on a new nano-periodic system for unifying and defining nanoscience.

Rudolf Urbanics

Head of in vivo lab.
MD., PhD.
Date of Birth: 8th of Oct. 1946
Place of work: Semmelweis University, Budapest, Nanomedicine Research and Education Center & SeroScience Ltd., Hungary
Phone +36208259691
E-mail urbanicsr@gmail.com

MD, PhD, Head of the in vivo laboratory of Nanomedicine Research and Education Center of Semmelweis University, and SeroScience Ltd., an immunotoxicity CRO, since 2008 in Budapest, Hungary.
He obtained MD diploma and the PhD degree at Semmelweis Medical School, Budapest, Hungary. He had teaching and research activity at the parent university and held in between various research/collaboration positions at MaxPlanck Institute of Systemphysiology, Dortmund, Germany (prof D.W. Lübbers), at University of Pennsylvania, Philadelphia, USA, Cerebrovascular Research Center (head prof. M Reivich, Dr. J. H. Greenberg), at Pennsylvania Muscle Institute (prof. A.P. Somlyo), in the Knoll AG, Central Nervous System Research Department, Ludwigshafen, Germany, working in the field of CNS regulation of blood flow/metabolism, ischemic/hypoxic disorders, stroke and chronic neurodegenerative disease animal models. R. Urbanics was invited lecturer of three SMi's conferences, London, delivering talks about ischemic-hypoxic as well as chronic neurodegenerative disease animal models.
He was the Deputy R&D Director and Head of CNS Pharmacology Department at Biorex R&D Co.(1997-2003), worked at IVAX/Drug Research Institute Budapest, as Scientific Adviser, Leading researcher in Safety and CNS Pharmacology and later in IVAX/Drug Research Institute, Subsidiary of TEVA as Head of In Vivo Pharmacology Group (2003-2008).

Currently, he is working with in vivo models of nano drug - nano carrier induced, complement activation related pseudoallergic reactions (CARPA), clarifying their immune-toxicological and safety hazards.

René Verloes

René Verloes is MD, PhD, MSc in Appl. Toxicology. He is Senior Director Early Development at Janssen Infectious Disease in Beerse, a J&J Company. He joined the Global Medical Department in April 2005. His responsibility is to oversee clinical studies as support for early phase drug development through proof of concept (Phase I up to/including Phase IIA studies). He has a major interest in discovery and developing new medicines for which there is a high medical need. René is a permanent member of the Research and Early Development Management Board of Janssen Infectious Disease.

René has been in the pharmaceutical industry since 1983 working for 15 years at UCB Pharma in early phases of drug development and with now marketed products (Zyrtec, Keppra, Xyzal).

Thereafter, he worked on pediatric and therapeutic vaccines for 8 years in the Medical & Regulatory Department of GSK Biologicals, Rixensart, Belgium. There, he worked on biologicals (vaccines, immuno-modulators, adjuvants) on prophylactic (pediatric, adult) vaccines and therapeutic cancer vaccines. He was in charge of clinical studies in Europe, US and involved in early clinical studies in Japan.

René has honor degrees as MD and as PhD in BioSciences from the Free University of Brussels.

He obtained a Master Degree in Toxicology from the University of Surrey, Guildford, UK.

He is lecturing on Safety Evaluation at the Free University of Brussels (ULB) Belgium and has a broad culture involving EU, US and Japanese medical and regulatory experience.

Viola Vogel

Professor of Biologically Oriented Materials, Department of Health Sciences and Technology, ETH Zürich, Switzerland.

EDUCATION

• 1987 Ph.D. (Physics, Physiology), JW-Goethe University, Frankfurt/M, Germany
• 1983 Diploma (Physics, Zoology), JW-Goethe University, Frankfurt/M, Germany
• 1981 Vordiplom (Physics, Biology), JW-Goethe University, Frankfurt/M, Germany

EMPLOYMENT

2012 - now	Professor, Department of Health Sciences and Technology, ETH Zürich, Switzerland
2004 - 2011	Professor, Department of Materials, ETH Zürich, CH
2002 - 2004	Professor, Department of Bioengineering, Adjunct in Physics, University of Washington, Seattle, USA
1997 - 2003	Founding Director, Center for Nanotechnology, University of Washington, Seattle, USA
1997 – 2002	Associate Professor, Department of Bioengineering, University of Washington, USA
1991 - 1997	Assistant Professor, Center for Bioengineering, University of Washington, USA
1988 - 1990	Postdoctoral Research Associate, Physics (Prof. Y. R. Shen), University of California, Berkeley, USA
1987 - 1988	Research Scientist, Max-Planck Institut für Biophysikalische Chemie, Göttingen, Germany (Department Selbstorganisierte Systeme, Prof H. Kuhn)
1982 - 1987	Research Assistant, Max-Planck Institut für Biophysikalische Chemie, Göttingen, Germany (Department Selbstorganisierte Systeme, Prof H. Kuhn)

HONORS

2010	Guest Editor, Lab on a Chip, 10th Anniversary Special Issue: Switzerland, August 2010.
2008 - 2013	ERC Advanced Grant, European Research Council
2006	Julius Springer Prize 2006 for Applied Physics
2005	Research Award, Philip Morris Foundation
2003 -	Fellow of the American Institute for Medical and Biological Engineering (AIMBE)
1993 – 1998	"First Award" from the Institute of General Medicine, National Institutes of Health, USA
1989 – 1990	Feodor-Lynen Fellowship/Alexander von Humboldt Foundation
1988	Otto-Hahn Medal of the Max-Planck Society, best Ph. D. Thesis at the Max-Planck Institute for Biophysical Chemistry

SELECTED MAJOR LECTURES (last 5 years)

• 2011 Marie Curie Lecture (Year of Chemistry), Chemistry as Innovating Science (CHAINS) Meeting, Amsterdam, NL
• 2011 Plenary Speaker, 37th Intern. Conference on Micro and Nano Engineering (MNE), Berlin, Germany
• 2011 Timoshenko Lectures and Visiting Scholar, Stanford University, Department of Mechanical Engineering, USA
• 2010 Plenary Speaker, 10th Intern. Conference on Nanostructured Materials, Nano 2010, Rome, Italy
2010 Keynote Speaker, World Congress of Biomechanics, Singapore
• 2010 Invited Lecturer, High Polymer Research Group Conference, 50th Anniversary, Pot Shrigley, Cheshire, Manchester, GB
• 2010 Keynote Speaker, MEMS 2010, IEEE, Hong Kong
• 2009 Evening Lecture, Deutsches Museum, München, Germany
• 2009 Plenary Lecture, E-MRS, Annual Meeting of the European Materials Research Society, Strasbourg, France
• 2009 After Dinner Talk, ESF Nanomedicine Conference, Sant Feliu de Guixols, Spain
• 2007 Lecture, Opening Ceremony for the New Building of the Fraunhofer Institute for Biomedical Technology, IBMT Potsdam-Golm, Germany
• 2007 40th Lacey Lectureship, Department of Chemistry and Chemical Engineering, California Institute of Technology (CalTech), Passadena, USA
• 2006 Plenary Lecturer, The 10th Anniversary International Conference on Miniaturized Systems for Chemistry and Life Sciences (microTAS), Tokyo, Japan
• 2006 Moderator, NanoEquity Europe 2006, How Nanotools Innovate the Life Sciences, Deutsche Börse, Frankfurt (German Stock Market), Germany

SELECTED SYNERGISTIC ACTIVITIES

2006 - 2010	Jury Member, German Ministry for Science and Education (BMBF), Innovationswettbewerb zur Förderung der Medizintechnik" (Competition Innovations in Medical Technologies), Germany
2004 - 2010	Gordon Research Conference Council, and Selection and Scheduling Committee Member
2003 - 2004	Human Frontiers Science Program, US Representative on the Council of Scientists
2006	Conference Chair, Annual Spring Meeting, Materials Research Society (MRS), San Francisco
2006	Organizing Chair, First IDEA League Summer School. Biotechnology and Bioengineering Applications in Medicine, Monte Verità, Ascona, Switzerland.
2003 - 2006	Member, European Academy, Section Nanotechnology Assessment

SCIENTIFIC ADVISORY BOARD MEMBERSHIPS

2012 - now	Rapporteur for the Max-Planck-Society, Physical Science Division, Germany
2011 - now	Hochschulrat (Board of Regents), Ludwig Maximillian University, Munich, Germany
2010 - now	Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, USA
2008 - now	SAB Chair, Center for Nanoscience (CeNS, Excellence Cluster Munich), Germany

2007 - now Chair since 2010, Max Planck Institute of Colloids and Interfaces (Golm), Germany
2005 - now Institute of Bioengineering and Nanotechnology (IBN, Biopolis), Singapore
2007 - 2011 Center for Nano Integration Duisburg Essen (CeNIDE), Germany
2004 - 2011 Member, External Research Advisory Board (FOKO B), Eidg. Material Prüfungsanstalt (EMPA), Dübendorf, Switzerland
2004 - 2008 Strategic Planning Commission, ETH Zurich

SELECTED PUBLICATIONS

• M. Chabria, S. Hertig, M. Smith, **V. Vogel**, Stretching fibronectin fibers disrupts the binding of bacterial adhesins by physically destroying the epitope, Nature Communication, 1 (2010) 135-139.
• Klotzsch E, Smith ML, Kubow KE, Muntwyler S, Little WC, Beyeler F, Gourdon D, Nelson BJ, **Vogel V**: Fibronectin forms the most extensible biological fibers displaying switchable force-exposed cryptic binding sites. Proc. Natl. Acad. Sci. USA 106 (2009) 18267-72
• K. E. Kubow, E. Klotzsch, M. L. Smith, D. Gourdon, W. C. Little, and **V. Vogel**, Crosslinking of cell-derived 3D matrices up-regulates the stretching and unfolding of new ECM assembled by reseeded fibroblasts." Integrative Biology, 1 (2009) 635-48
• I. Schoen, J. Ries, E. Klotzsch, H. Ewers, **V. Vogel**, Binding-Activated Localization Microscopy of DNA Structures, NanoLetters, 11 (2011), 4008–4011
• I. LeTrong, P. Aprikian, B. A. Kidd, M. Forero-Shelton,V. Tchesnokova, P. Rajagopal, V. Rodriguez, G. Interlandi, R. Klevit, **V. Vogel**, R. E. Stenkamp, E. V. Sokurenko, W. E. Thomas, Structural basis for mechanical force regulation of the adhesin FimH via finger trap-like b-sheet twisting, Cell 141 (2010) 645-655.
• W. E. Thomas, V. Vogel, E. Sokurenko, Biophysics of Catch Bonds, Annu. Rev. Biophys. 37 (2008) 399-416
• **V. Vogel**, M. P. Sheetz, Cell Fate Regulation by Coupling Mechanical Cycles to Biochemical Signaling Pathways, Current Opinion Cell Biology 21 (2009) 1-9

Yuri Volkov

PROFESSIONAL QUALIFICATIONS/ DISTINCTIONS

• M.D. (1985) 1st Moscow Sechenov Medical University, Russia
• Ph.D. (1995) Institute of Immunology, Moscow, Russia
• M.A. (2010, Jure Officii) Trinity College Dublin, Ireland
• F.T.C.D. (Fellow of Trinity College Dublin), 2010

POSITIONS/AFFILIATIONS

• Professor of Molecular Medicine, Department of Clinical Medicine, Trinity College Dublin.
• Principal Investigator, Institute of Molecular Medicine (IMM), Trinity College Dublin
• Principal Investigator, Trinity College Centre for Research on Adaptive Nanostructures and Nanodevices (CRANN), Trinity College Dublin.

RESEARCH INTERESTS AND EXPERTISE

Nanomedicine and biomedical applications of nanotechnologies, molecular mechanisms of immune system functioning in health and disease, cell adhesion and migration in inflammation and cancer, intracellular signalling and cytoskeletal dynamics, advanced cell and molecular imaging.

Prof. Yuri Volkov received his MD from the Moscow Medical University and subsequently a PhD in biomedical sciences at the Institute of Immunology, Moscow. He has been working at the Department of Clinical Medicine, Trinity College Dublin since 1995. His research interests for a number of years have been focused in leukocyte biology, mechanisms of inflammation and cell adhesion receptors functioning in immune defence and disease development. Among the main achievements in this area was the discovery and characterization of the crucial impact of intracellular phosphorylation enzyme of the protein kinase C family – PKC-beta for the process of T cell migration. Success of these studies has been made possible through the implementation of the cutting edge High Content Analysis (HCA) cell and molecular imaging technologies. As a Principal Investigator at the Institute of Molecular Medicine, Prof. Volkov has made a key contribution into establishing of the HCA Centre which represents a state-of-the art facility in the academic institution at the international level. Prof. Volkov has formed a large-scale interdisciplinary alliance between the Schools of Medicine, Physics, and Chemistry at Trinity College aimed at the development of new nanoscale molecular imaging and drug delivery systems. Prof. Volkov is also a Principal Investigator at the Trinity College's Centre for Research on Adaptive Nanostructures and Nanodevices (CRANN), where his group is pursuing the applications of nanomaterials for advanced research and medical diagnostics. Prof. Volkov currently coordinates a large scale EU FP-7 funded Consortium "NAMDIATREAM" (www. namdiatream.eu) which unites the expertise of 22 European academic, research, clinical and industrial partners towards the development of nanotechnological toolkits for early diagnostics and treatment monitoring of major types of malignant diseases. He is also a Lead TCD partner for EU FP-7 LSP "MULTIFUN" and "Celtic Alliance for Nanohealth" Ireland-Wales INTERREG Consortium.

SELECTED PUBLICATIONS

• Mohamed BM, Verma NK, Davies AM, McGowan A, Staunton KC,Prina-Mello A, Kelleher D, Botting CH, Causey CP, Thompson PR, Pruijn GJM, Kisin ER, Tkach AV, Shvedova AA, **Volkov Y**. Citrullination of proteins: a common post-translational modification pathwayinduced by different nanoparticles in vitro and in vivo. Nanomedicine, 2012 (in press). Doi: 10.2217/ NNM.11.177
• Movia D, Prina-Mello A, Bazou D, **Volkov Y**, Giordani S. Screening the cytotoxicity of single-walled carbon nanotubes using novel 3D tissue-mimetic models. ACS Nano, 2011, 22;5(11):9278-90.
• Kagan VE, Konduru NV, Feng W, Allen BL, Conroy J, **Volkov Y,** Vlasova II, Belikova NA, Yanamala N, Kapralov A, Tyurina YY, Shi J, Kisin ER, Murray AR, Franks J, Stolz D, Gou P, Klein-Seetharaman J, Fadeel B, Star A, Shvedova AA. Carbon nanotubes degraded by neutrophil myeloperoxidase induce less pulmonary inflammation. Nat Nanotechnol. 2010, 5(5):354-9.
• Williams Y, Sukhanova A, Nowostawska M, Davies AM, Mitchell S, Oleinikov V,Gun'ko Y, Nabiev I, Kelleher D, **Volkov Y**. Probing cell-type-specific intracellular nanoscale barriers using size-tuned quantum dots. Small. 2009, 5(22):2581-8.
• Jan E, Byrne SJ, Cuddihy M, Davies AM, **Volkov Y**, Gun'ko YK, Kotov NA. High-content screening as a universal tool for fingerprinting of cytotoxicity of nanoparticles. ACS Nano. 2008, 2(5):928-38.
• Conroy J, Byrne SJ, Gun'ko YK, Rakovich YP, Donegan JF, Davies A, Kelleher D, **Volkov Y**. CdTe nanoparticles display tropism to core histones and histone-rich cell organelles. Small. 2008, 4(11): 2006-15.
• Nabiev I, Mitchell S, Davies A, Williams Y, Kelleher D, Moore R, Gun'ko YK, Byrne S, Rakovich YP, Donegan JF, Conroy J, Sukhanova A, Cottel D, Gaponik N, Rogach A, **Volkov Y**. Nonfunctionalized nanocrystals can exploit a cell's active transport machinery delivering them to specific nuclear and cytoplasmic compartments". Nano Letters 2007, 7(11):3452-3461
• **Volkov Y**, Long A, McGrath S, NiEidhin D, Kelleher D. Crucial importance of PKC-beta (I) in LFA-1-mediated locomotion of activated T cells. Nature Immunology 2001, 2(6):508-514

Hans-Peter Vornlocher

Dr. Hans-Peter Vornlocher,
Managing Director Research
Axolabs GmbH, 95326 Kulmbach, Germany
e-mail: hans-peter.vornlocher@axolabs.com

PROFESSIONAL POSITIONS

since Nov. 2011 Managing Director Research Axolabs GmbH

2007 - 2011 Managing Director Research, Roche Kulmbach GmbH
2003 -2007 Vice President Research, Alnylam Europe AG, Kulmbach/Germany
2001 - 2003 Head of Research, Ribopharma AG, Kulmbach/Germany
2000 - 2001 Research Scientist, Max-Planck-Institute for Biophysical Chemistry, Göttingen
1998 - 2000 Research Scientist, Institute for Molecular Biology, University Marburg

EDUCATION

1995 - 1998 Post-Doc, Department of Biological Chemistry/School of Medicine, UC Davis
1991 - 1995 PhD-Thesis in Biochemistry, Department of Biochemistry, University Bayreuth
1984 - 1991 Diplom in Biology, University Erlangen-Nürnberg

SELECTED PUBLICATIONS

• Höbel S. et al. 2010 Polyethylenimine/small interfering RNA-mediated knockdown of vascular endothelial growth factor in vivo exerts anti-tumor effects synergistically with Bevacizumab. J Gene Med. 12, 287-300
• Akinc, A., et al. 2008 A combinatorial library of lipid-like materials for delivery of RNAi therapeutics. Nat. Biotechnol. 26, 561-9.
• de Fougerolles, A. et al. 2007 Interfering with disease: a progress report on siRNA-based therapeutics. Nat. Rev. Drug Discov. 6, 443-53.
• John, M., et al. 2007 Effective RNAi-mediated gene silencing without interruption of the endogenous microRNA pathway. Nature 449, 745-7.
• Vornlocher, H.-P. 2006 Antibody-directed cell-type-specific delivery of siRNA. Trends Mol. Med. 12, 1-3.
• Zimmermann, T.S., et al. 2006 RNAi-mediated gene silencing in non-human primates. Nature 441, 111-4.
• Soutschek, J., et al. 2004 In vivo silencing by RNA interference of an endogenous gene following systemic administration of modified siRNAs. Nature 432, 173-178.

Andreas Widmer

Prof. A.F. Widmer earned his MD degree in Switzerland, where he completed his fellowship in internal medicine in several hospitals. He founded the specialty infectious diseases as the secretary of the Swiss Society for Infectious Diseases back in 1998. He was trained in hospital epidemiology by R. Wenzel, in Iowa, IA, USA, where he completed his master degree in epidemiology at the University of Iowa. He is a core member in the "patient safety" program of the WHO, past President of the Swiss Society for Hospital Epidemiology, editorial advisory board member of the journal "Clinical Infectious Diseases". In addition, he is in the guidelines committee of the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Disease Society of America (IDSA), and treasurer of the European Study Group on Nosocomial Infections (ESGNI). His research interests are control of multiresistant pathogens such as MRSA, surgical site infections, in particular implant-associated infections, and C. difficile infections. His current position is deputy head of the division of infectious diseases and hospital epidemiology, and head of hospital epidemiology at the University Hospital Basel, Switzerland. He is President of "Swiss-NOSO", the National surveillance project for surgical site infections.

Kenneth Wong

Dr. Kenneth Wong is currently Clinical Assistant Professor at the Department of Surgery in the University of Hong Kong. He is an accomplished surgeon as well as a scientist with wide research interests, which include the application of nanomaterials in the field of wound repair and regeneration; tumor targeting and therapy; and genetic basis of congential anomalies.
He has contributed significantly in both clinical and basic science research and has so far over 70 indexed publications. He has also been invited to give more than 50 lectures.
He is an Assocaite Editor for Nanomedicine: Nanotechnology, Biology and Medicine and is a regular reviewer for many international scientific journals.

Marie-Andrée Yessine

Dr. Marie-Andrée Yessine has joined OctoPlus, a drug delivery and formulation company in the Netherlands, in February 2007 where she is now working as a Senior Development Scientist. During her five years spent at the company, she has been highly involved in the formulation development and scaling up of complex biopharmaceutical formulations for international clients. She has worked with small molecules, siRNAs, proteins, lipid-based nanocarriers, and microspheres for controlled release drug delivery.
Marie-Andrée holds a Ph.D. in pharmaceutical sciences, more specifically in the formulation of nucleic acid-based drugs into lipidic and polymeric nanocarriers, from the Faculty of Pharmacy of Université de Montréal, Québec, Canada.

Yuliang Zhao

• Professor, Deputy Director-General, National Center for Nanoscience and Technology of China, Beijing 100190.
• Professor, Director, Chinese Academy of Sciences Key Laboratory for Biomedical Effects of Nanomaterials, Beijing 100049
• Professor, Director, The Research Center for Cancer Nanotechnology,
(A Joint Research Center of Tianjin Cancer Hospital & Chinese Academy of Sciences) zhaoyuliang@ihep.ac.cn, zhaoyl@nanoctr.cn

Biography: Yuliang Zhao, Ph.D. is a Professor and Deputy Director-General of National Center for Nanoscience and Technology of China, the Director of Chinese Academy of Sciences Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, Institute of High Energy Physics, Chinese Academy of Sciences (CAS), and the Director of Research Center for Cancer Nanotechnology, CAS & Tianjin Cancer Hospital. His research interests span both basic and translational research including Nanotoxicology, Low-toxic nanomedicine for cancer therapy, Nanochemistry for lowering the toxicity of nanomaterials/nanomedicines, and Molecular Dynamics simulation of biochemical processes at nano-bio interface. His primary research focus is on the toxic properties of nanomaterials, and development of novel nanomedicine of low-toxicity (without delivery systems) for low-toxic cancer chemotherapy, especially, proposed the use of non-killing cell mechanism to inhibit cancer growth by nanostructure materials. He is the author of over 200 publications, 10 books, and 17 book chapters as well as an inventor on eight patents. He has delivered more than 150 invited talks and is now serving as Associate Editor/Advisory Editorial Board member for 7 SCI journals in USA and Europe.

CURRICULA VITAE OF POSTER SUBMITTERS AT CLINAM 5/12

Mihaela Aluas

I am specialized in solid state nuclear magnetic resonance spectroscopy (SS-NMR)-methods development and applications. I have obtained my PhD in Germany at Martin Luther University Halle-Wittenberg and I have experience as a postdoctoral researcher in U.S.A at the University Hawaii at Manoa and in Romania at Babes-Bolyai University Cluj-Napoca. I have a working experience of 10 years as researcher, 4 years as Wissenschaftlicher Mitarbeiter in Germany at Martin Luther University Halle-Wittenberg and I published 14 papers in ISI ranked journals (Hirsch Index 4).
I have published the book Solid-State NMR Spectroscopy on Polymer Blends (2007) and I am the editor of the book Advanced Experimental Methods for the Study and the Analysis of Bio-Nano-Systems (2012). I am currently implementing, as project manager, the project with the title Performant doctoral program for the professional development of highly qualified human resources in interdisciplinary scientific research funded by the European Social Fund (ESF) and the Government of Romania.

Mohammad Ali Amini

CONTACT ADDRESS

NO 12, Kaj alley, Ziba St., Shariati St., Postal code: 1948766771.
Cell: (+98) 9122720389
Email: ma.amini@kiau.ac.ir

CURRENT POSITION

Research Assistant in laboratory of Biotechnology, Islamic Azad University (IAU) and Pharmaceutical Biotechnology laboratory, Tehran University of Medical Sciences.

EDUCATION

Doctor of Veterinary Medicine (DVM), IAU-Karaj branch, Iran (2004-2011).

RESEARCH INTEREST

- Development and evaluation of pulmonary and nasal drug delivery systems
- Drug targeting
- Mucosal vaccine delivery systems
- Immunoadjuvants

PROJECTS

- Preparation and characterization of chitosan nanoparticles (with different sources and derivatives) containing FMD viruses as a nano-carrier for intranasal vaccine delivery in guinea pig
- Production and characterizations of a novel formulation for pulmonary delivery of budesonide using nebulizer
- Study of siRNA delivery with chitosan nanoparticles in cancerous cell lines
- Modeling the factors controlling the particle size in PLA-Budesonide nanosuspension, acetaminophen nanosuspension, and chitosan emulsion using Artificial Neural Networks
- Extraction and chemical characterization of chitosan from mycelium of Rhizopus nigricans and Mucor miehei

PUBLICATIONS

- Patent Registration: **M.A. Amini**, F. Tajdini, Production of Chitosan from Fungi, Certified by Department General of Companies and Industrial Property Registration, 2009. (Serial No.: A/87-002559, Ref. No. in Patent Register: 56054)
- A. Amani, **M.A. Amini**, H.S. Ali, P. York, Alternatives to conventional suspensions for pulmonary drug delivery by nebulizers- a review, Journal of Pharmaceutical Sciences, 2011; 100:4563-70.
- F. Tajdini, **M.A. Amini**, N. Nafissi-Varcheh, M.A. Faramarzi, Production, physiochemical and antimicrobial properties of fungal chitosan from Rhizomucor miehei and Mucor racemosus, International Journal of Biological Macromolecules, 2010; 47:180–183.
- M. Aghajani, A.R. Shahverdi, S.M. Rezayat, **M.A. Amini**, A. Amani, Preparation and optimization of acetaminophen nanosuspension through nanoprecipitation using microfluidic devices- an artificial neural networks study, Pharmaceutical Development and Technology, 2012 Jan 19 [Epub ahead of print].

Alexander Andriyanov

B.Sc.
Ha-Keren str., 22/2, Maale-Adomim, Israel
Office: +972-2-6758509.
Mobile: +972-54-6815103
Email: aleandr11@gmail.com

WORKING EXPERIENCE

2009 – Now. Hebrew University, Prof. Yechezkel Barenholz's Lab of Membrane and Liposome Research, Hadassah Medical School, Jerusalem, Israel
Laboratory technician

EDUCATION

2010 – Now M.Sc. Studying in Biochemistry, Metabolism and Endocrinology, Hebrew University Faculty of Medicine
2007 – 2010. B.Sc. in Biotechnology. Hadassah Academic College, Jerusalem, Israel.

SKILLS

- Liposome preparation
- Determination of the drug concentration
- Tissue culture
- Histopathology
- in vivo mouse/rat xenograft models

Rohidas B. Arote

Asst. Professor, Dept of Dentistry, School of Dentistry, Seoul National University, Seoul, South Korea 110-749
Email: rohi06@snu.ac.kr

EDUCATION

2010.12.01- present Assistant Professor, School of Dentistry, Seoul National University, Seoul, Korea
- 2009.03.01- 2010.11.30 Postdoctoral research fellow, School of Agricultural Biotechnology, Seoul National University, Seoul, Korea
- 2005-2009 Ph. D. School of Agricultural Biotechnology, Seoul National University, Seoul, Korea "Preparation and Characterization of Poly(ester amine) Derivatives as Gene Carriers" (Research Advisor: Prof. Chong Su Cho)
- 2000-2002 M.S. (M. Pharm) Department of Pharmacognosy and Phytochemistry, Prin. K. M. Kundnani college of Pharmacy, Mumbai University, Mumbai, MH State, India "A Composite Herbal Hair Care Formulation from Natural Sources" (Research Advisor: Prof. P. M. D'Mello)
- 1996-2000 B.S. (B. Pharm), P. D. V. V. P, Fds College of Pharmacy, Ahmednagar, Pune University, MH state, India

RESEARCH AREAS

1. Gene therapy
- Preparation and characterization of non-viral polymer-based gene delivery system
- Chemical modifications of polymers for cell-specific targeting (e.g. liver, tumor, antigen presenting cells), hydrophilicity and high transfection efficiency.
- Nanoparticle formulations for therapeutic delivery agents
- Plasmid DNA, RNA isolation, purification, in vitro cell study and in vivo animal study
- Application to cancer therapy using aerosol gene delivery

2. Drug delivery system

• Preparation and characterizations of controlled release drug carrier
• Chemical modification for cell specificity, hydorphilicity, and mucoadhesive property
• Research (Formulation Development) New Drug Delivery Systems. Conventional systems such as Tablets, Capsules, Liquid Orals, Ointments & Creams and Parenterals

PATENTS

• Synthesis and characterization of poly(ester amine) from polycaprolactone diacrylate and polyethylenimine as gene carrier, Patent, Korea, 10-0860416 (19.09 2008)
• Synthesis and characterization of poly(ester amine) from glycerol dimetharylate and polyethylenimine as gene carrier, Patent, Korea (filed).

AWARDS

• Best Researcher of the Year (2009) Award by Research Institute for Agriculture and Life Sciences, Seoul National University.
• Young Scientist Award (Juergen Springer Award for a Young Scientist)
• "Novel Biodegradable and Branched Poly(ester amine)s Based on Glycerol Dimethacrylate and Low Molecular Weight Polyethyleneimine as a Gene Carrier" for oral presentation in 16th POLYCHAR: World Forum on Advanced Materials, World Unity Convention Centre, Lucknow, India, February 17 – 21, 2008
• Brain Korea 21 (BK-21) Fellowship
Recipient of Brain Korea fellowship since 2008 to 2009
• Korea Science and Engineering Fellowship
Recipient of Korea Science and Engineering Fellowship since 2005 to 2008.

EMPLOYMENT/STUDIES

• College of Agriculture and Life Science, Seoul National University, Seoul, South Korea. (03/2005 to 02/2009: Graduate student)
• Successfully synthesized and characterized various biodegradable cationic polymers.
• Successful characterization of these biodegradable cationic polymers as gene carrier in vitro and in vivo applications.
• Preparation of nanoparticles for drug delivery purpose
• Ajanta Pharma Ltd, Mumbai, India. (02/2003 to 10/2004: Formulation & Development Officer)
• The complete formulation work from laboratory scale to scale up for final production.
• Preparation of Master Formula Records (MFR) and Batch Manufacturing Records (BMR) from the above results.
• Compilation of documents related to development, validation & scale up.
• Monitoring stability condition of products as per ICH Guidelines.
• Trouble shooting for manufacturing process.

Anthony Amaechi Attama

Professor
Head, Department of Pharmaceutics, UNN

Department of Pharmaceutics
Faculty of Pharmaceutical Sciences
University of Nigeria
Nsukka 410001, Enugu State, Nigeria
Tel.: +234-8077488245; 08036618204
E-mail: aaattama@yahoo.com; anthony.attama@unn.edu.ng, URL: http://www.unn.edu.ng/
Languages spoken: English, German and Ibo

EDUCATION

• 2005-2006 Post-doctoral Research Fellow, Institut für Pharmazeutische Technologie, Technische Universität Carolo Wilhelmina zu Braunschweig, Mendelssohnstr. 1, D-38106 Braunschweig, Germany.
• 2002 University of Nigeria Nsukka, Enugu State, Nigeria
• 1998 University of Nigeria Nsukka, Enugu State, Nigeria
• 1994 University of Nigeria Nsukka, Enugu State, Nigeria

INVITED LECTURES

• Speaker, Nanotechnology Drug Delivery Systems: The Magic Bullets of Our Time? Invited Lecture Presented at the Three-day Workshop tagged "Application of Nanotechnology in Industry" organized by National Research Council (NRC) of Egypt, Cairo Egypt, January 29th to 31st, 2012.
• 2nd Plenary Lecture, Drug Delivery: Exploring the Option of Nanotechnology, 11th Annual National Conference and Scientific Meeting of Nigerian Association of Pharmacists in Academia (NAPA) Faculty of Pharm. Scs., Ahmadu Bello University, Zaria, October 24th to 28th, 2011.
• 2nd Plenary Lecture, Incorporating Nanotechnology into Nigeria Healthcare Scheme, 10th Annual National Conference and Scientific Meeting of Nigerian Association of Pharmacists in Academia (NAPA), Faculty of Pharm. Scs., Nnamdi Azikiwe University, Agulu Campus, October 3rd to 7th, 2010.

SHORT LIST OF PUBLICATION

• Builders PF, Mbah CC, **Attama AA** (2012). Intrinsic and functional properties of a gelling gum from Dioclea reflexa: A potential pharmaceutical excipient. British Journal of Pharmaceutical Research 2(1): 50-68, 2012.
• Charles L, **Attama AA** (2011). Current state of nanoemulsions in drug delivery. Journal of Biomaterials and Nanobiotechnology (JBNB), 2, 626-639.
• Esimone CO, **Attama AA**, Ngwu G, Iloabanafo CA, Momoh MA, Onaku, LO (2011). Mosquito repellent activity of herbal ointments formulated with Occimum gratissimum oil. Journal of Pharmacy Research. 4(10) 3442-3444.
• Onaku LO, **Attama AA**, Okore VC, Tijani AY, Ngene AA, Esimone CO (2011). Antagonistic antimalarial properties of pawpaw leaf aqueous extract in combination with artesunic acid in Plasmodium berghei-infected mice. Journal of Vector Borne Diseases 48, 96–100.
• **Attama AA**, Uzor PF, Nnadi CO, Okafor CG (2011). Evaluation of the wound healing activity of gel formulation of leaf extract of Aspila africana Fam. Compositae. J. Chem. Pharm. Res. 3(3) 718-724.

Giora Beit-Ya'akov

PERSONAL DETAILS

Residential address:
Hazon-Tzion 10, Jerusalem
Date of birth: August 15, 1983
E-mail: giorabei@post.tau.ac.il

EDUCATION

• 2011-present M.Sc student - Electrical Engineering, Tel-Aviv University (TAU). Thesis: Multi-unit recording of neural response for epi-retinal electrical stimulation (advisor: Prof. Yael Hanein).
• 2007-2010 B.Sc. - Electrical Engineering (cum laude) and Physics, TAU.

ADDITIONAL ACADEMIC EXPERIENCE

• 2011-2012 Teaching assistant at the TAU School of Electrical Engineering (courses: introduction for micro-electro-mechanical systems; introduction for semi-conductors).
• 2009-2010 Research assistant at the TAU Micro and Nano-Systems laboratory (research topic: opto-electrical characterization of hetero-structures based on quantum-dots and carbon nano-tubes).
• 2008-2009 Research assistant at the TAU Electrical Discharge and Plasma laboratory (research topic: vacuum arc deposition of hard oxide coatings).

AWARDS

• 2012 scholarship from the TAU center for nanoscience and nanotechnology.
• 2011 scholarship from the TAU Marian Gertner Institute for Medical Nanosystems.

PUBLICATIONS

• I. Zukerman, V.N. Zhitomirsky, G. Beit-Ya'akov, R.L. Boxman, A. Raveh and S.K. Kim, Vacuum arc deposition of Al2O3-ZrO2 coatings: arc behavior and coating characteristics, J Mater Sci (2010) 45:63796388.

Sidsel Bering Olsen

Sidsel Bering Olsen, Master student
Group for Molecular Oncology
Institute for Molecular Medicine, University of Southern Denmark
J.B. Winsløws Vej 25/1, 5000 Odense-C
Phone: +45-6550-3977
Fax: +45-6550-3950
Email: sbolsen@health.sdu.dk

Language skills: Danish (Mother tongue), English

EDUCATION

• Aug. 2007 - Jun. 2010 Bachelor student of Biochemistry and Molecular Biology at the University of Southern Denmark, Denmark
• Jun. 2010 Ba. Scient from the University of Southern Denmark in Biochemistry and Molecular Biology
• Aug. 2010 - Oct. 2012 Master student of Biochemistry and Molecular Biology at the University of Southern Denmark, Denmark
• Aug. 2011 - Oct. 2012 Master thesis at the University of Southern Denmark, Institute for Molecular Medicine, Molecular Oncology, Odense, Denmark
(Head: Prof. PhD Jan Mollenhauer)
• Apr. 2012 Received funding from Kræftens Bekæmpelse

Ines Block

Dr. rer. nat. Ines Block
Date of birth: July 17th, 1979 (Bremerhaven, Germany)

EDUCATION AND POSITIONS

• October 1999 - March 2005 Studies of Biochemistry, Ernst-Moritz-Arndt-University of Greifswald, Germany

• August 2002 – February 2003 Research internship at the Chemistry Department of the New Mexico State University in Las Cruces (New Mexico, USA)
Research field: Development of electrochemical biosensors
• February 2005 Diploma in Biochemistry (equivalent to MSc), Research field: Application and optimization of pH-detectors for Ion chromatography
• April 2005 – March 2009 PhD candidate in the department of Chip-Based Peptide Libraries, German Cancer Research Center (DKFZ) in Heidelberg (Germany), and the Kirchhoff-Institute for Physics at the University of Heidelberg (Germany)
Research field: Generation and application of highly complex peptide arrays
• March 2009 PhD graduation (Dr. rer. nat.) at the Naturwissenschaftlich-Mathematischen Gesamtfakultät of the Ruprecht-Karls-University Heidelberg, Germany
• Since March 2009 Postdoc position in the Molecular Oncolgy Group of Prof. Dr. Jan Mollenhauer at the Institute of Molecular Medicine of the University of Southern Denmark in Odense, Denmark
Research Field: Systematic Identification of breast cancer genes and drug targets through targeted functional genomics; Development of a new Biochip-format for the molecular profiling of cancer stem cells

Therese Bormann

Therese Bormann, 3rd year Ph.D. student
University of Basel, Biomaterials Science Center, Basel, Switzerland.

University of Applied Sciences Northwestern Switzerland, Institute for Medical and Analytical Technologies, Muttenz, Switzerland

Therese is a third year doctoral student working within the SNF project "Porous shape-memory-scaffolds as mechanically active bone implants" at the fabrication and the physico-chemical characterization of complex-shaped NiTi-structures. Fabrication of porous specimens and scaffolds is done by the additive manufacturing method of selective laser melting (SLM). Aim of the project is the development of SLM-built NiTi-implants, which exhibit an advanced interaction with the surrounding tissue. The work is carried out in close collaboration between the Biomaterials Science Center, University of Basel and the University of Applied Sciences, Northwester Switzerland under supervision of Prof. Dr. Bert Müller (Uni Basel) and Prof. Dr. Michael de Wild (FHNW). The project consortium includes also two industrial partners.

Therese originates from Germany, where she did her diploma in materials science at the University Halle-Wittenberg. Her studies have been focused on biomedical materials and materials engineering. Therese conducted her diploma thesis at the Fraunhofer Institute for Mechanics of Materials in Halle/Saale, where she was also working for six month in the Business Unit of Biological and Macromolecular Materials. Therese is currently living in Basel, Switzerland, where most of her work is carried out.

Roberta Cavalli

Roberta Cavalli is currently an Associate Professor of Pharmaceutical Technology at the Faculty of Pharmacy of the University of Turin where she took her degrees in Pharmaceutical Chemistry and in Pharmacy, the PhD in Pharmaceutical Sciences and the Post-Doc.

She is in the Doctoral School in "Scienze della Natura e Tecnilogie Innovative" of the University of Turin and in the Post-graduate School of Hospital Pharmacy.

She is a member of CRS, AAPS, AFI, ADRITELF. INSTM, Italian Association of Chemistry and Technology of Cyclodextrins and the European Cyclodextrin Society.

She is involved in various national and international research team. The researches of RC are focused on the development of innovative nanodelivery systems for therapeutic and diagnostic purposes, as nanoparticles, micro- and nanobubbles, self-assembled nanostructures, micelles, vescicles, cyclodextrin derivatives and nanosponges using mainly lipids, polymers, either natural or synthetic, and cyclodextrins.

She is also involved in research projects for the formulation of particulate theranostic agents for anticancer drugs and for the design and characterization of new systems for gene delivery.
She developed systems for the delivery of DNA, siRNA and oligonucleotides. Novel antiviral and antiviral formulations were designed in her laboratory.

The activity of RC is evidenced by more 100 scientific papers on international journals, more than 20 industrial patents and many congress communications.

Alexander Chernysh

Chernysh Alexander M. Professor, Doctor of Biological Sciences.
Date of birth 22.08. 1941
Head of Laboratory "Biophysics of cell membranes," Institute of General Reanimatology of Russian Academy of Medical Sciences, Moscow, Russia.

SPECIALITY - BIOPHYSICS

The author of 86 scientific articles and two patents for inventions. The scientific interests : nanostructure of cell membranes, the methods of atomic force microscopy, calibrated electroporation, the effect of ionizing radiation on cell membranes, biophysics of ventricular fibrillation and cardiac defibrillation. The author of scientific monograph on the theory of ventricular fibrillation of the heart. Author of the textbook "Physics and biophysics for medical students" (5 editions). Author of laboratory works on biophysics for medical students.
e-mail: amchernysh@mail.ru

Hans Deyhle

Goldauerstrasse 37, 8006 Zuerich
Tel. +41 (0)44 3610416
email: hans.deyhle@psi.ch

2007 Diploma in experimental physics at the Swiss Federal Institute of Technology Zuerich.
Diploma thesis on artificial muscles based on electrically activated polymers at the Biomaterials Science Center (University of Basel, Switzerland).
2007-2009 scientific collaborator at School of Dental Medicine (University of Basel, Switzerland), in the field of dental re-mineralization and imaging by X-ray absorption tomography.
2009-present PhD student at Paul Scherrer Institute (Villigen, Switzerland) and Biomaterials Science Center on the topic of small angle X-ray scattering applied to human teeth.

Eleni Efthimiadou

date of birth: 28 December 1979
nationality: Greek
E-mail: elefth@chem.demokritos.gr

ACADEMIC BACKGROUND

• 2009-2011 Work as a Researcher in The NCSR "DEMOKRITOS", Institute Of Material Science, Laboratory of Sol-Gel. Athens, Greece
• 2005-1010 Master in Catalysis and Enviromental Chemistry, in The Open University of Greece. Patra, Greece.
• 2006- 2009 Doctor of Philosophy in The NCSR "DEMOKRITOS", Institute Of Physical Chemistry, Laboratory of Chemical Biology. Athens, Greece.
• 2004-2006 Master in Bioinorganic chemistry, in The NCSR "DEMOKRITOS" in Collaboration with University of Athens, Department of Chemistry, Greece.
• 1999-2004 Bachelor of Science in Chemistry, Department of Chemistry, The University of Athens, Greece.

PROGRAMS

• 1. 1-3-04 έως 30-6-04: Research in Paramagnetic Organometallic Compounds of Rare Earth Elements and study of these compound in helth, Program Frame: "E-1094"
• 2. 01-2007 to 03- 2009: Research in MRI Contrast Agents, Program Frame: «PEP Attikis, 1.2, Action 1.2.1».
• 3. 2007 - 2009: Research in Drug Delivery Systems, "Nanoscale Functionalities for Targeted Delivery of Biopharmaceutics", 'NMP' INTEGRATED PROJECT, Contract No NMP4-CT-2006-026723. European Union Program Frame.
• 4. 2009 - today: Research in Nanobiophrmaceutics, "IDEAS –NANOTHERAPY". A novel nano-container drug carrier for targeted treatment of prostate cancer.

PUBLICATIONS

24/ CONFERENCES PAPERS: 32/ H-Index: 14
SELECTED PAPERS
• **E. K. Efthimiadou**, M. E. Katsarou, M. Fardis, C. Zikos, E. N. Pitsinos, A. Kazantzis, L. Leondiadis, M. Sagnou, D. Vourloumis. Bioorganic and Medicinal Chemistry Lett. 2008, 18 (23), 6058-6061.
• **E. K. Efthimiadou***, A. Karaliota , G. Psomas. Journal of Inorganic Biochemistry. J. Inorg. Biochem, 2010, 104, (4), Pages 455-466.
• A. Chatzipavlidis, P. Bilalis, **E. K. Efthimiadou**, N. Boukos, and G. C. Kordas. Langmuir, 2011, 27, 8478–8485.
• Maria E. Katsarou, **Efthimiadou E.K.**, George Psomas and Dionisios Vourloumis*. Journal of Medicinal Chemistry, 2008, 51(3). 470-478.
• **Efthimiadou E. K.**, Tapeinos C., Bilalis P., Kordas G. J. Nanoparticle Research. 2011, 13, 6725-6736
• **E.K. Efthimiadou**, L.-A. Tziveleka, P. Bilalis, G. Kordas, International Journal of pharmaceutics,2012, 428, 134-142.

Johans Fakhoury

4-4440 Ch. Des Cageux
Laval, Québec
(514) 572-5099
johans.fakhoury@mail.mcgill.ca

EDUCATION

• 2004-2010 Ph.D. Experimental Medicine McGill University, Montréal, Québec
Thesis Title: Conservation and Divergence of Mouse and Human Telomerase Regulation
• 2000-2004 B.Sc. Honours Biochemistry, Minor in Middle-East Languages
First Class Honours in Biochemistry, McGill University, Montréal, Québec
Thesis TItle: Platinum phenanthroimidazole complexes as G-quadruplex DNA selective binders

PROFFESSIONAL EXPERIENCE

• September 2010 – Current Post-Doctoral Fellow, McGill University, DNA Nano-technology Laboratory, Montréal, Québec
Project Title: Specific and efficient delivery of cancer therapeutics using modular DNA nanotubes

PUBLICATIONS

• **Fakhoury J.**, Mclaughlin C., Sleiman H.F., Cellular Uptake of DNA Nanostructures is Shape Dependent. (2012) (Submitted-ACS Nano)
• Hamblin G., Carneiro K., **Fakhoury J.**, Bujold K., Sleiman H., Rolling Circle Amplification-Templated DNA Nanotubes Show Increased Stability and Cell Penetration Ability. (2012) (J Am Chem Soc. 2012 Feb 2)
• Carneiro K., Hamblin G., Hänni K., **Fakhoury J.**, Nayak M. ,Rizis G., McLaughlin G., Bazzi H. and Sleiman H. Stimuli-responsive organization of block copolymers on DNA nanotubes. (2012) (Accepted-Chemical Science)
• Castor K., Mancini J., **Fakhoury J.**, Weill N., Kieltyka R., Englebienne P., Avakyan N.,Langille A., Mittermaier A., Autexier C., Moitessier N., Sleiman H.F.(2011) Platinum(II) phenanthroimidazoles for targeting telomeric G-quadruplexes. (ChemMedChem. 2012 Jan 2;7(1):85-94)
• **Fakhoury, J.**, Marie-Egyptienne D.T., Londoño-Vallejo J.A., Autexier C. (2010). "Telomeric function of mammalian telomerases at short telomeres." J Cell Sci 123(Pt 10): 1693-1704.
• Kieltyka, R., Englebienne P., **Fakhoury, J.**, Autexier C., Moitessier N., Sleiman H.F. (2008). "A platinum supramolecular square as an effective G-quadruplex binder and telomerase inhibitor." J Am Chem Soc 130(31): 10040-10041.
• Kieltyka, R., **Fakhoury, J.**, Moistessier N., Sleiman H. F. (2008). "Platinum phenanthroimidazole complexes as G-quadruplex DNA

selective binders." Chemistry 14(4): 1145-1154.
• **Fakhoury, J**., Nimmo G. A., Autexier C. (2007). "Harnessing telomerase in cancer therapeutics." Anticancer Agents Med Chem 7(4): 475-483.

Sarah Fredriksson

Sarah Fredriksson finished her MSc in Biotechnology Engineering at Lund University and received her PhD submitting a Thesis with the title "GENE FUSION IN PROTEIN ENGINEERING - Design of novel peptides and bifunctional enzymes" in 1999. She has published scientific papers and is the author of several patent applications. Sarah Fredriksson research interests are novel nanostructures, preclinical imaging and protein engineering. She is the founder and CEO of Genovis AB, a Swedish company focusing on novel nanostructures for research and commercial use within the life science industry. She is also in partnership with Lund University in a project developing nanostructures for sentinel node detection and partner in the Luminescent Polymers for In Vivo Imaging of Amyloid Signatures (LUPAS) consortium.
Sarah Fredriksson is an active member of the board of Eijdo research AB, Sparbankstiftelsen Skånes Venture Capital Foundation, Lund University Board of Directors, member of the Board of Biomedical, Medical and Public Health Education at Lund University and member of the advisory board of the Nanometer Consortium (Lund).

Doron Friedman

Doron Friedman PhD, B.Pharm., M.Sci and PhD from the Hebrew University of Jerusalem School of Pharmacy in pharmaceutical sciences and a registered pharmacist.
Doron expertise is in bio-pharmaceutics, Drug Delivery Systems, pharmaceutical formulation, drugs oral absorption and bioavailability. Doron record encompasses academic and industrial experience in managing pharmaceutical R&D, products` development. Doron experience is in innovative delivery solutions for water insoluble and amphiphilic pharmaceuticals anhydrous emulsions and liposomes. Doron has published twenty pear reviewed papers and over sixty patents in the area of drug delivery systems. Doron is the inventor of Loteprednol etabonate eye drops, Lotemax™ formulation and various topical products.
Doron is a group leader at Prof. Barenholz labs and managing the research and development at Lipocure Ltd..

Simona Ghiani

Date of bith: 7th of November, 1979
Nationality: italian
Home address: via della Chiesa 16/2
Almese 10040 (TO)
Mobile: 0039 3280465489
Email: simona.ghiani@gmail.com

EMPLOYMENTS

• May 2008 to date **Employee at Bracco Imaging SpA,**
Description of work: Formulation and purification of pharmaceutical products, colloidal dispersions, emulsions and nanoparticles for imaging of pathological tissues in preclinical studies.
Physico-chemical characterization of magnetic resonance imaging agents and optical characterization of fluorescent probe.
• Nov. 2007-Apr. 2008 **Collaboration in LIMA laboratory of Bioindustry Park, Colleretto Giacosa (TO)**
Description of work: Organometallic synthesis and characterization of MRI contrast agents for the imaging of neurodegenerative diseases.
• Jun. 2007-Aug. 2007 **Internship in the research center of Kao Corporation, Tokyo – Japan (Japanese company leader in Healthcare and cosmetic products)**
Description of work: Study of physico-chemical properties of hair melanin and screening of new formulations to improve hair "volume-up".
• Mar. 2004-Nov.2004 **Collaboration at the University of Turin:**
Description of work: investigation of the metal complexes for the catalysis of hair melanin bleaching processes.

EDUCATION

• Oct. 2004-Oct.2007 **PhD** in Chemical Science, Università degli Studi di Torino
Project: "Chemistry of hair melanin: the role of metal ions in the bleaching process".
• Sept. 2001-Mar.2004 **Master of science:** Metodologie chimiche avanzate, Università degli Studi di Torino. Final grade 110/110 cum laude.
• 09/1998-09/2001 **Bachelor of chemistry:** Analytical chemistry.

ADDITIONAL INFORMATION

Expertise in pharmaceutical formulations as colloidal dispersions for diagnostic probe delivery. Expertise in chemistry of transition metal complexes, in particular their pharmacological application as imaging contrast agents. Expertise in cosmetic formulation and healthcare. Expertise in analytical technique: Inductively Coupled Plasma (ICP-MS, ICP-OES), Nuclear Magnetic Resonance (NMR), electrochemical techniques (polarography, cyclic voltammetry), UV-vis and fluorescence spectroscopy, chemical modeling and computational studies, Photon Correlation Spectroscopy. Participation in international cross-functional projects for the research and development of new medical technologies.

Anna Gisselsson

Genovis AB, POB 790, 220 07 Lund, Sweden
Telephone: +46-46-101236
E-mail: anna.gisselsson@genovis.com

EDUCATION

• Ph.D. degree in biochemistry at Lund university 2003
• Master degree in chemistry at Lund university 1998

PROFESSIONAL EXPERIENCE

• 2009 – Current	Research scientist and COO, Eijdo research AB, Genovis Group, Malmö/Lund, Sweden. Research and development of nanoparticles for molecular imaging
• 2009	CEO Eijdo research AB, Malmö, Sweden. Contract research within preclinical MRI
• 2006 – 2009	Research scientist in biochemistry, Imagnia AB, Malmö, Sweden. Contract research within hyperpolarized metabolic imaging
• 2004 – 2006	Research scientist in biochemistry, Amersham Health R&D AB, part of GE Healthcare, Malmö, Sweden. Research and development of clinical contrast agents
• 1998 – 2003	PhD student at the department of Plant Biochemistry, Lund university. Thesis: Violaxanthin de-epoxidase and the xanthophyll cycle

Johan Härmark

MSc
School of Technology and Health, Royal Institute of Technology
Phone number: + 46 8 524 810 72
jharmark@kth.se
Stockholm, Sweden

EDUCATION

- 2009 MSc Dissertation: "Fluorescent Labeled Media in Affinity Columns for Visualization and Direct Detection of Protein A Leakage", GE Healthcare, Uppsala, Sweden
- 2003-2009 Master of Science in Engineering Biology with Bioprocess Engineering as profile at Linköping University, Sweden

COURSES AND WORKSHOP

- 2010 Electron Microscopy, Postgraduate Course, Department of Structural Biotechnology, Royal Institute of Technology, School of Technology and Health, Stockholm, Sweden
- 2010 SCANDEM – High-Resolution Microscopy Meeting, Practical workshop by Steve Ludtke, Baylor College of Medicine, Houston on Computational TEM: Single Particle Image Processing using EMAN, Stockholm, Sweden

PROFESSIONAL EXPERIENCE

- 2010- Postgraduate student, Royal Institute of Technology, School of Technology and Health, Department of Structural Biotechnology – Electron Microscopy, Stockholm, Sweden
  Supervisors: Hans Hebert and Philip J.B. Koeck

Marion Helle

12, rue de Rome 54000 Nancy, France
Age: 26
phone: +33 (0)6.84.31.47.17
e-mail: m.helle@nancy.unicancer.fr

EDUCATION

Nancy-University - France

- 2010: University degree in Animal Experimentation of level 1 Faculty of Pharmacy
- 2006-2008: Master Research in Health and Life Sciences, Pharmacology speciality Faculty of Sciences
- 2003-2006: Bachelor in Biology Geology

PROFESSIONAL EXPERIENCE - FRANCE

- Since 2009: PhD student: Sentinel lymph node mapping with quantum dots: breast cancer application - Centre Alexis Vautrin / Dir.: Pr. F. Marchal / In collaboration with the "Laboratoire Photons et Matière", ESPCI, Paris
- CRAN, Nancy-University, CNRS - Health Engineering Project
- 2006: Master 2 training course: Comparative study of a treatment by PPAR alpha and PPAR gamma ligands on oxidative status of human colorectal cell HT29 / Laboratory of molecular engineering and pharmacological biochemistry - Metz

SPECIAL SKILLS

Scientific and Technique: Microscopy (conventional, of fluorescence), Spectroscopy (spectrophotometry, spectrofluorimetry), Fluorescence imaging in vivo, Histology (paraffined and frozen sections, staining), Cell culture (healthy and cancer cells in monolayer, toxicity tests), Molecular Biology (DNA and RNA extractions, RT-PCR semi quantitative and quantitative, Western-blot), Animal experimentation (organ collection, graft and excision of tumours, ways of injection: s.c., i.c, i.v.), High performance liquid chromatography, Flow cytometry
Informatics: Office, EndNote, Origin, ImageJ.
Languages: French (mother tongue), English (fluent)

PUBLICATIONS

• E. Cassette*, **M. Helle***, L. Bezdetnaya, F. Marchal, B. Dubertret, T. Pons Design of new Quantum Dot Materials for Deep Tissue Infrared Imaging. Advanced Drug Delivery Reviews, Article in submission
• M-A. D'Hallewin, J. Garrier, **M. Helle**, L. Bezdetnaya, F. Guillemin Animal models for photodiagnosis and photodynamic therapy. Israel Journal of Chemistry, Article in press
• E. Cassette, T. Pons, C. Bouet, **M. Helle**, L. Bezdetnaya, F. Marchal, B. Dubertret Synthesis and characterization of near infrared Cu-In-Se/ZnS core/Shell Quantum Dots for in vivo imaging. Chemistry of Materials, 2010 22 (22), pp 6117–6124

Simone E. Hieber

CURRENT POSITIONS

- Since 02/2007 Postdoctoral Fellow Biomaterials Science Center, University of Basel
- Since 02/2010 Academic Coordinator Biomedical Engineering, University of Basel

EDUCATION

02/2007 PhD Computer Science
Computational Science and Engineering Lab
ETH Zurich, Zurich, Switzerland
Thesis: Particle methods for flow-structure interactions

10/2001 Diploma Engineering Cybernetics
University of Stuttgart, Stuttgart, Germany
Thesis: Model-based optimization of the ethanol rate in S. Cerevisiae

05/2001 Master of Science Mathematics
Michigan Technological University, Houghton, MI, USA
Thesis: An investigation of the mesh dependence of the stochastic discrete droplet model applied to dense liquid sprays

RESEARCH ACTIVITIES

- Modeling and Simulation (e.g. particle methods, level sets, FEM)
- Image Processing (e.g. segmentation, registration)
- Biomedical Engineering (e.g. virtual surgery, cancer research)

TEACHING ACTIVITIES

- Computer Science for Mechanical Engineers
- Modeling and Simulation
- Numerical and Symbolic Computing

Margaret Holme

3rd year Ph.D. student

Biomaterials Science Center, University of Basel
Department of Chemistry, University of Geneva
University Hospitals of Geneva

Maggie is a final year doctoral student working under the supervision of Prof. Dr. Bert Müller at the Biomaterials Science Center, Basel. Her research focuses on the development of novel nanocontainers for the triggered release of vasodilators for symptomatic relief during heart attacks. The project is truly multidisciplinary and its scope spans from nanocontainer formulation and optimisation to in vitro experiments and imaging in collaboration with the University Hospitals of Geneva. She is currently living in Geneva where she conducts most of her current research at the Department of Chemistry under the supervision of Dr. Andreas Zumbuehl and at the Uni-

versity Hospitals under the supervision of Dr. Med. Till Saxer. Maggie originates from Scotland and graduated from Imperial College London in 2009 with a first class MSci in Chemistry, specialising in organic synthesis. During her studies she worked for a year as an intern in the R&D chemistry labs of F. Hoffmann La Roche here in Basel. Her Masters thesis was conducted at the Genetic Therapies Centre in the labs of Prof. Andrew Miller and involved the synthesis of novel fluorescent-tagged dinucleoside polyphosphates. She was shortlisted for the 2009 Salter's Prize, a national UK award that recognises students with the potential to make a positive contribution to UK chemical industry.

Rene in 't Zandt

Work address: Genovis AB, PO Box 790, Lund, Sweden
Telephone: +46-46-101239
E-mail: Rene.intZandt@genovis.com

CERTIFICATES

•Ph.D. degree in medical sciences at the University of Nijmegen (June 2000)
• Master degree in physics at the University of Nijmegen (June 1995)
• Bachelor degree in physics at the Technical School of Eindhoven (June 1992)

PROFESSIONAL EXPERIENCE

October 2009 – Current November 2010 – Current April 2009 – Sep 2009 April 2006 – Mar. 2009	Senior Scientist MRI Genovis Group. Contract research and development of molecular imaging using nanoparticles Senior Scientist MRI, Lund Bioimaging Center, Lund University CSO Eijdo research AB. Contract research within MRI MR-physicist / in vivo MR spectroscopist at Imagnia AB, Malmö, Sweden. Subject: hyperpolarized metabolic imaging.
April 2002 – March 2006	MR-physicist / in vivo MR spectroscopist at Amersham Health R&D AB, part of GE Healthcare, Malmö, Sweden. Subject: in vivo hyperpolarized 13C metabolic imaging.
January 2000 – December 2001	Post-doc at the Katholieke Universiteit Leuven, Belgium, Department of Electrical Engineering (ESAT). Subject: Advanced Signal Processing for Medical Magnetic Resonance Imaging and Spectroscopy. (TMR ERBFMRX-CT97-0160)
June 1995 – December 1999	Ph.D.-student at the University Hospital Nijmegen, the Netherlands, Department of Radiology, MR-spectroscopy. Subject: Cellular reprogramming in neuromuscular issues as a response to genetic lesions in the cellular network for energy homeostasis.

Aleksandra Maria Jaskot

Ph.D. Student,
Molecular Oncology Lundbeckfonden Center of Excellence NanoCAN
IMM - Institute for Molecular Medicine
University of Southern Denmark
JB Winsloews Vej 25, 5000 Odense C, DK
E-mail: ajaskot@health.sdu.dk
Date of birth: September 1st 1987

EDUCATION AND EXPERIENCE

• Ph.D. student since 01.02.2012 at the Lundbeckfonden Nanomedicine Center of Excellence NanoCAN, University of Southern Denmark in Odense. Ph.D. Title: "Optimization of novel nucleic acid-based drugs for personalized treatment of advanced metastatic melanoma." Supervisor: Prof. Dr. Jan Mollenhauer
• 10.2011 – 01.2012 Research Assistant at the Lundbeckfonden Nanomedicine Center of Excellence NanoCAN, University of Southern Denmark in Odense. Research field: optimization of siRNA design and testing of siRNA delivery to cancer cells.
• Master Degree obtained on 09.2011 in Molecular Bioscience at the Lundbeckfonden Nanomedicine Center of Excellence NanoCAN, University of Southern Denmark in Odense with a thesis: "Targeted functional analysis of a candidate gene set for breast cancer". Supervisor: Prof. Dr. Jan Mollenhauer.
• 01.2010 – 06.2010 Individual study activity in Prof. Dr. Jan Mollenhauer group at the Institute for Molecular Medicine, University of Southern Denmark:"Introduction into functional genomics methods in cancer research."
• 01/2010 – 06/2010 Erasmus Programme student on University of Southern Denmark in Odense.
• 01.06.2009 – 30.06.2012 Internship in the Department of Molecular Biology at the Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology Gliwice Branch. Research field: Cell culture of the mouse melanoma cell line and testing of drugs with anticancer properties.
• 2006 – 2010 Medical University of Silesia in Katowice, Poland. Studies in Biotechnology, Faculty of Pharmacy.

AWARDS

• 2012 Region Syddanmark Scholarship for Ph.D. students.

Hiba Kanaan

Address:P .O.b ox5 4866,J erusalem9 1547
Date of Birth: 6th March 1987
E-mail: hibakanaan87@gmail.com
Tel: +972-542039850

EDUCATION

2011 till now - A Master student at Hebrew University of Jerusalem, Lab of Membrane and Liposome Research, faculty of Biochemistry and Molecular Biology.

• 2004 – 2008	Hadassah College, Jerusalem B.A of Medical Technology
• 1992 – 2004	Rosary Sister School, Jerusalem

EXPERIENCE

• 2008 till today	Hebrew University, Lab of membrane and liposome Research Duties involved Lab assistance.
• 2006	Shaarei sedek hospital, Lab of Medical Genetics Duties involved 320 hours of training.
• 2000	3 months training at Washington University, Medical school, Lab of Medical Genetics.

Nour Karra

Place of Birth: Jaffa Tel-Aviv, Israel
Emails: Nour_Karra@yahoo.com, Nourk@ekmd.huji.ac.il.

EDUCATION

• 2007-present Ph.D. Candidate, The Institute for Drug Research, The School of Pharmacy, The Hebrew University of Jerusalem. Dissertation: "Antibody Targeted Drug Delivery Systems for The Improved Treatment of NSCLC". Supervisors: Prof. Simon Benita, (The Hebrew University) and Prof. Juergen Borlak (Hannover Medical School in Germany). Expected to complete the thesis in June 2012.
• 2007 M.Sc. Clinical Pharmacy (with distinction), The Hebrew University of Jerusalem.
• 2004 B.Pharm. Pharmacy (with distinction),The Hebrew University of Jerusalem.

PROFESSIONAL EXPERIENCE

Academic Appointments: Teaching Assistant, Department of Pharmaceutics, The Hebrew University of Jerusalem:
Pharmaceutical Preparations Laboratory instructor (2008-2011)
Pharmacotherapy and Clinical Pharmacy instructor (2005-2006)
Licensure and Certification: Israeli license for practicing pharmacy (2004).

HONORS AND AWARDS

• 2011 Travel Award by the Roni Izenberg Fund for outstanding research on lung cancer, awarded to attend the AAPS Annual Meeting and Exposition, Washington DC, USA (October 2011)
• 2010 Doctoral scholarship for outstanding Arab PhD students, awarded by The Council for Higher Education, The Planning and Budget Committee
• 2010 The Israel Association of University Women Award for outstanding PhD students
• 2009 Selected by the External Relations Department of the Hebrew University as a delegate and speaker of the Hebrew University Students at a fundraising gala held in Monaco
• 2006 Joseph Lachman Excellence Award for outstanding achievements in M.Sc. Studies, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem

PUBLICATIONS

• Peer-Reviewed Publications: "The ligand nanoparticle conjugation approach for targeted cancer therapy." **N. Karra** and S. Benita. Current Drug Metabolism, 2011 Sep 5, (Epub ahead of print, PMID: 21892918).
• Chapters in Books: **Nour Karra** and Juergen Borlak. "Nanomedicine and Nanotoxicology". In: "Nanostructured Biomaterials for Overcoming Biological Barriers" (Editors: Maria Jose Alonso and Noemi S. Csaba), Royal Society of Chemistry Publishing.

Hyunjin Kim

Address:
Molecular Imaging & Therapy Branch,
National Cancer Center,
323 Ilsan-ro, Goyang-si,
Gyeonggi-do 410-769, Korea.
E-mail: nalvi77@ncc.re.kr
Tel: +82-31-920-2522

EDUCATION

• M.S. Department of Life Science, Gwangju Institute of Science and Technology, Gwangju, Korea.
• B.S. Chemistry, Chonnam National University, Gwangju, Korea.

PUBLICATIONS

• A highly sensitive magnetite nanoparticle as a simple and rapid stem cell labelling agent for MRI tracking, Hyunjin Kim, Hyun-Mi Dae, Cheongsoo Park, Eun Ock Kim, Daehong Kim, In-Hoo Kim, Yun-Hee Kim and Yongdoo Choi, J. Mater. Chem., 2011, 21, 7742
• A fluorescent turn-on probe for the detection of alkaline phosphatase activity in living cells, Tae-Il Kim*, Hyunjin Kim*, Yongdoo Choi and Youngmi Kim, Chem. Commun., 2011, 47, 9825–9827

Joachim Koeser

• 2008-present Senior Scientist in the Nanotechnology Group of the School of Life Sciences at the University for Applied Sciences and Arts Northwestern Switzerland. The work there is related to medical applications of nanotechnology, like antimicrobial implant surfaces, nanoparticles, stimulus-responsive drug release, surface modifications and biosensors.
• 2003-08 Application development for cantilever based sensors at Concentris GmbH (Basel, Switzerland).
• 1999-2003 Post-Doc in the group of Prof. Ueli Aebi at the Biozentrum (Basel, Switzerland), working on the correlation of structure and function of the nuclear pore complex.
• 1995-99 PhD thesis at the German Cancer Research Center (Heidelberg, Germany) investigating the assembly of desmosomal cell adhesion structures.
• 1989-95 MSc in Biology, University of Heidelberg, Germany, and Trinity College Dublin, Ireland.

Elena K. Kozlova

Professor, Doctor of Physical and Mathematical Sciences.
Date of birth: 0.7.05.1958

Specialty - physics. Position - Senior Researcher in the Laboratory «Biophysics of cell membranes,» Institute of General Reanimatology of Russian Academy of Medical Sciences, Moscow, Russia.
The author of 65 scientific articles. The scientific interests: radiobiology, effects of ionizing radiation on the body and on the membrane of red blood cells, biophysical modeling, electroporation of the cells under physico-chemical factors, atomic force microscopy. Author of the textbook "Physics and biophysics for medical students". The member of European Association for Red Cell Research.
e-mail: waterlake@mail.ru

Xiaochun Li-Blatter

Xiaochun Li-Blatter has worked as scientific collaborator at the division of biophysical chemistry, Biozentrum of the University of Basel since 1997.

HER RESEARCH FOCUSES ON FOLLOWING FIELDS

• Quantitatively evaluate the mechanism of activation and inhibition of ATP binding cassette (ABC) transporters such as P-glycoprotein (ABCB1) and the breast cancer resistance protein (ABCG2). Enhance the understanding of the structure-activity relationship of substrates, modulators, and inhibitors of these transporters.
• Investigating the physicochemical properties of drugs; in particular by determining membrane partitioning constants, and establishing correlations between (passive) trans-membrane diffusion of drugs and active transport by ABC transporters.
• Intensively work towards an in-depth understanding of the monomer, micelle or vesicle forms of detergents and lipids. Goal: obtain function based solutions for increasing drug bioavailability.

• Understand the mechanisms of binding of cytosolic proteins to membranes and their roles in biological functions, for instance: talin peptide, TAT peptide, alpha-Synuclein and its mutants.

THE MAIN RESEARCH TOOLS

Isothermal titration calorimetry, differential scanning calorimetry, CD spectroscopy, UV and fluorescence spectroscopy, monolayer expansion technique, monolayer absorption technique, ultracentrifugation, dynamic light scattering, micro-electrophoresis and cytosensor, high throughput screening technique.
She got the diploma of organic chemical engineer at Beijing before.

Markus List

Since 12/2011 PhD student in the Group for Molecular Medicine at the University of Southern Denmark,
J.B. Winsløws Vej 25.1, 5000 Odense C,
Phone: +45 6550 3957
mlist@health.sdu.dk.

EDUCATION

• 05/2011 MSc in Bioinformatics, University of Tübingen, Germany
• 09/2008 BSc in Bioinformatics, University of Tübingen, Germany

PROFESSIONAL EXPERIENCE

• 2010-2011: DAAD stipend for an exchange semester at the University of Auckland, New Zealand, in order to conduct my master thesis in the field of the creation of dynamic models on metabolomics data.
• 2008-2010: Experience as software developer through a part-time job (2008-2010) at careon GmbH, Tübingen, Germany and a six months work placement (2010) at osthus GmbH, Mannheim, Germany.

CURRENT SCIENTIFIC ACTIVITY

I am working on my PhD thesis with the goal of developing bioinformatics tools for management and analysis of data stemming from both, high-throughput RNAi screening (HTS), and reverse-phase-protein-arrays (RPPA). A major goal is the integration of these and other data to gain novel insights in cancer development.

Sergey Lobov

Browitt Nanoparticle Laboratory,
Dept. of Applied Mathematics, RSPE
Rooms 1.01-1.03 and 1.07
Innovations Building
Bldg. 124 Eggleston Road
Australian National University
Canberra, A.C.T. 0200
AUSTRALIA
Ph: 61-2-61256390
Mob: 61-(0)40-5966348
E-mail: sergey.lobov@anu.edu.au
ResearcherID: http://www.researcherid.com/rid/A-8803-2011

EDUCATION AND CURRENT ACADEMIC APPOINTMENT

Graduated St.-Petersburg State University, Russia, with a degree Master of Science in Biology/Biochemistry. Received doctoral degree (PhD) in Medical Biochemistry, Umeå University, Sweden. Currently is a Research Fellow at Browitt Nanoparticle Laboratory, Research School of Physics and Engineering, Australian National University, Canberra, Australia.

RESEARCH INTERESTS

Biochemistry and genetic engineering applied in targeted nanoparticle imaging for research and clinical applications. The technology of carbon caged radioisotope Tm99 developed in Browitt Nanoparticle Laboratory, ANU, Australia is used as a vehicle and a basic principal for imaging. The chemical properties of the carbon cage are used in further biochemical reactions to provide composite nanoparticles that are employed to localise the particle to the area of interest. The poster presents characterisation of charge-dependent in vitro and in vivo interactions of the basic and composite nanoparticles, and provides evidence for clinical application of the method, and in particular imaging of lung perfusion.

Kirill Makedonski

Kirill Makedonski Ph.D., M.Sc.
Biochemistry, Immunology and Molecular Biology expert, Project manager.
Shabtay Hanegbi str., 49/9, Jerusalem.
Home: +972-2-6766279.
Mobile: +972-54-4734380
Email: k_makedonski@yahoo.com

WORKING EXPERIENCE

• 2010 – Now Hebrew University, Prof. Yechezkel Barenholz's Lab of Memrane and Liposome Research, Hadassah Medical School, Jerusalem, Israel
Research Associate – Deveoping of Delivery Systems for siRNA in vivo.
• 2007 – 2010 Hebrew University and NovoTyr Therapeutics, Prof. Alex Levitzki's lab Jerusalem, Israel
Postdoc fellow and project manager – Search and developing of novel IGF1 receptor inhibitors as new anticancer drugs, studying of molecular mechanism of inhibition.
• 2006 – 2007 Trisogen biotechnologies Ltd.P, Petach Tikva, Israel
Project manager – Molecular genetics research and development. Developing of the novel method of diagnosis of Down syndrome on the earlier stage of pregnancy.
• 2002 Hebrew University, Jerusalem, Israel
Teaching Assistant – course "Biochemistry of inherited material"
• 2002 -2006 Hebrew University, Dr' A. Taraboulos lab, Jerusalem, Israel.
Research Assistant. Studying PrPSc biology in animal models.
• 1997-1999 Hebrew University, Prof. S.Z. Ben Sasson lab, Jerusalem, Israel.
Research Assistant. Studying of specific protein kinase inhibitors effecto nt hef unctioninga ndd evelopmento ft hei mmunes ystem.

EDUCATION

• 2006 Ph.D in Biochemistry. Hebrew University, Jerusalem, Israel.
Dissertation: "The role of MeCP2 protein in regulation of gene expression"
• 1997 M.Sc. in Microbiology with excellence. Hebrew University, Jerusalem, Israel.
Thesis: "Role of signal strength transduced by T cell receptor and CD28 in proliferation and maturation of CD4+ T cells"
• 1995 B.Sc. in Biology. Hebrew University, Jerusalem, Israel.

SKILLS

• Molecular biology and biochemistry methods: Southern, Northern and Western blotting; transfection and transformation, cloning and ligation; PCR, Real time -PCR, RT-PCR; Gel shift, Footprinting, siRNA.
• Chromatin Immunoprecipitation Assay, Bisulfite method
• Immunostaining, FACS analysis
• Cell biology, Cell culture and Microbiology methods.
• Working and manipulations with laboratory animals; DNA microinjections
• HPLC experience
• Scientific writing

Jan Philipp Mehlich

Address: Nonnstraße 19, 53119 Bonn
Date of birth: October 9th 1981
Place of birth: Warendorf, Germany

EDUCATION

• 1988 – 1992 Primary school: Dechant-Wessing-Grundschule Hoetmar
• 1992 – 2001 Secondary school: Gymnasium Laurentianum Warendorf (Abitur 06/2011)
• 12/2001 – 09/2002 Civil service
• 10/2002 – 10/2011 Westfälische Wilhelms University Münster, Chemistry department
• 04/2007 – 10/2007 Diploma Thesis with Prof. Dr. B. J. Ravoo (Microcontact chemistry)
• 11/2007 – 07/2011 PhD course in the work group of Prof. Dr. B. J. Ravoo (DFG scholarship, IRTG Münster-Nagoya)
• Since 10/2010 Master course "Angewandte Ethik (Applied Ethics)", WWU Münster and Center for Nonprofit Management, Münster
• Since 11/2011Scientific co-worker at the Europäische Akademie Bad Neuenahr-Ahrweiler GmbH

ADDITIONAL EXPERIENCE AND PRACTICE

• 1999 Award in "Jugend forscht"
• 1999 Award in the Pupil's Competition of German Biology Association
• 04/2006 – 09/2006 Research projects at Seoul National University, South Korea (Materials
Sciences and Molecular Genetics)
• 09/2009 – 03/2010 Research stay at Nagoya University, Japan (IRTG exchange program)

LANGUAGES

English, fluent (9 years at school)
Latin, Latinum degree (5 years at school)
French, basics (2 years at school)
Korean, basics (1 year language course 2h/week)
Japanese, basics (language course, 150h)

Malin Mejàre

Since 2006 I am at Genovis AB, a life science company, in Lund, Sweden, where I currently hold a position as Chief Scientific Officer. Our focus is in developing novel nanostructures for multimodal imaging in preclinical use and enzymes for antibody characterization. I have a PhD from the department of Pure and Applied Biochemistry at Lund University, Sweden, in year 2000. The research focus in my thesis was protein engineering and development of novel affinity tags for use in biotechnological applications. Furthermore, I have experience as a research scientist in the field of antibody engineering at BioInvent International AB, a pharmaceutical company developing antibody drugs.

Beatriz Monteiro

Address: Rua Miguel Russel nº26 - 2ºE, 2855-120 Corroios (Portugal)
Telephone: (+351) 212552041
Mobile: (+351) 969166211
E-mail: beatriz.jc.monteiro@gmail.com
Nationality: Portuguese,
Date of birth: 10/11/1988

EDUCATION AND TRAINING

• 15/09/2006 - 05/02/2010 Bachelor in Celular and Molecular Biology
New University of Lisbon (Portugal) - Faculty of Science and Technology
• 15/09/2010 - 2012 Master in Biotechnology
Technical University of Lisbon (Portugal) - Instituto Superior Técnico

LANGUAGES

Portuguese, English, Spanish, Castilian

ADDITIONAL INFORMATION

• Volunteer for Food Bank campaign in Portugal.
• Member of Board of European Students of Technology (BEST) in Lisbon.

Carolin Müller

Nationality: German
Date of birth: 25.04.1982
Address: Nyvangsvej 19b, 5000 Odense C
Email: camueller@health.sdu.dk

EDUCATION / RESEARCH AND WORK EXPERIENCE

• 02/2011 – today PhD student at the University of Southern Denmark, Faculty of Health Science-; Institute for Molecular Medicine
• 10/2008 – 01/2011 Research Assistant (full-time position), University of Applied Sciences, Mannheim, Germany, Institute of Molecular and Cell Biology
• 10/2009 - 11/2009 Guest researcher; University of Southern Denmark, Odense; Research group Prof. Dr. Jan Mollenhauer
• 10/2003 - 08/2008 Studies of Biological Chemistry, University of Applied Sciences Mannheim, Germany
• Degree: Diplom-Ingenieur (Dipl. Ing.) Biotechnology
• Diploma Thesis: University of Applied Sciences Mannheim, Germany, in cooperation with German Cancer Research Center (DKFZ) Heidelberg, Germany
• 09/2002 - 08/2003 High school graduation
• 09/1998 - 01/2002 Chemical laboratory technician trainee at Carl-Engler-School, Karlsruhe, Germany

Liudmila Nikitina

EDUCATION

• February 2012- university assistant, Institute of Cell Biology, Histology and Embryology, Medical University of Graz, Austria
• February 2009-February 2012 postdoctoral fellow, Institute of Biochemistry and Molecular Medicine, University of Bern, Switzerland
• November 2007-October 2008 postdoctoral fellow, Institute of Cell Biology, Histology and Embryology, Medical University of Graz, Austria
• December 2006-October 2007 Research assistant, Research center for Obstetrics, Gynecology & Perinatology named after acad. Kulakov, Moscow, Russia
• December 2003-September 2006 Research Assistant, Biochemistry Department of Faculty of Medicine of Moscow State University, Moscow, Russia
• 2002-2005 PhD fellow, Biochemistry Department of Faculty of Medicine of Moscow State University, Moscow, Russia
• 1996-2002 Study of Human Medicine, Faculty of Medicine, Moscow State University, Russia

RESEARCH INTERESTS:

• Reproductive immunology
• Placental transport
• Placental angiogenesis
• Genetic predisposition to pregnancy complications
• Emryology, embryotoxicology
• Nanomedicine, nanotoxicology

Adkhamjon Paiziev

Citizenship: Uzbekistan
Date of Birth: 18/10/1950

Institute of Electronics Uzbek Academy of Science
Phone: (998-71)2623719
Fax:(998-71)2628767
E-mail: adxam_payziev@rambler.ru

DEGREES

• 2011-present time Head of Lab. Biophotonics (Medicine, Biology), Arifov Institute of Electronics Uzb. Acad. Sci.
• 2009-2011 Head of Appl. Phys. Group (Cotton physics and physiology), Arifov Institute of Electronics Uzb. Acad. Sci.
• 02/03/2009-26/05/2009 Visiting professor (Cell biology), Plant genetics and physiology, Hebrew University Jerusalem (Izrael)
• 2005-2006 Cell biologist (Plant cell wall morphology), Wageningen University, Lab. Cell Biology ,Wageningen and AMOLF FOM Institute, Amsterdam , The Netherlands
• 2004 Biophotons and cell physiologist (Biophotonics and agrophysics), TEMPUS Educational Program "Tempus" support high education. IMG-UZB1009-2004, Università di Catania, Italy.

POSITIONS HELD

• 2004-present Cell cytologist, Pathology Institute Health Ministry Republic of Uzbekistan
• 1999-present Microscopist, Uzbek Academy of Sciences, Institute of Electronics, Department of Applied Physics, Tashkent , Uzbekistan
• 2004-present Lecturer Agrophysics, Tashkent State Agrarian University, Department of Information Technology. Tashkent, Uzbekistan
• 1998-1999 Senior scientist, U.A.Arifov Institute of Electronics. Positron Diagnostics and Positron Tomography Group.Tashkent, Uzbekistan.
• 1975-1978 Ph.D. student, A. F. Ioffe Physical-Technical Institute Academy Sciences USSR , Leningrad. Department Physics. Leningrad, Russia

MEMBERSHIPS

• Member of European Microscopy Society
• Member of Open Textile Journal Editorial Board
• Member of Cellulose Journal Editorial Board
• Member of Int. Soc. Lab. Hematology

AWARDS

• Berend Houwen Award of European Hematology Association (EHA) (2010, The Brighton, UK)
• Award of Patent Office Republic of Uzbekistan "NEW INTELLECT" in nomination "The best invention of year 2010"

FIELD OF INTEREST

• Material science (positron spectroscopy of solids, semiconductors and composite materials, native fibers)
• Medicine (early diagnostics of cancer cells, morphology of alive cells, visualization of cells by light microscopy)
• Cotton science (physiology of cotton fibers, morphology and structure of cotton cell wall, textile properties of cotton fibers)

Simona Pinzaru

I am associate professor at the Department of Biomedical Physics, Theoretics and Molecular Spectroscopy from Babes-Bolyai University, with expertise in applied Raman spectroscopy methods development (SERS). I received my "Dr" title in 1998, under the joint scientific coordination of Professor Onuc Cozar from Babes-Bolyai University and Professor Wolfgang Kiefer from the University of Würzburg, Germany. I participated as postdoctoral researcher in many national or international research projects, focused on the applied spectroscopy techniques in biomedical, pharmaceutical and environmental field. Current research is focused on early cancer diagnostic and therapy monitoring using biocompatible nanoparticles. I have an experience of 20 years of research and education, 4 years as Wissenschaftlicher Mitarbeiter in Germany, at Bayerische Julius Maximilians-Universität Würzburg and I published 51 papers in ISI ranked journals (Hirsch Index 10). Researcher ID: A-4543-2011.

Sibyll Pollok

Institute of Photonic Technology Jena
Albert-Einstein-Strasse 9
D-07745 Jena, Germany
email: sibyll.pollok@ipht-jena.de
phone: + 49-3641-206309

EDUCATION

• 1997 – 2002 student of biochemistry at the Friedrich-Schiller-University Jena, Germany
• 2002 – 2007 PhD-student at the Leibniz Institute for Age Research Jena, Germany
• 2008 – 2011 PostDoc at the University of Applied Sciences Jena, Germany
• since 2012 PostDoc at the Institute of Photonic Technology Jena, Germany

Angela Riedel

Diploma in Molecular Biotechnology, Research Assistant, Group of Molecular Oncology, Institute of Molecular Medicine, University of Southern Denmark, J.B. Winsløws Vej 25/1, 5000 Odense-C
Phone: +45-6550-3977
Fax: +45-6550-3950
Email: ariedel@health.sdu.dk

EDUCATION

• Oct. 2003 - Sep. 2008 Student of Molecular Biotechnology at the University of Bielefeld, Germany
• Jan. 2006 - June 2006 Foreign term at the Royal Institute of Technology, Stockholm, Sweden
• Mar. - May 2006 Project thesis in the research group of PD PhD Peter Savolainen, Department of Molecular Biotechnology, KTH, Stockholm, Sweden
• Jan. - Sept. 2008 Diploma thesis in the research group of PD PhD Jan Mollenhauer, Division of Molecular Genome Analysis, German Cancer Research Centre (DKFZ), Heidelberg, Germany
• Dec. 2008 Graduate from the University of Bielefeld with a Diploma in Molecular Biotechnology
• Jan. 2009 – Dec. 2011 Employed as a Ph.D. student at the University of Southern Denmark, Institute for Molecular Medicine, Molecular Oncology, Odense, Denmark
(Head: Prof. PhD Jan Mollenhauer)
• Nov. 2010 Winner of the "Best Abstract Award" at the conference "Excellence in Oncology", Athens, Greece
• Jan. 2012 Employed as a research assistant at the University of Southern Denmark, Institute for Molecular Medicine, Molecular Oncology, Odense, Denmark

Nina Rogelius

Nationality: Swedish
E-mail: Nina.Rogelius@genovis.com
Telephone: + 46-46-12 99 45
Work address: Genovis AB, PO Box 790, Lund, Sweden.

CERTIFICATES

• PhD degree in neurobiology at the Lund university (June 2007).
• Master degree in molecular biology (January 2001). The Gothenburg university, Copenhagen university and Lund university.

PROFESSIONAL EXPERIENCE

• Jan 2008 - current Scientist at Genovis AB, Lund Sweden.
• Jan 2000 – Jun 2007 PhD student at Lund university, Sweden, the department of neurobiology. Project title; Progenitor cells in the postnatal central nervous system.
• Jun 1999 – Jan 2000 AstraZeneca AB, Lund, Sweden.

PUBLICATIONS

• **Nina Rogelius**, Cecilia Ericson, Cecilia Lundberg. "In vivo labeling of neuroblasts in the sub ventricular zone of rats". Journal of Neuroscience Methods 285-293, 2005.
• **Nina Rogelius**, Josephine B. Jensen, Cecilia Lundberg, Malin Parmar. "Retrovirally delivered Islet-1 increases recruitment of Ng2 expressing cells from the postnatal SVZ into the striatum". Experimental Neurology 388-398, 2006.
• **Nina Rogelius**, Josephine B. Jensen, Cecilia Lundberg, Malin Parmar. "Reprogramming of neonatal SVZ progenitors by Islet-1 and Neurogenin-2". Molecular and Cellular Neuroscience 453-459, 2008

Andrea Schönbächler

Date of birth: 17.September 1980
Place of birth: Einsiedeln (SZ)
Martial status: single / no children

DUCATION

• 2001 - 2007 ETH Zürich
Diploma in Food Engineering
Topic of thesis: Stability of plant ferritin under simulated in vitro pepsin digestion
• 1995 - 1999 Kantonsschule, Pfäffikon (SZ), Matura Typus C (Natural science)

CAREER RELATED WORK EXPERIENCE / WORK PLACEMENTS

• 2011- present PhD Student at the University of Basel, Department of Pharmaceutical Sciences, working at the FHNW HLS
• 2008 - 2010 Employee Assistant Quality Assurance, Estée Lauder AG, Lachen
• 2008 Temporary employee Product Safety Assurance, Frutarom, Wädenswil

SKILLS

Languages: German (native), English (fluent), French (intermediate)

Computer skills
PC software: MS Word, Excel, Outlook, PowerPoint, Access
Industrial software: SAP-ER /Oracle/ AS400/ LM7

Georg Schulz

Biomaterials Science Center, University of Basel
Tel. +41 (0)61 265 91 27
email: georg.schulz@unibas.ch

EDUCATION

• 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).
• 2008-present PhD student at Biomaterials Science Center on the topic of human brain imaging, in particular magnetic resonance imaging, phase contrast X-Ray computed tomography and small-angle X-ray scattering.

Roland Stauber

Univ.-Prof. Dr. Roland Stauber, Molecular and Cellular Oncology/ENT Department/ University Medical Center of the Johannes-Gutenberg University Mainz, Langenbeckstrasse 1, 55101 Mainz, Phone: +49 (0)6131-17-7002; Email: rstauber@uni-mainz.de

Date and place of birth: 7th June 1963 in Cham

EDUCATION

• 1984-89 Studies of Biology (Diploma), Julius-Maximilians-University Würzburg
• 1989-94 PhD with Prof. Dr. V. ter Meulen, Julius-Maximilians-University Würzburg
• 1994-97 Postdoctoral research with Prof. Dr. George Van de Woude, National Cancer Institute (USA)
• 1999 Habilitation and venia legendi, Medical Faculty University Erlangen-Nürnberg

POSITIONS

• 1997-2001 Head of Division "Dynamic Transport in Virology", Medical Faculty University Erlangen-Nürnberg
• 2001-2006 Head of Division "Translational Oncology" and Coordinator of the National Genome Research Net (NGFN1+2) CancerNet; Chemotherapeutic Research Institute-Georg-Speyer-Haus, Frankfurt am Main
• since 2006 W2 Professor, Faculty of Medicine, University Medical Center of the Johannes-Gutenberg University Mainz; Founder of the Mainz Screening Center (MSC)

AWARD AND HONOURS

• 1994-2000 DKFZ Fellowship "Infectiology" of the BMBF
• 1999 Habilitation Fellowship of the Walter und Sibylle Kalkhof-Rose-Stiftung
• 2010 Alexander Karl Prize for Cancer Research

REVIEWER

• EMBO Journal, Nature, Oncogene, ACS Nano, Nature Cell Biology, J. of Neuroscience, ACS Chemical Biology,
Cell Death and Disease, Celly Cycle
• Grant proposal of national and international funding agencies

RELEVANT PUBLICATIONS

• Tenzer, S., Docter, D., Rosfa, S., Wlodarski, A., Kuharev, J., Rekik, A., Knauer, S., Bantz, C., Nawroth, T., Bier, C., Sirirattanapan, J., Treuel, L., Zellner, R., Maskos, M., Schild, H., and **Stauber, R. H.** (2011). Nanoparticle Size is a Critical Physico-Chemical Determinant of the Human Blood Plasma Corona - A Comprehensive Quantitative Proteomic Analysis. ACS Nano doi.org/nn201950e.
• Kasper, J.; Hermanns, M. I.; Bantz, C.; Maskos, M.; **Stauber, R.**; Pohl, C.; Unger, R. E.; Kirkpatrick, J. C., Inflammatory and cyto-

toxic responses of an alveolar-capillary coculture model to silica nanoparticles: comparison with conventional monocultures. Part Fibre Toxicol 2011, 8, 6.

• **Roland H. Stauber**, Shirley K. Knauer, Negusse Habtemichael, Carolin Bier, Britta Unruhe, Simona Weisheit3, Stephanie Spange, Frank Nonnenmacher, Verena Fetz, Torsten Ginter, Sigrid Reichardt, Claus Liebmann, Günter Schneider, Oliver H. Krämer. A combination of a ribonucleotide reductase inhibitor and histone deacetylase inhibitors downregulates EGFR and triggers BIM-dependent apoptosis in head and neck cancer. Oncotarget 2012.

• Bier, C.; Knauer, S. K.; Klapthor, A.; Schweitzer, A.; Rekik, A.; Kramer, O. H.; Marschalek, R.; **Stauber, R. H.**, Cell-based analysis of structure-function activity of threonine aspartase 1. J Biol Chem 2011, 286, 3007-3017.

• Engels K., Knauer S., Loibl S., Fetz V., Hanker L., Harter P., du Bois A., Fisseler-Eckhoff A., Schweitzer A., Kommoss F., Hermanns I., Kleinert H., Nekljudova V., and **Stauber R.H.** (2008). NO-Signaling Confers Cytoprotectivity Through the Survivin Network in Ovarian Carcinomas. Cancer Research, 68(1), p. 5159 -66.

Triantafyllos Stylianopoulos

Dr. Triantafyllos Stylianopoulos holds a lecturer position in Chemical Engineering at the University of Cyprus and a visiting research fellow position at Harvard Medical School. He received a PhD degree in Chemical Engineer in 2008 from the University of Minnesota, USA. During his doctoral studies he was elected twice top doctoral student of the department. Dr. Stylianopoulos performed his post-doc work at the department of Radiation Oncology at Harvard Medical School and Massachusetts General Hospital, USA from 2008 to 2010.

His research work involves the combined use of state-of-the-art experimental techniques, such as intravital microscopy, and mathematical modeling in order to quantify in vivo the delivery of drugs to solid tumors, to explore the physiological barriers that hinder drug delivery and to create design rules for the optimal and homogeneous distribution of nanomedicines.

He has published over twenty publications in peer-reviewed scientific journals including three in Nature (Nature Medicine (ISI impact factor: 25.43), Nature Nanotechnology (ISI impact factor: 30.31), Nature Reviews Clinical Oncology (ISI impact factor: 10.78)) and four in the Proceedings of the National Academy of Science (ISI impact factor: 9.75). His work has been highlighted by Nature, Nature Materials, PNAS, and the American Association of Cancer Research. Two of his recent publications were selected by the "Faculty of 1000" among the top 2% articles in biology and medicine.

Dr. Stylianopoulos is a member of the European Foundation of Clinical Nanomedicine, the American Institute of Chemical Engineers, and the European Society of Biomechanics.

Farzaneh Tajdini

Contact Address: IAU-Karaj branch, Moazen Boulevard, Gohardasht, Karaj, Iran.
Email address: farzaneh.tajdini@kiau.ac.ir

UNIVERSITY EDUCATION

Doctor of Veterinary Medicine (DVM) (Tehran University, Iran)
Ph.D. (Mycology, Islamic Azad University-Science and Research branch, Iran)

POSITION

Assistant Professor (Faculty of Veterinary Medicine, Islamic Azad University, Karaj branch)
Director of University Scientific Associations (Islamic Azad University, Karaj branch)

RESEARCH INTEREST

Her laboratory focused on the clinical aspect of Mycotic diseases in cattle. However, she has shifted her interest to the use of Biotechnology in clinical trials recently. In the way that she extracted and characterized the new source of chitosan from fungi and used it as nano-carrier for drug and vaccine delivery systems.

SELECTED PROJECTS

• Preparation and evaluation of fungal chitosan nanoparticles containing FMD viruses as a new carrier for mucosal vaccine delivery.
• Production, physiochemical and antimicrobial properties of fungal chitosan from Rhizomucor miehei and Mucor racemosus.
• Evaluation of antifungal effects of standard essence of Seeberi & compare with Nystatin drug in growth of Candida albicans isolated in raw cow's milk.
• Comparison of high effect concentration of essences include of Myrtus communis and Natamycin drug on growth Trichosporon isolated in raw cow's milk.

Jakub Trojnar

Nationality: Polish. Date of birth: 3rd of February 1987. Address: Elsdyrløkken 112F, 5210 Odense NV. Email: jtrojnar@health.sdu.dk. Mobile: +45 501 07 195

EDUCATION

• 2004-2006: Secondary School no.VIII in Katowice, Poland. Classes with extended English and Russian courses.
• 2006-2010: Medical University of Silesia in Katowice. Poland. Main field of studies: Biotechnology (Department of Pharmacy). Further areas of studies: Biochemistry, Molecular Biology, Biology, Microbiology, Proteomics, Genetics.
• 2010: Erasmus student at the University of Southern Denmark in Odense. Area of studies: Biochemistry and Molecular Biology. Courses: Molecular mechanisms in tumor biology, Advanced Ecotoxicology, Biomolecular Mass Spectrometry, Recombinant human protein kinases: expression, purification, characterization. Individual study activity (400 h): Introduction into functional genomics methods in cancer research in the group "Molecular Oncology" of Prof. Dr. Jan Mollenhauer at the Institute for Molecular Medicine
• August 2010: Master student in Molecular Bioscience on University of Southern Denmark in Odense.
• September 2010: Research internship at Wastewater treatment plant in Katowice.

LANGUAGE SKILLS

• **English**, fluent in speaking and writing (extensively trained due to summer holiday employments in Holland and England).
• **German**, basic knowledge.
• **Polish**, native language

Member of Young European Biotech Network Organization.

Keren Turjeman

Born, March 14, 1979, Jerusalem, Israel.
Married, 1 child.
Phone: 972-50-9888867.
E-mail: Kerenbarhum@Gmail.com

EDUCATION

• 2006-present: Department of Biochemistry and Molecular Biology, Institute for Medical Research Israel-Canada (IMRIC), Hebrew University–Hadassah Medical School, Jerusalem. Ph.D. research in the Laboratory of Membrane and Liposome Research of Prof. Yechezkel Barenholz. Research project: sterically stabilized nano-liposomes having favorable pharmacokinetics and controlled drug release rate for medical applications.

• 2005-2006: M.Sc. (Summa Cum Laude) – Department of Biochemistry and Molecular Biology, Institute for Medical Research Israel-Canada (IMRIC), Hebrew University–Hadassah Medical School, Jerusalem. Research in the Laboratory of Membrane and Liposome Research of Prof. Yechezkel Barenholz. Research project: sterically stabilized nano-liposomes having favorable pharmacokinetics and controlled drug release rate for medical applications.
• 2001-2005: B.Sc. (Summa Cum Laude) – Department of Food Engineering and Biotechnology, Technion - Israel Institute of Technology, Israel.

TECHNICAL EXPERIENCE

In vitro techniques including liposome preparation, optimization and characterization using chemical, physical and bio-chemical methods such as RP-HPLC, atomic absorption spectrometry, mass spectrometry, electron paramagnetic resonance spectroscopy and fluorescent methods. Cell culture techniques including primary tissue culture and different cell-lines, bioassays for cytokines, cell proliferation assays, cytotoxicity assays and microscopy. In vivo studies in several murine models: experimental autoimmune encephalomyelitis (EAE), adjuvant-induced arthritis (AIA), SOD1 mice, mdx mice and P. berghei Anka model for cerebral malaria (CM).

EXPERIENCE

• 2007-present: Teaching Assistant at the Institute for Medical Research Israel-Canada (IMRIC), Hebrew University–Hadassah Medical School. Structure and function of biomolecules, workshops on purification and analysis of proteins.
• 2003-2004: Research at the Department of Food Engineering and Biotechnology, Technion - Israel Institute of Technology, in the laboratory of Prof. Sima Yaron.
Research project: The multiple antibiotic resistance (mar) operon in Salmonella Virchow.
• 1999-2001: ECI-Telecom, Final quality control (Exline team).
• 1990-1992: Israel Defence Forces, military police. Rank upon discharge: Sergeant.

Mehmet Hikmet Ucisik

Mehmet Hikmet UCISIK, Dipl.-Ing.
Department of Nanobiotechnology
University of Natural Resources and Life Sciences (BOKU) Vienna
(+43) 1 / 47654-2247

EDUCATION

• Present: University of Natural Resources and Applied Life Sciences, Vienna, Austria
PhD. Study in Department of Nanobiotechnology, Vienna Institute of Biotechnology (VIBT). Research Title: "Generation of Nanocarrier Systems with S-Layer Proteins", Research Interests: S-Layer Proteins, Surface Characterization, Cell Targeting, Drug Delivery using Emulsomes and Liposomes. Started in October 2009
• Transferred: Istanbul Technical University (ITU), Department of Molecular Biology & Genetics; PhD. in Program of Molecular Biology, Genetics and Biotechnology. Started at February, 2007; Ended the courses in January, 2008 GPA: 3.94/4.00
• August, 2006: TU Delft, Department of Biotechnology, Delft, The Netherlands; 2004/2006 MSc. Biochemical Engineering Program; Accomplished his thesis work in group of Bioprocess Technology GPA: 7.1 over 10
• July, 2004: Bogazici University, Chemical Engineering Department, Istanbul, Nation's Top-Ranked University and Engineering Faculty; 4-year BSc. Chemical Enginering Program GPA: 2.87/4.00
• June, 2000: German High School – "Deutsche Schule Istanbul", Istanbul, Turkey; Graduation with 2 degrees: German High School Diploma, "Abitur"; Grade: 2.8/4.0; Turkish High School Diploma; Grade: 7.88/10.00

PERSONAL INFORMATION AND LANGUAGE SKILLS

Male; Single; Has Turkish Nationality; Born in Istanbul in April 23, 1981. Fluent in English and German; native language Turkish

EXPERIMENTAL AND TECHNICAL SKILLS

Fermentation: Batch and Continuous; **Protein Purification:** chromatography techniques; **Metabolite Analysis** including enzymatic analysis and HPLC; **Molecular Biology Techniques:** Plasmid Extraction, Isolation and Cloning: **Polymerase Chain Reaction** (PCR), **SDS Gel Electrophoresis; Protein Characterization Studies: Surface Plasmon Resonance** (SPR), **Scanning Electron Microscopy** (SEM), **Transmission Electron Microscopy** (TEM), **Fluorescence Microscopy**, Zeta-Sizer; **Cell Culture Experiments**. **Conceptual Design of Up- and Down-Stream Processing** including **In-Situ Product Removal Process** and **Crystallization** Unit Operation; **Economic Feasibility** Studies; **Scale-Up**.

ATTENDED COURSES AND SEMINARS

• Workshop on Nanotechnology, Middle East Technical University, Ankara, Turkey, Sept 1-2, 2008:
• Medical Nanotechnology, Summer Short Course by Prof.Dr.Melik Demirel (Associate Professor of Engineering at Penn State University), Koc University, Istanbul, Turkey, June 22-26, 2009.
• Bionano Workshop, Waldemar Petersen Haus, Hirschegg, Austria Feb 7-13, 2010 (Poster Presentation),
• Summer School of International Center for Materials Research Program (ICMR) on 'Nanoscale Science of Biological Interfaces', University of California Santa Barbara (UCSB), USA, June 19 - July 1, 2010 (Poster Presentation)
• 2nd Annual Meeting of the Austrian Association of Molecular Life Sciences and Biotechnology (ÖGMBT), Vienna, Austria, September 27-29, 2010 (Poster Presentation)
• 2nd International Congress BioNanoMed 2010 – Nanotechnology in Medicine & Biology, Danube University Krems, Krems, Austria, November 2-3, 2010 (Poster Presentation)
• Linz Winter School 2011, Linz, Austria, February 1-3, 2011 (Poster Presentation)
• International Summer School on "Cellular Systems", Heidelberg, Germany, August 24-30, 2011 (Oral Presentation)
• 5th International Congress on Pharmaceutical Engineering (ICPE), Graz, Austria, Sep 29-30, 2011 (Poster Presentation)

Prabitha Urwyler

Dr. Prabitha Urwyler, Post Doc
Biomaterials Science Center, University of Basel

Prabitha is currently working as a Post Doc at the Biomaterials Science Center of the University of Basel. Her research focuses on measuring contractile cell forces to identify nano- and microstructures for tailored medical implant surfaces. She successfully defended her doctoral thesis entitled "Polymeric micro-cantilever sensors for biomedical applications" in Jan 2012.
Prabitha originates from India and obtained her Bachelor of Technology (B.Tech) in Computer Engineering from the Mangalore University, Karnataka, India in 1995. From Sept 1995 – 1997, she worked as a software programmer for Melstar Information Technologies Ltd, Mumbai, India. She continued working as a software developer at the Swiss News Agency (SDA – ATS), Bern, Switzerland until Sept 2008. Moving from software to biomedical engineering, she pursued her masters in 2006, which earned her M.Sc in Biomedical Engineering from the University of Bern in 2008.

Lijun Wang

Dr Lijun Wang is current Team Leader of NanoBiology research group in the Institute for Medical Science and Technology (IMSaT) at the University of Dundee, UK. She received her B.S. degree and MD from Peking University Health Science Centre, China, and the PhD in Molecular Physiology from the University of Dundee. She held a lectureship at Peking University Health Science Centre before she joined the University of Dundee. Dr Wang has broad knowledge and research experience in Cell Biology and Biochemistry including cell signalling and intracellular traffic, regulation of cell growth and cell death, and regulation of cell metabolism. Over the last few years Dr Wang has switched her research interest to the ever fast emerging and expending nanoscience and nanotechnology area and her research group is focused on the biology, toxicity and application of nanostructures in drug delivery, regenerative medicine, and diagnostic & therapeutic imaging.

RECENT SELECTED PUBLICATIONS

• Hoskins C, Min Y, Gueorguieva M, McDougall C, Volovik A, Melzer A, Cuschieri A, and **Wang L**. (2012) Hybrid gold-iron oxide nanoparticles as a multifunctional platform for biomedical application. J Nanobiotechnol. (Under review)

• Liu D, **Wang L**, Wang Z, and Cuschieri A. (2012) Different cellular response mechanisms contribute to the length-dependent cytotoxicity of multi-walled carbon nanotubes. Nanoscale Res Lett. (Under review).

• Riggio C, Calatayud MP, Hoskins C, Pinkernelle J, Sanz B, Torres TE, Ibarra MR, **Wang L**, Keiholf G, Goya GF, Raffa V, and Cuscheri A. (2012) Poly-l-lysine-coated magnetic nanoparticles as intracellular actuators for neural guidance. Int J Nanomed. (In press)

• Hoskins C, Cuschieri A, and **Wang L**. (2012) Cytotoxicity of polycationic iron oxide nanoparticles: Common endpoint assays and alternative approaches for improved understanding of cellular response mechanism. J Nanobiotechnol. (In press)

• Chen S, Hoskins C, **Wang L**, MacDonald MP, and Andre P. (2012) A Water-Soluble Temperature nanoProbe based on a Multimodal Magnetic-Luminescent nanoColloid. Chem Commun. 48:2501.

• Hoskins C, **Wang L**, Cheng W-P, and Cuschieri A. (2012) Dilemmas in the reliable estimation of the in-vitro cell viability in magnetic nanoparticle engineering: which tests and what protocols? Nanoscale Res Lett. 7:77.

• Gourevich D, Gerold B, Arditti F, Xu D, Liu D, Volovick A, **Wang L**, Medan Y, Gnaim J, Prentice P, Cochran S and Melzer A. (2012) Ultrasound activated nano-encapsulated targeted drug delivery and tumour cell poration. Adv. Exp. Med. Biol. 733:1350.

• Chen S, **Wang L**, Duce S, Brown SI, Lee S, Melzer A, Cuschieri A and Andre P. (2010) Engineered biocompatible nanoParticles for in-vivo imaging applications. J Am Chem Soc 132:15022.

• **Wang L**, Wang Z, Frank TG, Brown SI, Chudek JA and Cuschieri A. (2009) Rapid and efficient cell labelling with MRI contrast agent by electroporation in the presence of protamine sulphate. Nanomed (Lond) 4:305.

Zsoka Weiszhar

She graduated as a molecular biologist with MSc degree in immunology at the Eotvos Lorand University (Budapest, Hungary) in 2003. She completed her PhD studies at the Semmelweis Medical University (Budapest, Hungary) in 2010, and is currently preparing her PhD thesis about the role of complement system in different allergic processes including nanomedicine induced pseudoallergy. Formerly, she managed the HLA typing diagnostic and research unit of the National Insitute of Rheumathology (Budapest, Hungary). She obtained a 3-year research fellowship at the 3rd Department of Internal Medicine of Semmelweis University and a short-time DAAD fellowship in Bayreuth, Germany. She became experienced in clinical studies as coordinator / subinvestigator on asthma and COPD at the Pulmonology Clinic of Semmelweis University (Budapest, Hungary).

Since 2008, she has been working at the In vitro Immunotoxicology Department of SeroScience Ltd, where her task is to investigate in vitro complement reactogenicity of different parenteral drug compounds, nanomedicines and experimental nanostructures.

Pierre-Léonard Zaffalon

521, rue des Ecureuils
F-74520 Valleiry
+ 33 (0)6 07 26 36 44
zaffalo4@etu.unige.ch

PERSONNAL DETAILS

Born September 5, 1986 in Geneva, Switzerland. Swiss and French citizenship.

EDUCATION

• Since April 2009 Ph.D. thesis in bioorganic chemistry under the supervision of Dr. Andreas Zumbuehl.
Ph.D. thesis title: Synthesis of Artificial Phospholipids (Thesis defense: expected in December 2012).

• 10/2004-03/2009 Master in Chemistry, University of Geneva, Switzerland.
Organic Chemistry Supervisors: Dr. Andreas Zumbuehl, Prof. Stephan Matile.
Master thesis title: The Synthesis of 1-Hexadecylphosphocholine.

• 2001-2004 Secondary School, Saint-Julien-en-Genevois, France.
Scientific Baccalaureate (distinction: very good).

PUBLICATIONS

• F. Loosli, D. Alonso Doval, D. Grassi, **P.-L. Zaffalon**, F. Favarger, A. Zumbuehl Chem. Comm., 2012, 48, 1604-1606.

• I. A. Fedotenko, M. N. Holme, R. Tanasescu, **P.-L. Zaffalon**, A. Zumbuehl Chimia, 2011, 65, 859-862.

• **P.-L. Zaffalon**, E. Stalder, I. A. Fedotenko, F. Favarger, A. Zumbuehl Tetrahedron Lett., 2011, 52, 4215-4217.

• **P.-L. Zaffalon**, Andreas Zumbuehl Synthesis, 2011, 778-782.

• I. A. Fedotenko, **P.-L. Zaffalon**, F. Favarger, A. Zumbuehl Tetrahedron Lett., 2010, 51, 5382-5384.

INVITED/CONTRIBUTED TALKS

• **Pierre L. Zaffalon** "Study of Hydrogen Bond on the Assembly and Packing of Acylethanolamine Derivatives", Geneva Chemistry & Biochemistry Days 2012, University of Geneva, Geneva (Switzerland), January 20, 2012.

• **Pierre L. Zaffalon** "Synthesis of Artificial Phospholipids", Oppolzer Lectures 2011, University of Geneva, Geneva (Switzerland), January 28, 2011.

• **Pierre L. Zaffalon** "A Versatile Reagent for Phospholipids Synthesis", TiTech/UniGE Symposium 2010, University of Geneva, Geneva (Switzerland), November 25-26, 2010.

INTERVENTION ABSTRACTS FOR CLINAM 5/12

SIGNIFICANCE OF ANGIOGRAPHY FOR CANCER THERAPY WITH MAGNETIC NANOPARTICLES

CHRISTOPH ALEXIOU [1], S. Lyer1[1], S. Dürr1[1],R. Tietze1[1], Jenny Mann[1], E. Schreiber[1], T. Engelhorn[2], T. Struffert[2], A. Dörfler[2]

[1]ENT-Department, Section for Experimental Oncology and Nanomedicine (SEON), University Hospital Erlangen, 2Department of Neuroradiology, University Hospital Erlangen

INTRODUCTION

Magnetic nanoparticles are used for diagnostic as well as therapeutic purposes in medicine. The visualization of the particles on the one hand and their carrier-function for a focused application of pharmaceutics on the other hand are crucial components for tumor therapy with Magnetic Drug Targeting (MDT).
In this study the vascularization of the tumor and the body region, where the tumor was situated, was examined by flat-panel angiography before, during and after the application of MDT.

MATERIAL AND METHODS

In this study 10 animals, with VX2-tumors implanted subcutaneously at the left hind limb, were treated with Magnetic Drug Targeting. For the visualization of the tumor supplying vasculature and the respective tumors, flat-panel-angiography and -CT (Artis zee floor and syngo DynaCT, Siemens Healthcare, Forchheim, Germany) was performed directly before, during and after the application of chemotherapeutic-bound iron oxide nanoparticles (Fig. 1).

RESULTS

Comparing the animals, tumor vascularization was very heterogeneous. In most of the cases the tumors were supplied from the Arteria femoralis and the Arteria saphena and showed highly different intratumoral vessel architectures.

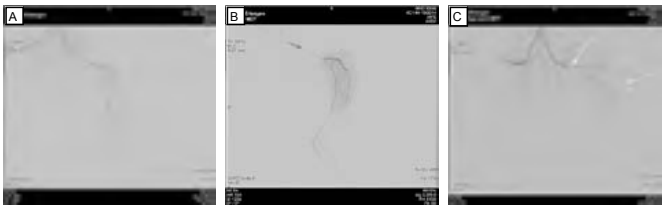


Fig. 1: 2D-Angiography of the hind limbs (New Zealand White rabbits) A) before MDT, B) during MDT with arterial access and C) after the application.

CONCLUSION

The tumor supplying vasculature is a very important parameter for a successful tumor therapy with magnetic particles by MDT. Therefore it is crucial to get detailed knowledge about the location of the tumor and its supplying vessels in advance of the intraarterial application and the adequate positioning of the external magnetic field.

ACKNOWLEDGEMENTS

This study was supported by the Else Kröner-Fresenius-Stiftung, Bad Homburg v.d.H. and the Spitzencluster Medical Valley of the German Ministry of Education and Science (FKZ: 01EX1012B).

NANOPARTICLES FOR THE THERAPY AND DIAGNOSIS OF ALZHEIMER'S DISEASE

DAVID ALLSOP

Prof.; Division of Biomedical and Life Sciences, Faculty of Health and Medicine, Lancaster University, Lancaster LA1 4YQ, UK.

There are currently 35 million people worldwide who suffer from Alzheimer's disease (AD) and this number is set to rise to around 120 million by 2050. Current treatments have only limited symptomatic benefits and are not targeted at underlying primary disease mechanisms. Considerable evidence suggests that the conversion of the Aβ peptide from monomer into aggregated forms in the brain is a key event in the pathogenesis of AD. Inhibition of Aβ aggregation, especially in its early stages, could have a major impact on the progression of this disease. We are working on nanoparticles as a vehicle for transporting Aβ aggregation inhibitors into the brain. One of these inhibitors is curcumin, which is being pursued within the 'NAD' Project, financed by the EU Framework 7 Program (agreement no: CP-IP 212043-2) which is co-ordinated by Prof. Massimo Masserini of University of Milano-Bicocca, Italy. A second inhibitor, being pursued at Lancaster University, is based on a retro-inverso peptide (see Reference 2) modified for brain penetrance. These nanoparticle derivatives have proved to be very effective inhibitors of Aβ aggregation. In principle, they could also be modified for use as imaging tools. We have found that liposomes derivatized with the retro-inverso peptide are particularly potent inhibitors of both Aβ oligomer and fibril formation. There have been some high profile failures of various drugs candidates aimed at inhibiting the formation or aggregation of Aβ in recent years. Our nanoparticle-based therapies are an alternative approach to more conventional drugs that will soon enter animal testing and can hopefully succeed in human clinical trials.

1. Taylor M., Moore S., Mourtas S., Niarakis A., Re F., Zona C., Ferla B., Nicotra F., Masserini M., Antimisiaris S.G., Gregori M. & Allsop D. (2011) Effect of curcumin-associated and lipid ligand functionalised nanoliposomes on aggregation of the Alzheimer's Aβ peptide. Nanomed: Nanotech. Biol. Med. 7, 541–550.
2. Taylor M., Moore S., Mayes J., Parkin E., Beeg M., Canovi M., Gobbi M., Mann D.M.A. & Allsop D. (2010) Development of a proteolytically stable retro-inverso peptide inhibitor of β-amyloid oligomerization as a potential novel treatment for Alzheimer's disease. Biochemistry 49, 3261–3272.

MASTERING THE DEVELOPMENT OF VIROSOMES FOR VACCINATION

MARIO AMACKER

Pevion Biotech AG, Worblentalstrasse 32, CH-3063 Ittigen, Switzerland

Pevion's virus-like particle (VLP) vaccine technology, called virosomes, is designed specifically for the development of safe and effective subunit vaccines. Virosomes are particularly well suited for addressing new vaccine indications. Pevion has the in-house expertise and capability to develop its candidate vaccines according to industry standards, including a state-of-the-art and scalable GMP manufacturing process. The transition of our current vaccine candidate against recurrent vulvovaginal candidiasis (RVVC) from the bench-scale to the CTM production will be reviewed, with focus on upscaling and change implementation.

NANOPARTICLES IN CANCER IMAGING

THOMAS L. ANDRESEN

Technical University of Denmark, Center for Nanomedicine and Theranostics, Department of Micro- and Nanotechnology, 2800 Lyngby, Denmark. Thomas.andresen@nanotech.dtu.dk

Nanoparticles are well established as effective drug delivery systems in vivo and have potential in biomedical imaging as a diagnostic tool. We have recently developed a highly efficient method for utilizing liposomes as imaging agents for positron emission tomography (PET) giving high resolution images and allowing direct quantification of tissue distribution and blood clearance. Our approach is based on remote loading, of a copper-radionuclide (64Cu), into preformed liposomes and copper entrapment by an encapsulated copper-chelator. We show that the 64Cu-liposomes provide quantitative in vivo imaging of healthy and tumour-bearing mice using PET. We also studied the liposome systems in canines with spontaneous tumors and showed that 64Cu-liposomes have potential as a

new diagnostic tracer in cancer diagnostics. The study showed that the EPR effect is substantial in a number of spontaneous tumors in canines. The 64Cu-liposomes resulted in very high tumor to muscle ratios and promising tumor to liver ratios in relation to using these systems as a diagnostic tool in the clinic. We further envision that 64Cu-liposomes will be an important tool for evaluating liposome performance in future and may become an important tool in selection of cancer patients for nanoparticle based chemotherapy.

ANALYSIS OF MICRO- AND NANO-SIZED PARTICLES WITH A TUNABLE NANOPORE SENSOR FOR THE PRECISE ENGINEERING OF DRUG DELIVERY SYSTEMS FOR NANOMEDICINE APPLICATIONS

DIMITRI AUBERT

Ph.D., Director Izon EMEA
Izon Science Ltd. Begbroke Centre for Innovation & Enterprise, Oxford University Begbroke Science Park, Sandy Lane, Yarnton, OX5 1PF, United Kingdom, E-mail: dimitri.aubert@izon.com
Fax: +44-1865-598-999 Tel: +44-1865-309-666
Web: www.izon.com

Precise measurements, with particle-by-particle detail, of a range of engineered and biological particles, i.e. liposomes, PLGA, lipids, micelles, virus-like-particles, polymers, viruses, bacteria, protein-conjugates, exosomes, vesicles will be described.

Particles are transported through a flexible pore via electric field and/or with pressure, for rapid and detailed determination of particle concentration (particles/mL for dosage), accurate size, aggregation levels, size distribution and relative surface charge distribution, all determined simultaneously.

Experimental parameters are adjusted in real-time for mapping how different populations within particle mixtures respond to externally applied conditions for high-resolution and powerful analysis of particle physical properties and their dynamic behaviour, i.e. to assess the level of surface modification (PEG-lyation) of drug delivery carriers.

The ability to individually interrogate each particle addresses the shortcomings of ensemble systems such as dynamic light-scattering and also of static systems using electron microscopy.

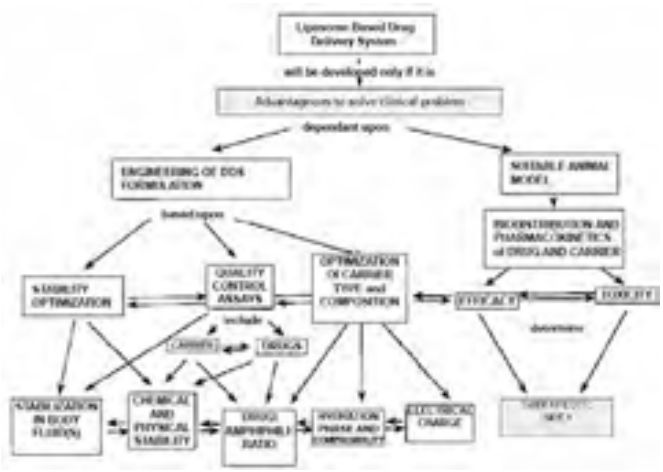
This also enables the quantification of the dynamic behaviour of particle mixtures, such as aggregation/fragmentation of particles, and surface modification changes to particles. Research work utilizing tunable pore sensors in virus quantification, pathogen interaction dynamics, medical diagnostics and drug delivery systems are presented.

ENABLING FULL UTILIZATION OF LIPOSOME-BASED THERAPIES

YECHEZKEL (CHEZY) BARENHOLZ

Laboratory of Membrane and Liposome Research, IMRIC, Hebrew University-Hadassah Medical School, Jerusalem 91120, Israel

The anticancer drug Doxil® (doxorubicin in liposomes) was the first liposomal drug and the first nano-drug approved by the US FDA (November 1995). Liposomes' "history" started in 1965 when they were first described by Dr. A.D. Bangham. Then liposomes were proposed and used as the main model for biological membranes. Five years later, due to their biocompatibility and versatility, combined with their ability to encapsulate and carry a broad spectrum of drugs, they were proposed as a promising drug delivery system. However, due to major scientific and technical issues, it took liposomes >25 more years to mature into >12 FDA-approved drugs. Based on lipid composition, size distribution, and morphology, liposomes can fit a broad spectrum of medical applications. While long-circulating nano-liposomes with high drug retention are superior for systemic treatments, larger conventional liposomes are better for local treatments. Today there is extensive evidence from animal and human studies that for systemic treatment of diseases in which the vasculature at the disease site is leaky and porous, long-circulating nano-liposomes, when loaded with suitable drugs, enable passive targeting to the disease site, referred to as enhanced permeability and retention (EPR) effect which may result in unique therapeutics, as was demonstrated for Doxil in cancer therapy (rev. in Barenholz, Y., J. Controlled Release, in press, 2012). The need to use nano-size liposomes introduces the major drawback of too low a drug-to-lipid ratio and therefore a drug dose that is below the therapeutic level. This was overcome by the use of remote (active) drug loading, while long circulation time and RES avoidance was achieved by using pegylated nano-liposomes. The requirements and considerations for designing liposomes to be used for local treatments, such as prolonged local analgesia, are very different, as long retention time at the site of administration is needed, combined with slow but sufficient controlled drug release. In both systemic and local treatments the success in fabrication of efficacious and tolerable liposomal formulations requires a multidisciplinary approach combined with optimal utilization of the disease's biological "Achilles' heel". The multidisciplinary approach requires an extensive in-depth knowledge of the relevancy of the following parameters: (i) lipid biophysics, which includes the application of the concept of lipid "additive packing parameter", lipid phase structure, which includes manner of packing, and lipid head group hydration, and (when needed) steric stabilization -- all these are obligatory to enable optimal selection of lipid for liposome fabrication; (ii) how to optimize selection of drugs based on their suitability to be efficiently loaded into, and released from, the desired liposomes; (iii) how fit the liposomes used are to the biological needs and to the anatomy and physiology related to the specific disease. A cross-talk between the above parameters, pharmacy, pharmacology, and medicine is a must.
The way we approach such a complex system that summarizes all the preclinical studies required is described by the following Concept Map (prepared with the help of Dr. Hanna Barenholz).



The presentation will highlight a few different liposomal drugs based on nano- and micro-size liposomes designed and formulated in Barenholz's Lab and show how their therapeutic performance is related to the above parameters.

DOXIL®: FROM GENERICS TO IMPROVED THERAPEUTICS

YECHEZKEL (CHEZY) BARENHOLZ

Laboratory of Membrane and Liposome Research, IMRIC, Hebrew University-Hadassah Medical School, Jerusalem 91120, Israel

Doxil®, the first FDA-approved nano-drug (1995), is based on three unrelated principles: (i) prolonged drug circulation time and avoidance of the RES due to the use of PEGylated nano-liposomes; (ii) high and stable remote loading of doxorubicin driven by a transmembrane ammonium sulfate gradient, which also allows for drug release at the tumor; and (iii) having the liposome lipid bilayer in a "liquid ordered" phase composed of the high-Tm (53 ºC) phosphatidylcholine, and cholesterol. The latter helps to maintain the gradient

and also enable to achieve a zero order slow drug release at the tumor site. Due to the EPR effect, Doxil is "passively targeted" to tumors and its doxorubicin is released and becomes available to tumor cells by as yet unknown means. Doxil success demonstrates the obligatory need for applying an understanding of the cross talk between physicochemical, nano-technological, and biological principles. However, in spite of the large reward, ~2 years after Doxil-related patents expired, there is still no FDA-approved generic "Doxil" available. The reason for as yet lack of Doxil-like approved generics will be discussed.

In retrospective it is obvious that in spite of Doxil success story it has few side effects (especially Foot and Hand syndrome) that if overcome or reduced will be highly beneficial and may improve Doxil performance.

The reasons for these side effects and means to reduce them will also be discussed.

LIPOVITO, A NOVEL 2 CHEMOTHERAPEUTICS IN 1 LIPOSOME ANTI-CANCER NANO-DRUG: FROM COMPUTATION AND MODELING TO DESIGN AND ANIMAL STUDIES

YECHEZKEL BARENHOLZ[A,*], Daniel Zucker[&a], Alexander Andrianov[&a], Ariel Steiner[&b] and Uri Raviv[b,*]

[a] Department of Biochemistry and Molecular Biology, IMRIC, The Hebrew University-Hadassah Medical School, Jerusalem 91120, Israel [b] The Institute of Chemistry, Edmond J. Safra Campus, Givat Ram, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

It is obvious today that a single drug cannot cure cancer, and therefore improving cancer therapy and progressing toward cancer cure requires combined therapy using two or more drugs in a way that the therapeutic effect will be synergistic. However, for dugs given as is (the conventional way) the chances to achieve this goal are slim, as each of the drugs has its own bio-fate. Encapsulation of two drugs in one long circulating nano-liposome may overcome this major drawback as such a liposome can take advantage of the EPR effect and accumulate at the tumor site loaded with the two desired chemotherapeutics. If the long circulating nano-liposomes are designed well the drugs' release can be controlled to achieve the desired release kinetic order and rate (see Fig. 1) so that the efficacy of the combination therapy will be optimal.

Based on in silico research and modeling, we selected the combination of topotecan (TPT) and vincristine (VCR). The ratio-dependent synergy between these two drugs was evaluated, focusing on the in vitro - in vivo correlation (IVIVC).

The interaction between the drugs was evaluated in tissue culture by median effect analysis. Certain ratios of combined drugs were synergistic, whereas others were antagonistic, implying that the most efficacious combinations should be at a specific fixed drug ratio. For in vivo evaluation, long-circulating pegylated nanoliposomes co-remote-loaded simultaneously with both drugs by transmembrane ammonium sulfate gradient were developed. VCR and TPT were successfully co-encapsulated at therapeutically relevant levels as two drugs in one long circulating nanoliposome combination (LipoViTo). These liposomes were characterized in vitro for their size distribution, morphology (by cryo transmission electron microscopy) drug to lipid mole ratio, intraliposome drug phase (using cryo transmission electron microscopy and X ray diffraction (using both SAXS and WAXS), studying kinetic order and rate of the release of each of the two remote loaded drugs.

The nanoliposomes controlled the drugs' "biofate" and maintained a fixed drug ratio in vivo, allowing us to explore the IVIVC. Pharmacokinetics and biodistribution studies showed that LipoViTo delivers the two drugs simultaneously to the tumors, where they are released at a predefined zero-order kinetics, at the desired drug ratio and a slow rate (Fig.1).

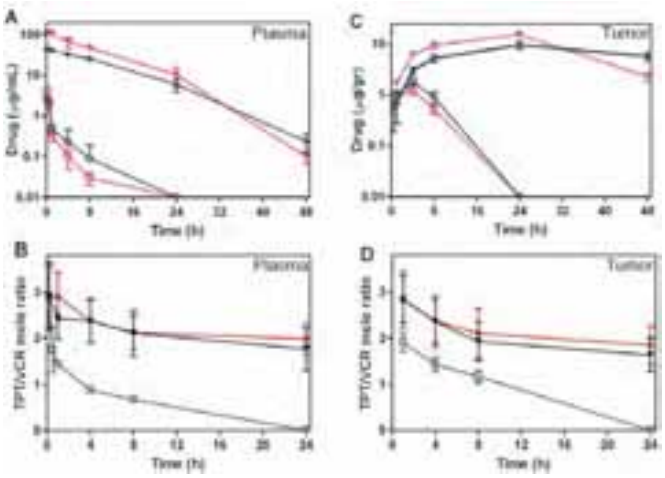


Fig 1. Concentrations and ratios of TPT and VCR in mice with Daoy tumors, after i.v. administration. A and C: Concentrations of TPT and VCR after administration of free TPT 10 mg/kg (□), free VCR 2 mg/kg (○), nSSL-TPT 5 mg/kg (▼) and nSSL-VCR 2 mg/kg (♦) in plasma (A) and tumor (C). B and D: Drug mole ratios in plasma (B) and tumor (D) for TPT and VCR after simultaneous i.v. administration of both drugs in a TPT/VCR mole ratio of 2.9. Three modes of administration were compared: free drugs (□), LipoViTo (●), and a mixture of nSSL-TPT with nSSL-VCR (▼). From Zucker and Barenholz JCR, 2010.

LipoViTo were more efficacious than the free drugs and liposomes with one agent, singly or in combination, in two tumor models in mice. Toxicity studies in mice revealed good tolerability of the LipoViTo (For more details see Zucker et al J controlled Release 2010 & 2012).

To summarize: chemotherapy regimens can be improved by co-encapsulation of two drugs' combination in long circulating pegylated nanoliposomes. There is low correlation of the synergy ratio dependence between the in vitro and the in vivo (low IVIVC).

SUBMICRON (NANO) EMULSIONS IN DRUG TARGETING AND DELIVERY IN OPHTHALMOLOGY

SIMON BENITA[1], Tal Hagigit[1], Muhammad Abdulrazik[1], Francine Behar-Cohen[2], Frederic Lallemand[3] and Jean-Sebastien Garrigue[3]

[1] The Institute for Drug Research of the School of Pharmacy, Faculty of Medicine The Hebrew University, Jerusalem, Israel . [2]INSERM, U872, Team 17, Université René Descartes Sorbonne Paris Cité, [3]Novagali Pharma SA, Evry Cedex, France.

Ophthalmic diseases are most commonly treated with a topical eye drop instillation comprised of conventional aqueous solutions. In addition to being limited to watersoluble molecules, these hydrophilic solutions are rapidly eliminated via the nasolacrimal drainage, resulting in poor ocular penetration of the drug and reducing it to less than 3% of the administered dose. Consequently, novel pharmaceutical approaches should be sought to overcome these drawbacks and expand the range of potential active ingredients (including those of a lipophilic nature) which remain longer on the ocular surface, provide sustained therapeutic concentrations, and also meet the regulatory criteria for market approval. Nanotechnologies which have shown potential to circumvent ocular barriers and facilitate the transport of active ingredients through the ocular tissues are currently considered the best solution for improving the ocular delivery of ophthalmic drugs, even though products reaching the market are still rare. This is probably due to the regulatory requirements and technical obstacles encountered in the scale-up and manufacturing processes of these complex and sophisticated dosage forms. Nevertheless, nanotechnology remains a promising approach for ophthalmic drug delivery. Compared to currently available approaches for administering eye drops, nanocarriers with bioadhesive properties (e.g. cationic nanoemulsions) are more efficient at delivering the appropriate concentrations of bioactive molecules to the eye. The mechanism

underlying the bioadhesiveness of nanocarriers is an electrostatic interaction which prolongs the residence time on the ocular surface. To create such an electrostatic interaction with the negatively-charged cells of the ocular surface, the carrier should be positively charged. This is the essence and advantage of the innovation of the cationic nanoemulsion technology developed in our laboratory and licensed to Novagali Pharma more than a decade ago.

The first stage of development by Novagali, following an initial proof-of-concept study carried out at the School of Pharmacy of the Hebrew University of Jerusalem, was to formulate the nanoemulsion with a cationic agent, an oil phase and surfactant compliant to US and EU pharmacopeias. Most recently, Lallemand and Colleagues published a review (1) describing the main steps in the successful development of cationic nanoemulsions from formulation to evaluation in clinical trials. A major challenge of the formulation work was selecting a cationic agent with an acceptable safety profile that would ensure a sufficient ocular surface retention time. Then, toxicity and pharmacokinetic studies were performed to show that the cationic emulsions were safe and well tolerated. Even in the absence of an active ingredient, cationic emulsions were observed in preclinical studies to have an inherent benefit on the ocular surface. Moreover, clinical trials demonstrated the efficacy and safety of cationic emulsions loaded with cyclosporine A in patients with dry eye disease. Ongoing studies evaluating latanoprost emulsion on patients with ocular surface disease and glaucoma suggest that the beneficial effects on reducing ocular surface damage may also extend to this patient population. The culmination of these efforts has been the marketing of Cationorm®, a preservative-free cationic emulsion indicated for the symptomatic treatment of dry eye. The primary relevant results will be presented.

It is now established that appropriate cationic nanoemulsion formulations can sustain the ocular delivery of lipophilic molecules and improve their efficacy. Attention and efforts are now being concentrated in exploiting the ocular therapeutical potential of cationic nanoemulsions as nanocarriers of polyanionic macromolecules such as siRNAs and antisense oligonucleotides (ODNs) for the treatment of severe eye diseases. Age-related macular degeneration (AMD) is the most common cause of vision loss in the elderly in the western world and its prevalence increases with age. It is characterized by the appearance of drusen on the macula, accompanied by choroidal neovascularization or geographic atrophy. Anti-angiogenic ODNs are considered important therapeutic macromolecules for treating AMD. However, ODN-based therapy is compromised by the rapid degradation of ODNs in biological fluids and by their inability to efficiently cross cellular membranes due to their hydrophilic and polyanionic character and large molecular structure. There is a need for efficient ODN delivery to intraocular tissues. The local permeability of ODNs into the eye can be improved by combining them with innovative cationic nanoemulsion formulations (2) as illustrated in Fig.1. Antisense oligonucleotides (ODNs) specific for VEGFR-2-(17 MER) and inhibiting HUVEC proliferation in vitro were screened for improved AMD treatment. One efficient sequence was selected and incorporated in different types of nanoemulsions, the potential toxicity of which was evaluated on HUVEC and ARPE19 cells (2).

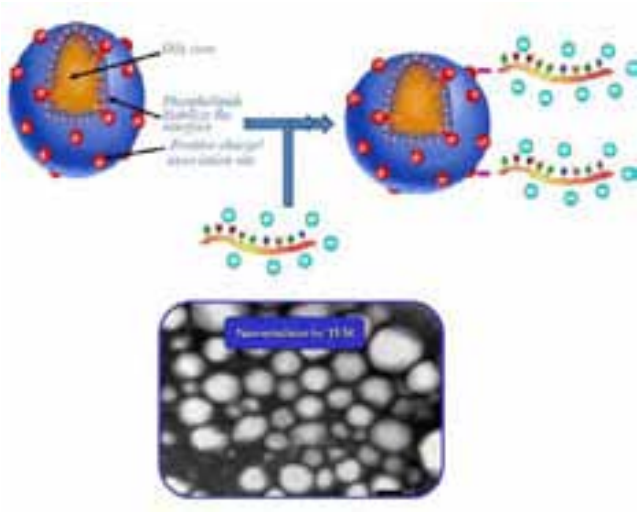


Fig. 1: TEM morphological characterization of cationic nanoemulsion constituted from internal oil droplets at the nano scale range dispersed in a continuous aqueous phase. The ion paired complex formation with antisense oligonucleotide is mediated by an electrostatic attraction as reported in reference 2.

Our results showed that below 10 µl/ml, a 2.5% mid-chain triglycerides cationic DOTAP nanoemulsion was non-toxic on HUVEC and retinal cells. This formulation was therefore chosen for further experiments. In-vitro transfection of FITC ODN17 in ARPE cells using DOTAP nanoemulsions showed that nanodroplets do penetrate the cells. Furthermore, ODN17 is released from the nanoemulsion after 48 h and accumulates in the cell nuclei. The biofate and tissue distribution of ODN17-associated cationic nanoemulsions following local and intravitreal administration have been carried out. Marked therapeutic ODN17 levels in the retina and other targeted tissues were detected 72 hours post intravitreal injection (3). It was also shown that ODN17 is protected from degradation for at least 72 hours post injection.

The efficiency of the ODN nanoemulsion in a pharmacological corneal neovascularization rat model and in a mouse model of retinopathy of prematurity (ROP) was assessed. High significant corneal neovascularization inhibition efficiency was elicited (Fig. 2). In the ROP mouse model, the ODN17 in PBS induced a 34% inhibition of retinal neovascularization when compared to the aqueous-vehicle-injected eyes. A significantly higher inhibition of vitreal neovascularization (64%) was observed in the group of eyes treated with ODN17 nanoemulsion. No difference of neovascularization was observed between blank nanoemulsion, scrambled ODN17 nanoemulsion, vehicle or non-treated eyes (2).

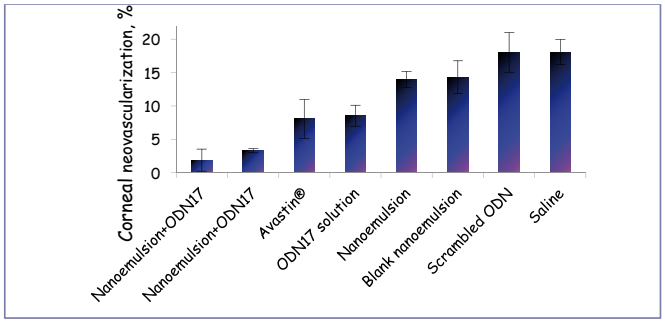


Fig. 2: Effect of various formulations on the cornea vascularization following induction of corneal neovascularization using a silver nitrate cauterization technique. Topical application of 50 µl from various formulations immediately following cauterization. Corneal photographs were taken on the 7th day and quantitatively analyzed using NIH imageJ software (N=6 rats). Data were statistically analyzed by one way ANOVA (P<0.01) as reported in reference 2.

The overall results indicate that cationic nanoemulsions can be considered a promising potential ocular delivery system and an effective therapeutic tool of high clinical significance in the prevention and forthcoming treatment of ocular neovascular diseases.

REFERENCES

• 1. F. Lallemand, P. Daull, **S. Benita**, R. Buggage and J-S Garrigue. Successfully improving ocular drug delivery using the cationic nanoemulsion, Novasorb. J. Drug Del., doi:10.1155/2012/604204.

• 2. T. Hagigit, M. Abdulrazik, F. Valamanesh, F. Behar-Cohen and **S. Benita**. Ocular antisense oligonucleotide delivery by cationic nanoemulsion for improved treatment of ocular neovascularization: An in-vivo study in rats and mice. J. Control. Rel., doi:10.1016/j.jconrel.2011.11.022.

• 3. T. Hagigit, M. Abdulrazik, F. Orucov, F. Valamanesh, M. Hagedorn, G. Lambert, F. Behar-Cohen and **S. Benita**. Topical and intravitreous administration of cationic nanoemulsions to deliver antisense oligonucleotides directed towards VEGF KDR receptors to the eye, J. Control. Rel., 145, 297- 305 (2010).

DIAGNOSTIC NANO-INTERFACES FOR CANCER DETECTION

FRANÇOIS BERGER*°, A Bouamrani°, Affyf Zaccharia°, C Salas°°,M Charavel°°, C N Dempsey***, F Dumas Bouchiat***, D Givord***, M Cosnier*, P Caillat*, R campagnollo*, D Ratel
CEA-LETI*-INSERM°-UJF+-CHU Grenoble- CNRS Louis NEEL***- °°LIP/PC2S- CLINATEC

Availability of molecular biomarkers usable for early detection and indicative of prognosis and therapeutic response prediction is a clinico-biological priority. The availability of a non invasive strategy to catch the low concentrated/and or tissue proteomic compartments as well as to none invasively monitor tissue response to therapy is a crucial issue. In the medico-biological state of the art, this is by now performed by invasive biopsy, and the success for the validation of non-invasive peripheral biomarkers has been low. The existence of major serum proteins, the large dynamic range of blood proteins as well as sample prep difficulties explain the relative failure of peripheral biomarkers discovery. Similarly, peritumoral tissues remain poorly accessible making difficult the annotation of the crucial "environment relapse" mechanisms and related targets.
A technological breakthrough is essential for that, which we provided through the introduction of specific micro/nano devices and strategies devoted to in vivo biomarker harvesting.
We developed the protool strategy implementing a direct micro-invasive but non-lesional bio-harvesting approach. Enhanced properties were obtained using silicon materials, surface chemistry and micro-nanostructuration. Preclinical toxicology was demonstrated as well as proof of concept in big animal models. The initial device was developed for brain pathology and extended to several other tumor locations.
Micro-beads harboring peptides libraries, as well as an alginate micro-nano-bioreactor was developed to catch the low concentrated proteome in pathological fluids such as serum or CSF. We also explored at the preclinical level the potential of biomarker harvesting using magnetic nano-beads harboring specific surface modifications. A consortium of biologists, technologists, nanoscientists and physicians provided synergistic competencies to reach these objectives.
This work paved the way to go deeper in biomarker harvesting, supporting the concept of "biomarker tissue-interface" and demonstrating the importance of micro-nanotechnologies in this field. Moreover, new strategies are now available to re-invent the old blood puncture and to explore pathological area providing to opportunity to find new theranostic biomarkers and targets for therapy.
Validating safely and quickly innovative technologies at the interface with the human body is mandatory. The development of new proof of concept "early phase" trial strategies needs to be supported, using multimodality in specific clinical trial environments. An adequate patient psycho-societal and acceptance evaluation is also mandatory.

NEUROTECHNOLOGY AND NETWORK DYNAMICS: NEUROCARE

PHILIPPE BERGONZO
Project Coordinator

Medical implants can repair the nervous system following an accident or disease, notably to correct the loss or impairment of eyesight (through retinal degeneration) or hearing (through damaged cochlea). Traumatic spinal injuries, drug-resistant epilepsies, psychiatric disorders and chronic neurodegenerative pathologies can also be treated (in the cortex) with such reconstructive approaches. NeuroCare aims to create better retinal, cortical and cochlear implantable devices through the use of improved interfacing between the electronic implants and living cells.
The NeuroCare concept involves high-quality, low-cost, carbon-based materials, used as therapeutic neuro-interfaces, namely nanocrystalline diamond and graphene. These materials are well-adapted for use in medical implants, because they (i) offer a wide range of electronic properties (metal, semiconductor and insulator), (ii) are bio-inert, (iii) can be used on flexible substrates and (iv) are physically robust. The project starts spring 2012 but does benefit from the notable advances developed during the DREAMS-FP6 project, that included the fabrication of diamond-based micro-electrodes for retinal implants (DREAMS – FP6).

STATE OF THE ART IN TARGETING LIGANDS

ROY BICKNELL

Different approaches to targeting will be discussed. Most utilise antibodies but there are other opportunities such as aptamers. Modification of antibodies can be very diverse. Usually antibodies are modified for a particular application such as imaging or therapy. These can include anything from radionuclides, photosensitizers, to microbubbles and nanoparticles. The various approaches will be reviewed with appropriate recent examples taken from the literature.

IMAGE ANALYSIS, AN INDISPENSABLE TOOL FOR THE FUTURE OF MEDICINE

GERD BINNIG

In clinical routine many different applications nanotechnological methods are well established, although in most cases they are not classified as such. Some of them are based on nature's nanotechnology and therefore were developed in the framework of cell biology, systems biology or molecular diagnostics. Many different methods in histopathology represent such cases where certain proteins or genes in the tissue are labelled by antibodies or other molecules which act as "intelligent" nano-probes. The binding of such nano-probes even when imaged with relatively low resolution techniques (e.g. optical or PET) still represents the visualization of nano-functionality. These methods have contributed to great progress in the understanding and diagnosis of cancer. However, most of these images are inspected visually. Digitalization of images in radiology is common practice and is becoming increasingly common in pathology. This digitization presents an opportunity to add a new dimension to healthcare. Through intelligent quantification image analysis can unveil the tremendous information hidden in all kind of medical or biological images. In a way image analysis can be viewed as a conversion of arrays of pixels, i.e. images, into minable data. Modern data mining tools correlate those data with other data like gene expression data or clinical outcome data and in this way create new biological and medical knowledge. The knowledge created can be used to develop more advanced labelling and analysis methods and again new data are available for data mining, and so on. A prerequisite for this conversion of medical images into minable data is the use of context driven image analysis methods that can deal with complex images.

I-ONE - BIO-ARTICIFICIAL ORGANS

FABIO BISCARINI
Prof. Dr.; Project Coordinator

I-ONE is focused on exploiting for flexible organic electronics for the development and testing of Active Multifunctional Implantable Devices (AMIDs) to treat Spinal Cord Injury (SCI). The use of flexible organic electronics devices will advance the state-of-the-art of implantable devices for SCI from passive to active layouts that will promote nerve regeneration by a combination of local stimuli delivered on demand, will sense inflammation, and will control the immune-inflammatory response. The biomedical impact of the project will be demonstrated in vitro and in vivo. In vitro, the neural therapeutic plasticity induced by the I-ONE device will be evaluated on stem cells, which will be differentiated to neural progenitor cells, and then to neural cells. In vivo, the study of neural plasticity will

be transferred to endogeneous stem cells by implanting the I-ONE device into a contusion SCI animal model. I-ONE will acquire the knowledge and the technology required to regenerate the nerve in the niche of the injury.

COMBINING LIGHT AND TARGETED NANOPARTICLES IN ONCOLOGY – THE TARGET-PDT EUROPEAN PROJECT APPROACH

PATRICK BOISSEAU[1], A-C Couffin[1], F. P. Navarro[1], C. Frochot[3,4], T. Bastogne[2,4], S. Gräfe[5], A. Weihe[5], I. Texier[1], F. Vinet[1], C. Maake[6], H. Walt[6], V. Albrecht[5], B. Fuchs[7] and M. Barberi-Heyob[2,4]

[1] CEA LETI MINATEC, 17 rue des Martyrs, 38054 Grenoble Cedex; [2] CRAN, Nancy Université, UMR 7039 CNRS, Centre Alexis Vautrin, Brabois, Avenue de Bourgogne, F-54511 Vandoeuvre-les-Nancy Cedex; [3] DCPR, Nancy-Université, ENSIC, 1, rue Grandville BP 20451 F-54001 Nancy Cedex; [4] GdR CNRS 3049 «Médicaments Photo-activables Photochimiothérapie»; [5] Biolitec AG R&D Winzerlaer Straße 2, D-07745 Jena; [6] University Hospital Zurich, Institute of Anatomy, Winterthurerstraße 190, CH-8057 Zurich; [7] University Hospital Zurich, Department of Orthopedics, Forchstraße 340, CH-8008 Zurich

Photodynamic therapy (PDT) is a non or minimally invasive treatment therapy relying on the ability of a photoactive non-toxic drug (PS) to generate upon activation with light, free radicals or singlet oxygen. These reactive species can oxidize cellular constituents, eventually leading to cell death. PDT has already shown great promise to improve treatment options for cancer patients due to its distinct advantages, including the strictly focused application, the biocompatibility with other treatment modalities, the option for repeatability, the excellent cosmetic or functional outcome and the fast recovery. However, current protocols display certain methodological flaws, hampering a widespread clinical use of PDT as an anti-cancer modality. Key problems are e.g. related to a suboptimal specificity of the PS to the tumor region, resulting not only in low local drug doses in cancer cells, but also in an unwanted photosensitivity of healthy tissue.
With the aim to increase the efficacy of PDT for clinical applications in oncology, the presentation focuses on the development of a novel nanocarrier-based approach.
Preclinical developments will present the benefits of the encapsulation of the photosensitizers into Lipidots® on their physical and chemical properties. In vitro evaluation of these PS loaded Lipidots® will be commented.
We anticipate that this concept will strongly improve PS transport and targeting, consequently enhancing PS concentrations at the tumor site even after systemic application. By revisiting conventional PDT using nanotechnology based PS delivery systems, we will set the stage for a better control of the therapy and more comfort for cancer patients.

Fig 1: various concentration of photosensistizers-loaded Lipidots® Credit: CEA-leti

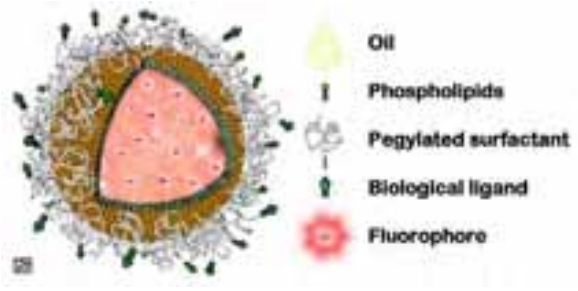


Fig 2: schematic view of Lipidots® nanocarrier. Credit: CEA-Leti

PLAYING GAMES TO HELP PEOPLE UNDERSTAND EMERGING TECHNOLOGIES

DONALD BRUCE

How do we get people thinking constructively and creatively about emerging areas of science like nanomedicine, human enhancement, or synthetic biology? How can the average citizen get his or her head round such unfamiliar (and maybe scary!) scientific ideas, with reliable and understandable information, and also be stimulated to think for themselves about their wider implications - what's known, what's uncertain? what aspects would be acceptable, what might not be? does it cohere with our values and our societies, or conflict? and so on.
For 10 years the Democs card game, devised by the New Economics Foundation, has offered a unique and effective way of enabling general publics to explore novel issues like these for themselves, in their own social settings. It is now in use in many countries and languages on topics as diverse as cloning and climate change. This interactive session will show how the game works - with a practical example for you to do – with ways it might be useful also in your context. Some emerging findings will be given about people's attitudes to nanomedicine and human enhancement from playing games in EC projects NanoBio-Raise and Ethentech. Games will be available for you to examine and play afterwards.
Democs is a group discussion, for 6-8 people, using cards as the source of information and as the stimulus for reflection and debate. Over 1-2 hours it takes people through basic factual information, and some of the ethical and social issues, posing questions, giving different viewpoints, using case studies to illustrate dilemmas. It invites people to come to their own conclusions, and also vote on potential policies or applications. It's free and suitable for 16 years and upwards, it assumes no prior knowledge of the subject, and can be played anywhere where you have a table, some chairs, some friends, and some coffee.

TRANSBUCCAL NANOFORMULATED INSULIN FOR TREATMENT OF MEAL-DEPENDENT HYPERGLYCEMIA

FRITZ R. BÜHLER[1], Jan Mous[2], Mark Schobel[3], and Thomas Rademacher[4],
[1]ECPM University of Basel, Switzerland, [2]Pharmida AG Basel, Switzerland, [3]MonoSol Rx, Warren, NJ USA, [4]Midatech Ltd, Oxford UK

Subcutaneous administration is mostly used for treatment of insulin-dependent diabetes mellitus. Oral administration of insulin is susceptible to hydrolytic degradation by acid and enzymes in the gastrointestinal tract. Insulin inhalation results in variable bioavailability. The buccal mucosa is available for insulin dosage forms bypassing portal circulation and first pass metabolism in the liver. In addition, better patient compliance and diabetes control is expected because insulin dose can be easily administered around meal time.
Glucose clearance rates could be improved by attaching non-modified monomeric insulin on glycan-coated gold nanoparticles (GNP < 3.5 nm) and administering through the buccal membrane using

muco-adhesive Pharm Film®. In various healthy and diabetic animal models including diabetic Rhesus monkeys, transbuccal insulin showed 20-30% bioavailability and had a faster onset and shorter duration of action compared to subcutaneous insulin administration. Numerous safety assessments including acute and 28-day repeated dosing in different species showed no adverse reactions or mutagenicity/genotoxicity at high doses of GNP.
Most recently, in a randomized, double-blind, placebo-controlled, single dose-escalating, First-in-Human study in 27 normal male volunteers the administration of transbuccal insulin in three cohorts with 5, 10 or 20 IU insulin strips vis-à-vis placebo strips and a control 5 IU Insulin aspart injected with 1 week interval, was well tolerated and safe. The results showed a very fast onset of action and dose-dependent increase of plasma insulin concentrations as well as glucose consumption using a standard euglycemic glucose clamp technique. For the first time, transbuccal Midaform™-insulin PharmFilm® administration showed a safe and dose-dependent glucose homeostatic effect and thereby provides a novel, non-injectable insulin administration for the treatment of diabetes.

THE COMBINATION OF IMAGING, DIAGNOSTICS AND TARGETED THERAPY – GATEWAY TO PERSONALISED MEDICINE

VINCENZO COSTIGLIOLA

Changing long-held beliefs is never easy. As a consequence of the accumulating clinical data and knowledge about the epidemiology and pathological mechanisms of the most frequent causes of morbidity and mortality, we are currently reconsidering our view of the origins and progression of cardiovascular, oncologic and neurodegenerative diseases.

Optimistic versus pessimistic prognosis for healthcare sector depends much on diagnostic, preventive and treatment approaches, which healthcare systems will preferably adopt in the near future. PPPM offers great promise for the future practice of medicine .
We are currently considering a new diagnostic and therapeutic approach combining the personal profile, screening protocols, diagnostic biomarkers and imaging.

IN VIVO HUMAN RECEPTOR IMAGIN FOR GASTRIC CANCER

DAXIANG CUI

Herein we reported that BRCAA1 monoclonal antibody conjugated fluorescent magnetic nanoprobes was prepared and characterized, and was used for gastric cancer in situ targeted imaging and hyperthermia therapy. Results showed that as-prepared fluorescent magnetic nanoprobes could target imaging in vivo gastric cancer cells by fluorescent imaging and magnetic resonance imaging. Under external magnetic field, as-prepared nanoprobes could kill tumor cells. In conclusion, as-prepared BRCAA1-monoclonal antibody-conjugated fluorescent magnetic nanoprobes have great potential in applications such as gastric cancer targeted imaging and therapy in near future.

Keywords: fluorescent magnetic nanoprobes; imaging; therapy; targeting

GLUTATHIONE PEGYLATED LIPOSOMES TO SAFELY ENHANCE THE DELIVERY OF DRUGS TO THE BRAIN; EXPERIMENTAL PROOF FROM IN-VIVO MODELS

MARCO DE BOER, Jaap Rip, Chantal Appeldoorn, Linda Chen, Rick Dorland, Burt van der Boom, Joan van Kregten, Pieter Gaillard, and Fredrik Lonnqvist

to-BBB technologies BV, Leiden, the Netherlands

Background
Many CNS diseases remain insufficiently treated due to poor drug efficiency and/or insufficient concentrations of the drug in relevant brain tissues. Thus, although several promising drug candidates are available for various CNS disorders there is a need to increase their ability to effectively cross the blood-brain barrier (BBB) to make them useful in clinical practice. The endogenous tripeptide glutathione (GSH) is found at high levels in the brain and is actively transported across the blood-brain barrier [1]. It has now been demonstrated that glutathione PEGylated liposomes (G-Technology®) are able to mediate safe targeting and enhanced delivery of encapsulated drugs to the brain [2].

Glutathione PEGylated liposomal doxorubicin – the lead product based on the G-Technology
The preclinical development of glutathione PEGylated liposomal doxorubicin (2B3-101), i.e. a CNS-targeted product based on the clinical experience from Doxil (PEGylated liposomal doxorubicin) in the treatment of non-CNS indications, was presented at CLINAM last year [3]. 2B3-101 has been shown to be superior to Doxil in increasing the brain uptake of doxorubicin and in reducing brain tumor growth in experimental models. Furthermore, the GLP toxicity studies showed no major differences between 2B3-101 and Doxil; no cardiotoxicity and neurotoxicity was observed. The ongoing clinical trial is designed to determine the safety, tolerability and pharmacokinetics of 2B3-101 in patients with solid tumors and brain metastases or recurrent malignant glioma.

Strengthening the G-Technology platform
To explore the full potential of the G-Technology as a brain drug delivery platform, and generate additional evidence to support its anticipated added value for the treatment of brain diseases, we have encapsulated several small molecules and peptides and investigated the brain uptake, as well as the efficacy, of the encapsulated drugs. In this effort we have used in vitro cell uptake studies, in vivo microdialysis to determine pharmacokinetics (PK) and brain uptake of the tested drugs and an animal model of multiple sclerosis (MS) to determine the efficacy of one of these drugs.

Liposomes
For the preparation of liposomal formulation, lipids and methods are used that resemble the GMP production of 2B3-101. In short, glutathione PEGylated (GSH-PEG) liposomes were prepared by post-insertion of GSH-PEG-DSPE micelles (4 mol%) into preformed vesicles (HSPC:Cholesterol:PEG-DSPE) containing Dextran-FITC (FD4), methylprednisolone (MP) or a peptide (DAMGO). In addition, PEG-DSPE micelles were used as a control reflecting the effects of non-targeted PEG liposomes.

Glutathione enhances uptake of liposomal FD4 by rat brain endothelial cells
The uptake of GSH-PEG liposomes containing FD4 was significantly higher compared to PEG liposomes in RBE4 cells, using FACS analysis. Similarly, the uptake of FD4 was also higher in the GSH-PEG liposomes when analyzed by fluorescent microscopy.

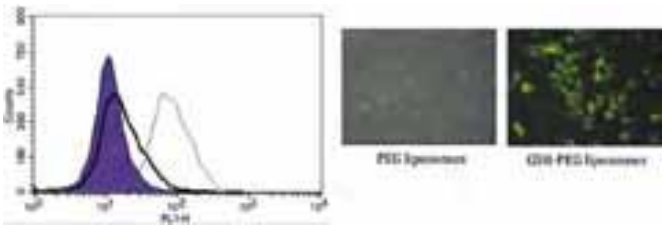


Figure 1. Fluorescence in RBE4 cells treated with GSH-PEG or PEG liposomes containing FD4.
Left: Untreated cells in purple, PEG liposomes black line, GSH-PEG liposomes gray line.
Right: Fluorescent microscopy pictures of the analyzed cells.

When comparing the effects of liposomes of different sizes, the differences in FD4 uptake between GSH-PEG and PEG liposomes were largest in the 100 nm, smaller in the 150 nm liposomes and absent using 200 nm liposomes. Experiments performed at 4°C

showed a far lower FD4 uptake in the GSH-PEG liposomes compared to the experiments performed at 37°C, suggesting an active uptake into these cells.

The brain uptake of DAMGO was enhanced by glutathione PE-Gylated liposomes

Using the anti-nociceptive peptide DAMGO, as a model peptide drug, the unbound drug concentrations were measured in the striatum and in the femoral veins of rats by use of microdialysis. In addition, plasma was sampled to measure the total concentrations of DAMGO, including the encapsulated DAMGO.

The steady-state plasma concentrations of unbound DAMGO were comparable following administration of free peptide (708±131 ng/mL) and GSH PEG liposomal peptide (768±143 ng/mL), respectively. However, the free peptide entered the brain only to a limited extent. The ratio between unbound drug concentration in brain interstitial fluid and blood, (Kp,uu) was 0.09±0.04, while GSH-PEG liposomes increased the brain exposure to DAMGO significantly to 0.21±0.17 ($p<0.05$).

GSH-PEG liposomes improved the efficacy of methylprednisolone in an animal model of MS

Initially, the PK and brain uptake of GSH-PEG liposomal methylprednisolone (MP) was compared to free MP in healthy rats. The free MP was cleared from the circulation within minutes, while the levels of GSH-PEG liposomal MP in the circulation remained significant for at least 8 hours. Furthermore, the brain uptake of MP increased 6.5-fold after the administration of GSH-PEG liposomal MP vs. free MP.

In the rat model of MS that was used, i.e. experimental autoimmune encephalomyelitis (EAE), a single dose of free MP given at the disease onset was not effective, while GSH-PEG liposomal MP significantly reduced the symptom score (total EAE clinical signs) to 42±6.4% of the saline control and was also more effective compared to PEG liposomes, as depicted in Figure 2 below.

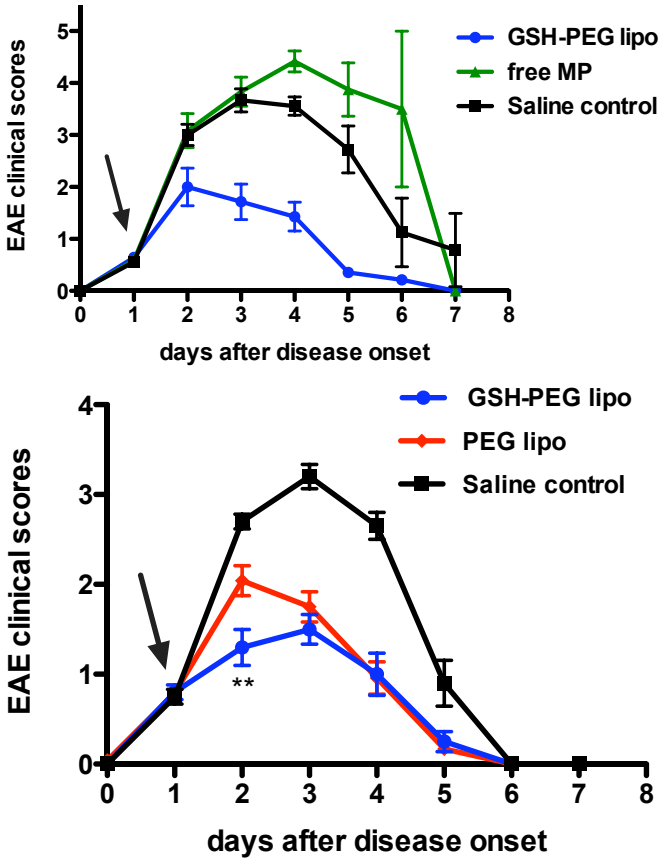


Figure 2: Efficacy of GSH-PEG liposomal MP administered at disease onset. Left: Rats (n=6/7 per group) received 1 dose of 10 mg/kg GSH-PEG liposomal MP (GSH-PEG lipo) or 10 mg/kg free methylprednisolone (free MP) or saline. Right: Rats (n=10/12 per group) received 1 dose of 10 mg/kg GSH-PEG liposomal MP (GSH-PEG lipo) or 10 mg/kg PEG liposomal MP (PEG lipo) or saline. ** $P<0.01$ GSH-PEG lipo vs. PEG lipo at the first day after treatment.

Conclusion

The mechanistic in vitro and in vivo studies performed to date have demonstrated that glutathione PEGylated liposomes (G-Technology®) offer a promising platform that could be used to safely enhance the delivery of small molecule and peptide drugs to the brain. This has now been shown for doxorubicin, methylprednisolone as well as an anti-nociceptive model peptide, DAMGO.

REFERENCES

• Gaillard, P.J.; Visser, C.C.; et al. Drug Discov. Today: Technol. 2011, doi:10.1016/j.ddtec.2011.12.002.

• Rip, J.; Appeldoorn C.C.M.; et al. Presented at: Pharmacology and Toxicology of the Blood-Brain Barrier: State of the Art, Needs for Future Research and Expected Benefits for the EU. Brussels, Belgium. 11-12 February, 2010.

• Gaillard, P.J.; Appeldoorn C.C.M.; et al. Presented at: 4th European Conference for Clinical Nanomedicine. Basel, Switzerland, 23-25 May, 2011.

BARRIERS TO THE CLINICAL DEVELOPMENT OF NANOMEDICINES

NEIL DESAI

In recent years, nanotechnology in medicine has emerged as a rapidly developing field to improve the delivery of a multitude of therapeutic moieties and to increase sensitivity, speed and specificity of diagnostics. Nanotechnology using lipid, polymer and protein-based nano constructs provides a new paradigm of rational delivery of therapeutic and diagnostic agents to the disease sites, which offers numerous potential benefits, including enhanced targeting and penetration, personalization of therapies, increased solubility and bioavailability of therapeutic molecules, improved pharmacological, pharmacokinetic and biodistribution profiles, reduced systemic toxicity, and controlled drug release. The unique physical properties of nanomedicines enable their adaptation for diverse routes of administration, including oral, intravenous, intraperitoneal, periadventitial, transdermal, nasal, and pulmonary delivery. Despite these advantages, only a relatively small number of nanomedicines have been approved for clinical use. The complexity of nanoparticles as multi-component three dimensional constructs can present significant challenges to the design of reproducible manufacturing processes, demonstration of efficacy and safety of nanotechnology-based agents in patients, and regulatory approval. Both the benefits and hurdles of nanotherapeutics are aptly demonstrated in the clinical development of nanoparticle albumin-bound nabTM-paclitaxel (ABRAXANE®), which demonstrates strong anti-tumor activity in a number of different cancer types including pancreatic cancer.

IMMUNOTOXICITY EVALUATION OF NANOMEDICINES

JACQUES DESCOTES

Nanomedicines consist of a wide variety of nanostructures with variable characteristics including size, shape, and surface properties. Therefore, immunotoxicological heterogeneity is likely to be a hallmark of nanomedicines and a critical hurdle for immune safety evaluation. Nanoparticles/materials have been shown to exert a range of immunological effects, such as interactions with cells of the immune system, inflammatory responses, activation of the complement cascade, or facilitation of antigen-specific hypersensitivity reactions. However, conflicting or opposite findings have been reported so that no definitive conclusion can be made to predict the existence or lack of immune-mediated adverse effects with most candidate nanomedicines at the present time.

Immunotoxicity evaluation must focus on the 4 following categories of immunotoxic effects: immunosuppression, immunostimulation,

hypersensitivity and auto-immunity. So far, only few nanoparticles/materials have been found to be inadvertently immunosuppressive. Current guidelines, such as ICH S8 or ISO TS10993-20, could be used as a starting point to propose strategies for assessing the immunosuppressive potential of nanomedicines. Short-term repeat-dose toxicity studies may be suitable provided consistent quality of the tested nanomedicines can be adequately documented. Including at least one immune function assay in such studies is deemed to be critical due to our limited knowledge on the immunotoxicity potential of nanomedicines and the reported immunological effects of various nanoparticles/materials. A major difficulty is the urgent need to evaluate and presumably adapt current assays, especially in vitro or ex vivo immune function assays, to the context of nanomedicines. Available data suggest that nanomedicines may exert immunostimulatory properties, e.g. cytokine release, which can be evaluated using dedicated in vitro or ex vivo assays. The facilitating role of nanoparticles in hypersensitivity reactions to unrelated allergens has been reported. This potential can be addressed by dedicated safety pharmacology studies. Hypersensitivity reactions may be either immune or non-immune mediated. Nanomedicines can be suspected to be intrinsically immunogenic or as a consequence of inadvertent adsorption or intended binding of various chemicals/proteins. Predicting the immunogenicity of nanomedicines may be less tricky than for small-molecular-weight pharmaceuticals or biologicals as nanomedicines are not expected to be humanized and reactive metabolites unlikely to be generated. Non-immune-mediated hypersensitivity reactions due to direct complement activation have been described with liposomes, but rarely if ever with other nanoparticles/materials. It is important to stress that complement activation may not result in clinically significant adverse reactions. Assays and animal models including systemic or passive cutaneous anaphylaxis, basophil activation, histamine release or complement activation can be used case by case. Finally, auto-immunity, the 4th category of immunotoxic effects, is beyond reach of preclinical prediction for the time being.
Available data suggest that nanomedicines can exert immunological effects, which may result in immunotoxic clinical consequences. Therefore, a systematic non-clinical evaluation of the immunological safety of nanomedicines is recommended. Most assays and animal models currently used in immunotoxicity evaluation are presumably applicable, at least to some extent, although adaptation to nanomedicines may prove to be required. In any case, the specificities and modalities of the immunotoxicity evaluation of nanomedicines remain to be fully characterized and validated for regulatory purposes.

LIPOSOMES AS LUBRICANTS, A NEW APPROACH FOR OSTEOARTHRITIS – ONGOING TRIALS

YANIV DOLEV
Dr. med. vet.; CEO, Moebius Medical Ltd., Israel

Moebius Medical is developing a lubricating, intra-articular injectable solution for diarthrosis (synovial joints).
The healthy articular cartilage is smooth and has low friction -- allowing the bones in a joint to glide smoothly over one another upon movement. The osteoarthritis cartilage is thinned, eventually completely worn out, resulting in a «bone against bone» joint, reduced motion, and pain.
We studied and compared liposomes of various lipid compositions either in the form of small unilamellar vesicles (SUV) or large multilamellar vesicles (MLV) using a cartilage-on-cartilage model that has been developed in order to assess the lubrication capabilities and wear and tear reduction
DMPC/DPPC-MLV was found to be the best bio-lubricant and the best anti-wear protector in these models (Sivan et al 2009, Verberne et al 2010). The mechanism of action of these liposomes involves hydrophilic lubrication related to high level of PC head group hydration at the liquid disordered (LD) phase and its unique softness at temperatures slightly above the solid ordered (SO) to LD phase transition temperature (Sivan et al 2009). Intra-articular injection of radiolabeled of DMPC/DPPC-MLV demonstrated prolonged durability (more than 28 days) in the joint (Yaniv et al in preparation). Local toxicology studies proved that intra-articular injection of a high dose of DMPC/DPPC-MLV in rabbits and rats is safe. A series of studies according to ISO-10993 confirmed its high biocompatibility.
A «First in man» study of this DMPC/DPPC-MLV is now being conducted at Hadassah Medical Center / Orthopedic Department.

REFERENCES

• Sivan, S., Schroeder, A., Verberne, G., Merkher, Y., Diminsky, D.,Priev, A., Maroudas, A., Halperin, G., Nitzan, D., Etsion, I., Barenholz, Y., "Liposomes Act as Effective Biolubricants for Friction Reduction in Human Synovial Joints"; Langmuir, 26, 1107-1116, 2010.
• Verberne, G., Schroeder, A., Halperin, G., Barenholz, Y., Etsion, I., "Liposomes as potential biolubricant additives for wear reduction in human synovial joints", WEAR, Elsevier, 268, 1037-1042, 2010.

CYCLOSPORIN PRO-NANODISPERSION LIPOSPHERE FORMULATION

ABRAHAM J. DOMB, Avi Avramoff, Wahid Khan, Anna Elgart and Amnon Hoffman
School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel

Oral delivery is the most preferred route of drug administration due to convenience, patient compliance and cost-effectiveness. For certain drugs it is difficult to achieve satisfactory bioavailability levels via oral administration due to the harsh environment and crossing barriers of the gastrointestinal (GI) tract, particularly for biomacromolecules. Studies on the uptake of NP by the oral route after oral gavage in rats has clearly shown that uptake by endothelial enterocytes and the M-cells in Peyer's patches is size dependent. The key factors for improved oral bioavailability are: particles size below 100nm, particles decorated with an amphiphilic surface-phospholipid, cholesterol derivatives, vitamin D or K etc. and the stability of the particle loaded with the active agent in the GI system.
The preparation and characterization of an oral pro-nanodispersion liposphere formulation for cyclosporin, a water insoluble peptide drug with limited bioavailability is described.
Pro-nanodispersion formulations were prepared by dissolving cyclosporin A (CsA) in a clear solution of solid fat, dispersing agents (Tween, Span, Phospholipids) and ethyl lactate as amphiphilic solvent. The oily formulation spontaneously form nanoparticles upon addition to aqueous media. The particle size of the formed nanoparticles is dependent on the formulation components and the composition.
Human studies indicated a correlation between the particle size and the bioavailability. Formulations that provide similar cyclosporine blood level to Neoral have been developed and is in clinical use (Deximune, Dexcel Pharma).. The formulation is delivered in a soft gelatin capsule of 25, 50 and 100 mg cyclosporine per capsule.

NANO-TONOMETRY: A NEW MEASURE FOR STRUCTURAL REORGANIZATION OF THE EYE CAUSED BY GLAUCOMA

RUTLEDGE ELLIS-BEHNKE
Medical Faculty Mannheim of the Ruprecht-Karls University of Heidelberg, Germany
Department of Brain & Cognitive Sciences, M.I.T., USA

Glaucoma is currently the leading cause of irreversible blindness; and one of the major risk factors for developing glaucoma is elevated intraocular pressure (IOP). In this study, an elevated IOP of approximately 120mmHg was introduced into the right eye ball of Sprague-Dawley rats for approximately 1 hour in vivo, while the left eye ball was kept at a normal state. Nanoindentation performed on the harvested tissues in vitro showed that the elastic modulus of normal corneas was 2.75 to 3.33 MPa, whereas that of corneas suffer-

ing from elevated IOP was significantly higher at 4.89 to 5.58 MPa. Scanning electron microscopy (SEM) imaging suggested that the collagen fibrils subjected to the elevated IOP became thinner; and on relaxing from being strained with an elevated IOP, their directionality became more random. Such observations are consistent with the fact that the collagen fibrils inside the cornea were strained and elongated by the elevated IOP. This may cause strain-stiffening effects in them and therefore result in the observed increase in elastic modulus of the cornea.

POLYPID - A LIPID-AND-POLYMER-BASED NOVEL LOCAL DRUG DELIVERY SYSTEM: FROM PHYSICO-CHEMICAL ASPECTS TO THERAPY OF BACTERIALLY INFECTED BONES

NOAM EMANUEL, Yosef Rosenfeld1, Or Cohen, Yaakov H. Applbaum. David Segal and Yechezkel Barenholz

PolyPid technology is a novel drug delivery system (DDS) that was planned to control the release of many drugs and biological agents over prolong period of time. It was developed in order to fulfill the major deficiencies of the two known and well established drug delivery systems, polymers and lipids. By the combination of the selected lipids with the selected polymers PolyPid has succeeded to achieve a novel DDS with unmatched performance to both. This DDS is based on the self-assembly of pharmaceutically known polymeric and lipids components into a highly organized fatty nano-scale derived super-molecular structures. The lipids used are mainly synthetic phospholipids and cholesterol. The release rate of the drug can be pre-programmed by the selection of the specific polymer and lipid composition, where the drug molecules are fully integrated in the final well organized structures. The PolyPid formulation structure was characterized by different physical methods including differential scanning calorimetric (DSC), SEM and X-ray diffraction. It was demonstrated that each one of the major component significantly contributing to the final organized structure as well as to its performance as DDS.

PolyPid technology platform enables to entrap a large variety of either a single or combination of drugs(s) (small molecules, peptides, proteins and nucleic–acids based drugs) and to release them in a pre-programmed zero-order kinetics profile for the desired time, in the preset range of several days to several months. The drugs reservoir is fully protected against biological destruction as well as against hydration, particularly important when long lasting activity of sensitive drugs is required.

BonyPid™

The first PolyPid technology based product is BonyPid™ which is designed to serve in the orthopedic field. Bacterial infection of bone may result in bone destruction and is difficult to cure due to its poor accessibility to systemically-administrated antibiotic. Together with that, the currently available local delivery systems are not sufficiently effective due to their high burst and short lasting effect. Therefore, prolonged and controlled local delivery of antibiotics to the bone tissue can play a major role in the treatment of acute and chronic (osteomyelitis) bone infections.

The BonyPid™ is based on the commonly used bio-degradable and biocompatible bone-void-filler particles. These particles are coated with a fine layer of the PolyPid based biodegradable formulation which include Doxycycline (Doxy), a potent and a broad-spectrum antibiotic that is commonly used to treat a variety of infections. Upon in-vivo hydration the entrapped Doxy is released over a predefined period of 30 days (Figure 1). The pre-set zero order kinetic release profile was pre-designed to achieve long lasting and sufficient local drug concentrations. The coating surface is gradually disintegrated layer by layer thereby releasing the antibiotic into the surrounding tissue, while the bone-void-filler scaffold remains and supports bone recovery.

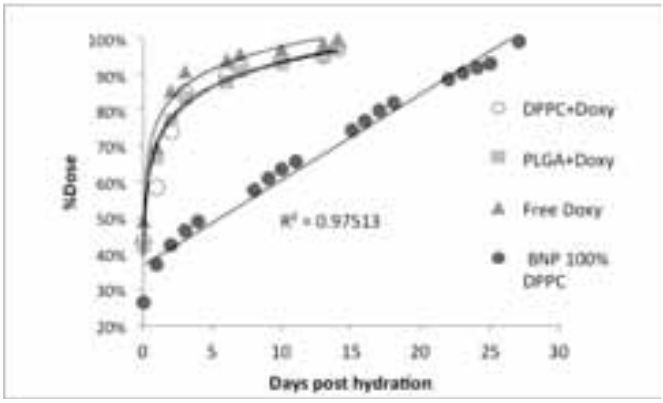


Figure 1: The release profile of Doxycycline (Doxy) from BonyPid (BNP) vs. coating that only contains the polymer (PLGA) or the phospholipid (DPPC). The accumulated time-dependent release profile of Doxy from freshly hydrated BNP granules. BNP granules were hydrated with 5% FBS solution at 37°C. The solution was replaced by the fresh FBS solution daily and the concentration of the intact Doxy in the daily collected supernatant was determined by HPLC. The accumulated release of Doxy was normalized to the initial entrapped amount (% of dose) and plotted over time in days post initial hydration.

BonyPid™ has been tested pre-clinically in infected rabbit's tibiae model. It was clearly demonstrated that the therapeutic efficacy of BonyPid™ is very high in both acute and chronic infections models as well as significantly advantageous over the non-formulated free drug. The high safety profile of BonyPid™ was demonstrated in vitro as well as in vivo.

Many of the compound and comminuted severe open fractures are both contaminated as well as missing bone and therefore require bone grafting. The timing of bone grafting remains an unsettled issue. Due to the danger of infection in bone grafting it is recommended only between one to six months post fracture surgery procedure. Therefore, bone grafting requires a second operation, when the surgeon considers the infection to be under control. Due to its dual activity as a bone substitute that can facilitate bone recovery and its ability to eradicate local bacterial contamination we believe that bone grafting by BonyPid™ following trauma has the ability to be used successfully as an immediate grafting.

MARKET PERSPECTIVES ON NANOCARRIERS

SANDRA ERB

The breakthrough of a new technology depends on the existence of a viable market that opens investment opportunities. Although business analysts find it difficult to estimate the volume and growth rates of the nanomedicine market, it is expected to be a billion dollar market with rapid growth potential. However, companies have found it difficult to commercialize products. This presentation will discuss the obstacles for nanocarriers and what is needed to overcome them.

OPENING ADDRESS OF THE CANTON OF BASEL

CHRISTOPH EYMANN

Basel, a major European hub for healthcare research, welcomes you once again to the 5th European Conference for Clinical Nanomedicine. This scientific meeting focuses on patients but deploying the new tools that nanotechnology has created. Nanomedicine encompasses not only targeted delivery of known drugs, but offers new mechanisms of therapy without the use of known therapeutics. Basel and its industrial infrastructure are devoted to helping patients with untreated diseases and it is an appropriate setting for such a meeting and as such it welcomes you as pioneers. We are proud that you

render us the capital of Nanomedicine during these days.
This meeting despite its size has an informal and friendly atmosphere which does much to enhance global communication. Our science based city is a perfect background to such an event with its venues, museums and galleries. This conference was founded in Basel five years ago and we are proud that it returns annually. That is why the Canton of Basel has supported this year the CLINAM Foundation in difficult economical times to ensure the continuity in Basel of this by now well-known conference.

500 years ago Basel was already famous for innovation in Medicine. In 1526 Philippus Theophrastus Aureolus Bombast von Hohenheim later called "Paracelsus" came to Basel and was appointed the "Town Doctor" by the municipal authorities, with an additional and provocative role in Medicine at the University. Sadly the conservative Faculty of Medicine prohibited him from lecturing, since it was rumoured, as it happens correctly, that he was a revolutionary. After a long quarrel between the municipal authorities and the faculty, finally interceded by Erasmus of Rotterdam who was at the time also living in Basel, Paracelsus was allowed to give lectures. Before starting the series of lectures Paracelsus proclaimed that he will revolutionize medicine and free it from malpractice. "Most doctors" he proclaimed "have damaged patients since they have interpreted uncritically the words of Hippocrates, Galenos and Avicenna". He condemned dogmas replacing them by the slogan "experimenta ac ratio", meaning "experiments and sound mind".

He declared: "It is the duty of clinicians to understand nature and its secrets, to acquire knowledge of diseases and its causes and to be open-minded to new therapies. He ended his proclamation with "Good bye Ladies and Gentlemen - and take my suggestions for the medicine of today" His lectures terrified the old faculty of medicine and they chased him out of town - nevertheless his ideas had now been published and were freely discussed. His great success in Basel was that he led medical research away from doctrine to medicine based on observing patients. This again led to the development of herbal and synthetic drugs with proven success in therapy.

So, we sincerely hope that you will emulate Paracelsus, by thinking of better ways to treat patients, but using the new science of "Nano". For those who are worried I can guarantee that the magistrates and the university have changed a lot and that you will no longer be chased out of town for your outlandish ideas!

I say that both as the Basel Minister of Education and also as a member of the University Council – so you see the municipal authorities and the faculties have grown together in 500 years!

Although you will work hard I hope that you enjoy your stay and will get some glimpses of Basel outside the Congress Centre. And that you will return every year to the CLINAM Conference to live up again to what Hohenheim said: "experimenta ac ratio".

UNDERSTANDING IMMUNOTOXICITY OF NANOMATERIALS: FOCUS ON INFLAMMASOME ACTIVATION

BENGT FADEEL
Prof., M.D., Ph.D.; Division of Molecular Toxicology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

Engineered nanomaterials may have an impact on the innate or adaptive immune system, our principal defense system against foreign intrusion; such interactions, in turn, could result in adverse outcomes or could potentially be exploited for therapeutic gain eg. for vaccination. The recognition or non-recognition of engineered nanomaterials by immune-competent cells may determine not only the toxicological effects of such materials but also their fate in the body (biodistribution, biodegradation). However, understanding the physico-chemical properties (eg. size, shape, surface charge) that drive interactions of nanoparticles with biological systems remains a key challenge. We believe that important lessons can be learned from immunology and the interaction of microorganisms with the immune system and we postulate that there are common pathways that are triggered by biological 'particles' and artificial nano-objects i.e. engineered nanomaterials. Conversely, we propose that engineered nanoparticles may be useful as probes with which to dissect biological mechanisms. The inflammasome is a macromolecular, cytosolic complex in phagocytic cells that senses 'danger' and initiates the inflammatory response through caspase-1-dependent processing of pro-interleukin (IL)-1-beta leading to secretion of the active, pro-inflammatory cytokine, IL-1-beta. Studies in recent years have defined a number of inflammasome agonists ranging from microbial molecules, to environmental agents, most notably asbestos, silica and various types of nanoparticles. This presentation will discuss nanomaterial-induced inflammasome activation.

FURTHER READING

• Feliu N, Fadeel B. Nanotoxicology: no small matter. Nanoscale. 2010 Dec 1;2(12):2514-20.
• Nyström A, Fadeel B. Safety assessment of nanomaterials: implications for nanomedicine. J Control Release. 2012 Jan 25. [Epub ahead of print]

ARE BRONCHIAL CELLS A SHIELD TO LUNG TOXICITY OF NANOMEDICINES?

ELIAS FATTAL
UMR CNRS 8612, Faculty of Pharmacy, Univ Paris-Sud, Châtenay Malabry, 92290, France

Despite the ongoing debate about the safety of nanomedicines and especially the wide interest raised by their lung administration for the treatment of various diseases, little information is available on their effect towards the airway epithelial barrier function. We carry out study to evaluate the damages induced to the pulmonary epithelium upon exposure to biodegradable poly(lactide-co-glycolide) (PLGA) nanoparticles (NPs). For this purpose we have used an in vitro model of Calu-3 cell to mimic the bronchial epithelial barrier. Positively and negatively charged as well as neutral PLGA NPs were obtained by coating their surface with chitosan (CS), poloxamer (PF68) or poly(vinyl alcohol) (PVA), respectively. The role of NP surface chemistry and charge on the epithelial resistance and mucus turnover was investigated. MUC5AC was used as a marker of mucus production. It was shown that the interaction with mucin reduced the penetration of CS- and PVA-coated NPs while the hydrophilic PF68-coated NPs were able to diffuse across the mucus barrier leading to a higher intracellular accumulation. NPs did not interfere with the formation and maintenance of tight junctions, with the exception of CS-coated NPs which caused a transient but reversible decrease of the trans-epithelial electrical resistance (TEER). NPs did not increase the MUC5AC mRNA expression or the protein levels regardless of their surface properties. Moreover, non inflammation was observed as evaluated by measurement of proinflammatory cytokines. These in vitro results highlight that biodegradable PLGA NPs may not harm the integrity and function of the bronchial airway barrier and demonstrate the crucial role of NPs surface properties to achieve a controlled and sustained delivery of drugs via the pulmonary route.

TOWARDS A MAGIC BULLET AGAINST MALARIA: PAUL EHRLICH REVISITED

XAVIER FERNÀNDEZ-BUSQUETS[1,2,3], Patricia Urbán[1,2,3], Juan José Valle-Delgado[1,2,3], Ernest Moles[1,2,3], Joana Marques[1,2,3], Patricia Marín-García[4,5], María Belén Jiménez-Díaz[6], Cinta Díez[1,2,3], Elisabetta Ranucci[7], Amalia Díez[4], Antonio Puyet[4], Iñigo Angulo-Barturen[6], José M. Bautista[4] and Joan Estelrich[3,8]

1. Nanobioengineering Group, Institute for Bioengineering of Catalonia, Barcelona, 2. Nanomalaria Group, Barcelona Centre for International Health Research, Barcelona, 3. Nanoscience and Nanotechnology Institute, University of Barcelona, 4. Departamento de

Bioquímica y Biología Molecular IV, Universidad Complutense de Madrid, 5. Departamento de Ciencias Morfológicas y Biomedicina, Universidad Europea de Madrid, 6. Diseases of the Developing World, GlaxoSmithKline, Tres Cantos, Madrid, 7. Department of Industrial and Organic Chemistry, Università degli Studi di Milano, 8. Departament de Fisicoquímica, Facultat de Farmàcia, University of Barcelona

Paul Ehrlich was a revolutionary immunologist, the father of haematology, and the creator of the field of chemotherapy. Ehrlich's dream of the "magic bullet" – his term – that would seek out and specifically destroy invading microbes or tumor cells is now not only a reality but a major aspect of clinical medicine. However, a century later the implementation of this medical holy grail continues being a challenge in three main fronts: identifying the right molecular or cellular targets for a particular disease, having a drug that is effective against it, and finding a strategy for the efficient delivery of sufficient amounts of the drug in an active state exclusively to the selected targets.

At present, administration methods of antimalarial drugs release the free compound in the blood stream, from where it can be significantly removed by many tissues and organs, thus reducing its availability for Plasmodium-infected erythrocytes. Due to this lack of specificity regarding the target cells, current oral or intravenous delivery approaches for most antimalarial drugs require high doses. However, unspecificity of toxic drugs demands low concentrations to minimize undesirable side-effects, thus incurring the risk of sublethal doses favouring the appearance of resistant pathogen strains. Targeted nanovector systems can fulfill the objective of achieving the intake of total doses sufficiently low to be innocuous for the patient but that locally are high enough to be lethal for the malaria parasite.

We work on the development of antimalarial drug-carrying nanovectors specifically targeted to Plasmodium-infected red blood cells (pRBCs). Our first immunoliposomal prototype delivers its contents exclusively to pRBCs containing the P. falciparum late forms trophozoites and schizonts **(Figure 1)**, and improves on average tenfold the efficacy of the antimalarial drugs chloroquine and fosmidomycin **(Figure 2)**. Using chloroquine concentrations well below its IC50, and by modifying parameters such as liposome size, density of targeting antibodies on the liposome surface, targeted antigen, and intraliposomal drug concentration, we approach 100% of parasitemia reduction both in vitro and in vivo using a murine model for P. falciparum malaria. We will discuss our current work aiming at the improvement of the nanovector through modification of (i) the targeting element: better antibodies, non-protein molecules such as DNA aptamers and polysaccharides, (ii) the encapsulated drug(s), and (iii) the type of nanocapsule, making special emphasis on polymeric structures. Our objective in the short term is the design of a nanostructure adequate to enter the preclinical pipeline as an economically affordable new antimalarial therapy.

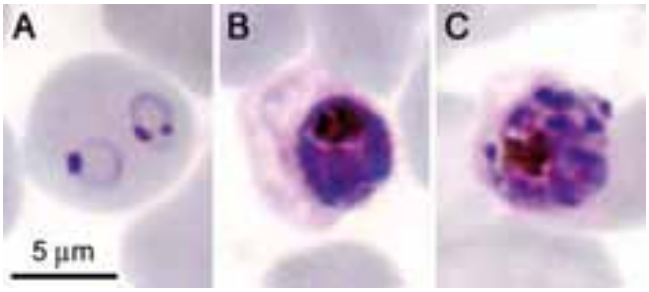


Figure 1: Giemsa staining of P. falciparum-infected RBCs. At the ring (A), trophozoite (B), and schizont (C) stages of P. falciparum. From [1].

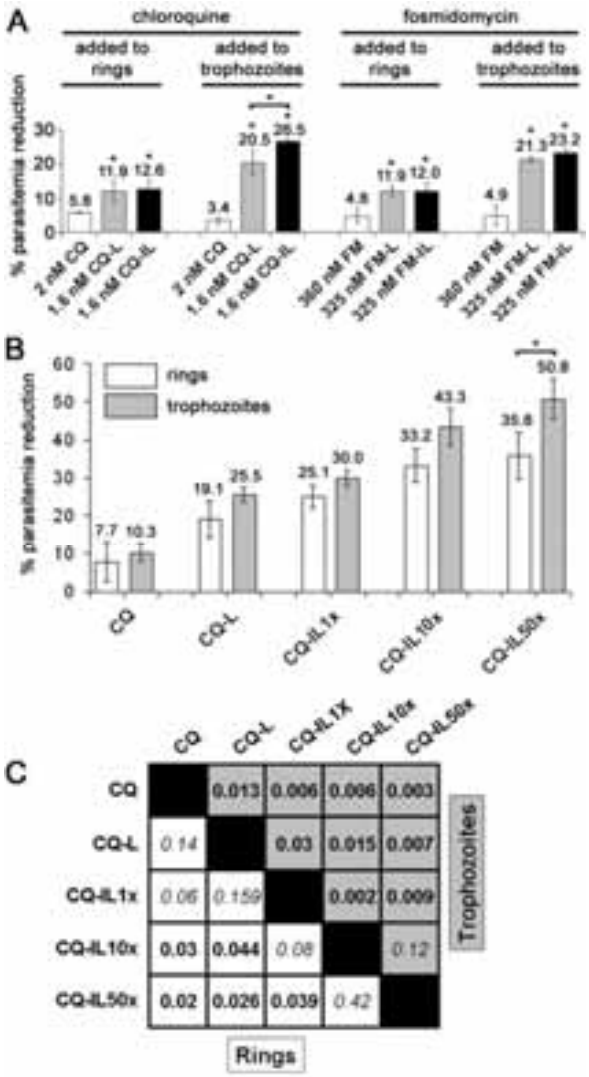


Figure 2: Growth inhibition assays. (A) Effect on P. falciparum viability of chloroquine (CQ) and fosmidomycin (FM), free or encapsulated in liposomes (L) or immunoliposomes (IL) and added at the ring or the trophozoite stage. The values express percentage of reduction respective to the parasitemia of controls without drug added. Asterisks on top of individual bars indicate significant differences ($p \leq 0.05$) with the corresponding control sample of the non-encapsulated drug. (B) Effect on P. falciparum viability of increasing amounts on chloroquine-loaded immunoliposomes of a monoclonal antibody targeted to trophozoites. The amount of chloroquine added to the culture was 4 nM in all samples. 1×, 10×, and 50× correspond respectively to 5, 50, and 250 estimated antibody molecules per liposome. The asterisk indicates a significant difference ($p \leq 0.05$). (C) Grid showing the p values within the trophozoite and ring samples in the experiment from panel B. Bold numbers indicate a significant difference ($p \leq 0.05$). From [1].

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REFERENCES

Urbán P, Estelrich J, Adeva A, Cortés A, Fernàndez-Busquets X: Study of the efficacy of antimalarial drugs delivered inside targeted immunoliposomal nanovectors. Nanoscale Research Letters 2011, 6:620.

TOPICAL INTERFERON ALPHA FOR PREVENTION OF CERVICAL CANCER IN PHASE III CLINICAL TRIALS

MARIANNA FOLDVARI
School of Pharmacy, Waterloo Institute of Nanotechnology, University of Waterloo, Waterloo, ON. Canada

School of Pharmacy, Waterloo Institute of Nanotechnology, University of Waterloo, Waterloo, ON. Canada
Human papillomavirus (HPV) causes benign and malignant infections of the anogenital tract. Cervical cancer, caused by high-risk HPV types 16, 18, 31 and 45, is the second most common cancer in women and the fifth most common cancer overall. Topical delivery of macromolecules is a novel concept and its practical implementation is limited by the lack of effective delivery system. Recent progress in the field may provide new opportunities to develop formulations that can be applied non-invasively onto skin or mucosa to achieve localized treatment of dermatological and infectious diseases. Biphasic vesicles (nanoemulsion, representing a new class of dermal and mucosal delivery system for protein drugs, as a cream formulation is an alternative non-injectable dosage form to deliver interferon alpha for the treatment of HPV infections. We have developed biphasic vesicles as a topical intravaginal delivery system for interferon alpha (IFNα; 19kDa). In the Phase II study, 41 women with cytologically confirmed, HPV-induced low-grade squamous intraepithelial lesions (LSIL, PapIIw to Pap IIID) of the cervix were studied across four clinical sites. In the treatment group twenty women received Topical Biphasix™-IFNa-2MU, self-administered intravaginally three times per week for a period of 6 weeks with a follow-up evaluation at 12 weeks. In the placebo group 21 women received no treatment. The preclinical development of Biphasix™-IFNa indicated excellent dermal safety and product stability. Clinical trial results in patients with LSIL/CIN1 indicated that 46.7% of the women in the treated per-protocol population had their abnormal Pap smears revert to normal during the 12 week period, compared with only 15.8% of the untreated women. Topical Biphasix™-IFNa was shown to be safe and effective in the treatment of LSIL/CIN1 as evidenced from the clinical responses, and was recently approved for Phase III studies by the FDA.

TAILORING LIPOSOME FORMULATIONS FOR CANCER CHEMOTHERAPY

ALBERTO GABIZON
Prof. Dr. med.; Shaare Zedek Oncology Institute and Hebrew University-School of Medicine, Jerusalem, ISRAEL

Most of the currently used anti-tumor agents have problematic toxicities compromising efficacy, and often resulting in life-threatening events. Liposomes can provide effective control of the release rate and of the tissue distribution of many of these agents. These pharmacokinetic changes have a major pharmacodynamic impact with attenuation of toxic effects and protection of sensitive tissues. Polyethylene-glycol coating of liposomes results in inhibition of liposome uptake by the reticulo-endothelial system and significant prolongation of liposome residence time in the blood stream. A hallmark of these long-circulating liposomal drug carriers is their enhanced accumulation in tumors. The mechanism underlying this passive targeting effect is the phenomenon known as enhanced permeability and retention. Developments in drug loading technology have improved the efficiency and stability of drug entrapment in liposomes, particularly with regard to cationic amphipathic drugs such as anthracyclines, vinca alkaloids, and camptothecin analogs. An example of liposome formulation with demonstrated clinical added value is pegylated liposomal doxorubicin (PLD), which has demonstrated clinically a unique pharmacokinetic profile and a favorable safety profile with an impressive reduction in cardiac toxicity and proven efficacy against various malignancies and can be considered as the first anti-cancer nanomedicine approved for clinical use. Co-encapsulation of two active agents in the same liposome is another potentially valuable approach in liposome delivery. We developed a pegylated liposome formulation of a dissociable salt of alendronate, a commonly used amino-bisphosphonate, with doxorubicin (PLAD). PLAD is a very stable formulation with a long-circulating pharmacokinetic profile similar to PLD. PLAD was tested in vitro and in vivo, and was found to have greater anti-tumor activity than PLD, particularly in a multidrug-resistant mouse tumor model. Another approach applicable to liposomal drug delivery combines the concept design of a stable and long-circulating liposome with chemical modification of a drug to form a lipophilic prodrug with strong association to the liposomal bilayer. This is the case of a lipophilic prodrug of mitomycin-C activated by thiolytic cleavage. Thiolytic cleavage takes place in the tissue micro-environment with negligible activation in plasma thus preventing drug activation and drug leakage in the blood stream. Pegylated liposomal mitomycin-C prodrug is more effective and less toxic than conventional chemotherapy in of various human tumor models. In summary, liposome-based systems offer a vast array of potential applications in the delivery of cancer chemotherapeutic agents which may result in a substantial improvement of the therapeutic index. (Supported by a Professorship award of the Israel Cancer Research Fund)

CONVERGING TODAY'S BUILDING BLOCKS TO ETHICAL MEDICINE - WHEN IS NANOMEDICINE "NOW"?

ROGÉRIO GASPAR
Prof. Dr.; Faculty of Pharmacy University of Lisbon and iMed.UL Research Center (Portugal)

Current developments in the research landscape of nanomedicines brought the attention to the fact that as an already well established area of clinical practice, it now faces also some questions previously addressed by new chemical entities and biologicals (1-5).
Advances brought by nanomedicines in oncology and infectious diseases are now expanding both within theses clinical areas and also looking at their use to other less targeted clinical situations.
The advances in clinical practice brought by nanomedicines in the last 30 years will allow for significant improvements in the next phase, sustained by both solid basic research and an increased amount of clinical data compiled across different technologies and therapeutic areas.
The innovation in materials science as to meet clinical standards already established for already approved medicinal products that went through the challenge of regulatory approval for both clinical experiences (under clinical trials) but also for marketing authorisation and routine clinical use.
Nanomedicine is bringing converging sciences to an adequate platform of technologies that will allow to provide better health care but also enable the design and clinical use of innovative solutions to unmet clinical needs.
We envisage a near future where bridging diagnostics and therapeutics through nanotechnology-based tools brings the promise of personalised medicine as an attainable goal at an adequate cost for both society and patients.

BIBLIOGRAPHY

• (1) **Gaspar, R** (2007), Regulatory issues surrounding nanomedicines: setting the scene for the next generation of nanopharmaceuticals, Nanomedicine 2(2), 143-147 , DOI 10.2217/17435889.2.2.143
• (2) **Gaspar, R**. and Duncan, R. (2009) Polymeric carriers: preclinical safety and the regulatory implications for design and development of polymer therapeutics. In: Vicent, M. J. and Duncan, R. (Eds. Theme Issue: Polymer Therapeutics: Clinical Applications and Challenges for Development), Advanced Drug Delivery Reviews 61, 1220-1231, DOI: 10.1016/j.addr.2009.06.003
• (3) **Gaspar, R**. (2010) Therapeutic Products: Regulating Drugs and Medical Devices. Chapter 14, in International Handbook on Regulating Nanotechnologies edited by Graeme A. Hodge, Diana M. Bowman and Andrew D. Maynard. 640 pp, ISBN 978 1 84844 673 1

• (4) Duncan, R. and **Gaspar, R**. (2011), Nanomedicine(s) under the microscope, Molecular Pharmaceutics, 8 (6), 2101–2141, DOI: 10.1021/mp200394t
• (5) Videira M. and **R Gaspar** - Patentability and Intellectual Property Issues Related to Chitosan-Based Biopharmaceutical Products – Chapter 25 in Sarmento, Bruno & das Neves, Jose (editors) "Chitosan-based Systems for Biopharmaceuticals: Delivery, Targeting and Polymer Therapeutics" John Wiley and Sons Ltd. ISBN: 978-0-47097-832-0 (Hardback, 584 pages), Publication date: March 2, 2012

NANOMEDICINE: CONVERGING NANOTECHNOLOGY AND BIOTECHNOLOGY

EHUD GAZIT
Department of Molecular Microbiology and Biotechnology
Tel Aviv University, Tel Aviv 69978, Israel

The discipline of nano-medicine is one of the most fast growing fields of nanotechnology. Nano-medicine is based on the long-term tradition of biotechnology and drug development together with new concept of nano-science and nanotechnology. Indeed, Doxyl, liposome-encapsulated doxorubicin – the first nanotechnology-based drug, represents the first convergence of nano-science and nanotechnology. Concept of liposome physical chemistry and the self-assembly of lipid molecules together with drug development tools were used for the introduction of new and effective way for cancer treatment. Current advances in the field of organic and inorganic self-assembly processes and products provide new tools for nano-medicine development. The availability of novel nano-scale organic scaffolds, advanced natural and synthetic matrix materials, "smart" advanced materials, grafted copolymers and more allows the design and synthesis of new diagnostics and therapeutics tools. Moreover, inorganic quantum dots and contrast agents lead to the development of advanced diagnostics of earlier and more accurate diagnosis of diseases.

HIGH-SPEED ATOMIC FORCE MICROSCOPY HAS COME OF AGE: SEEING CELLULAR MOTORS AT WORK

CHRISTOPH GERBER

Atomic Force Microscopy technologies have come of age and are ubiquitous in many applications in life sciences. Apart from high resolution 3 D images of samples very close to their native state various technological developments has turned the technique into a multifunctional nanoscopic toolbox.Single molecule spectroscopy and highly selective and sensitive diagnostic devices showing great potential in system biology and preventive medicine to evaluate treatment response efficacy in personalized medicine are prominent examples.
Time resolved recent developments shows the power of high speed AFM in observing the cellular machinery at nanometer and millisecond resolution. Based on micro sized cantilever with a low Q and operated at high frequencies in the dynamic mode, high speed AFM provide excellent spatial resolution into the time domain of chemical activity. Current technology utilized by structural biologist produces static snapshots of proteins but seeing them in action is the ultimate goal. HS-AFM visualizes the stepwise movement of the motor proteins myosin V . Hand over hand real time movement in 36 -nanometer steps along the active filament is revealed.The energy required is generated by the hydrolysis of ATP to ADP Examining processes of F0F1- ATPase the rotary enzyme that synthesizes ATP ,the universal fuel of cells, is an other example where the cellular machinery is shown in action with HS- AFM at nanometer and milli- second resolution .This new developments is set to take prominent place in the field of biomolecular imaging and beyond.

NOVEL IMAGE-GUIDED NANODRUG PARADIGMS FOR ENHANCED CANCER THERAPY

NAHUM GOLDBERG
Prof. S., MD, FSIR; Vice-Chairman for Research; Section Head, Image-guided Therapy, Dept. of Radiology, Hadassah Hebrew University Medical Center, Jerusalem, Israel

Over the last two decades interventional radiology techniques have made a major impact on the minimally-invasive treatment of cancer. For example, image-guided tumor ablation is a cutting-edge technique that is now being used clinically worldwide over 150,000 times / year to treat an expanding spectrum of small, focal tumors in normal and cirrhotic liver, the kidney, bone, lung, and other organs. This is achieved by coagulating the tumor using short duration (4–15 min), high temperature heating (< 50°C) via needle electrodes from sources such as radiofrequency, microwave, and laser. Potential benefits include reduced morbidity and mortality compared to standard surgical resection and the ability to treat non-surgical patients. However, clinicians have been conventionally unable to achieve complete ablation in many cases, particularly at margins of tumors larger than 3 – 5 cm. This presents a substantial barrier toward clinical efficacy. Fortunately, this presentation will demonstrate how one can rationally combine radiofrequency (RF) and other methods of thermal ablation with adjunctive nanotherapies to improve tumor destruction to overcome this barrier and effectively improve local tumor control and overall survival. Accordingly, we will present our initial work combining RF with liposomal doxorubicin that has not only validated our step-wise approach of characterization, optimization, and demonstration of improved survival in animal models with rapid translation into the clinic, but has also identified key mechanisms that we can further manipulate to improve therapeutic outcomes. This includes not only improving targeted delivery of agents in nanocarriers to RF ablated tumors; but also modifying liposome construction and content to enable the nanocarriers to synergistically interact with thermal ablation. Specific mechanisms thus far identified include increasing cell stress to induce cellular apoptosis in zones immediately adjacent to the ablation, and downregulation of heat-shock protein (HSP) production in a more peripheral viable peri-ablational zone. Accordingly, our recent promising efforts using liposomal paclitaxel and GLA enriched liposomes to amplify apoptotic pathways and the use liposomal and now micellar quercetin preparations to down regulate HSP-70 will be highlighted. Finally, the use of interventional radiologic techniques to increase focal drug deposition by targetinga nanopreparations to specific sites in vivo will be addressed.

CANCER NANOTECHNOLOGY – FROM INNOVATION TO CLINICAL TRANSLATION - VIEW FROM THE NCI ALLIANCE FOR NANOTECHNOLOGY IN CANCER (NIH) IN THE UNITED STATES

PIOTR GRODZINSKI
Ph.D.; Director, NCI Alliance for Nanotechnology in Cancer
National Cancer Institute

Nanotechnology will provide novel, paradigm shifting solutions to medical problems. In oncology, nanomaterials are enabling targeted delivery of imaging agents and therapeutics to cancerous tissue; nanoscale devices are providing for multiplexed sensing in early disease detection and therapeutic monitoring.
Nanotherapeutics are capable of increasing treatment effectiveness while limiting side effects. Next generation of nanotherapies for cancer is expected to use active targeting of tumor-specific cell markers to deliver entirely new modalities of cancer treatment, including triggered release of cytotoxic molecules, genetic material, heat, or cellular disruption. Given that positive cancer outcomes are associated so closely with early detection, another important goal of cancer nanotechnology efforts is to improve diagnostic capabilities, through in vivo imaging contrast enhancement and in vitro device develop-

ment. Magnetic resonance imaging, ultrasound, positron emission tomography (PET) will all benefit from the development of these new contrast agents. Furthermore, those constructs can be made 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 performing theranostic functions of diagnosis and subsequent treatment. Advances in microfluidics and nanodevices will greatly accelerate the genetic and proteomic analysis of cancer subtypes in vitro and the monitoring of markers of early or premalignant stage cancer and premetastatic disease.

In order to further these research goals, NCI formed a program called Alliance for Nanotechnology in Cancer which was initiated in 2004. The Alliance funds Centers of Cancer Nanotechnology Excellence, the development of nanotechnology platforms, and two training programs: Cancer Nanotechnology Training Centers and Path to Independence Awards. 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.

The nine Centers of Cancer Nanotechnology Excellence are:

• Carolina Center of Cancer Nanotechnology Excellence at the University of North Carolina, • Center for Cancer Nanotechnology Excellence and Translation at Stanford University, • Center for Cancer Nanotechnology Excellence at Johns Hopkins University, • Center for Translational Cancer Nanomedicine at Northeastern University, • Dartmouth Center for Cancer Nanotechnology Excellence at Dartmouth College, • MIT-Harvard Center of Cancer Nanotechnology Excellence, Nanomaterials for Cancer Diagnostics and Therapeutics at Northwestern University, • Nanosystems Biology Cancer Center at California Institute of Technology, • Texas Center for Cancer Nanomedicine at the University of Texas Health Science Center.

This presentation will describe the current advances of cancer nanotechnology, future strategies and prospects of the field, and details behind the organization of the Alliance.

Recipients of NCI Alliance for Nanotechnology in Cancer Awards in 2010

"THE IMPACT OF NANOTECHNOLOGY ON DNA SEQUENCING"

MICHAEL HEHENBERGER

Ph.D.; IBM T.J. Watson Research Center, Yorktown Heights, NY

For over sixty years, IBM Research has been at the forefront of basic science and done groundbreaking work in physics, chemistry, nanotechnology and computer science. In the early 1990's, computational biology was added as a new discipline, thus positioning IBM as a partner for significant Life Sciences partnerships, suh as the DNA Sequencing collaboration with Roche Diagnostics.

In 2010, Roche and IBM agreed to work together on a revolutionary new approach to DNA Sequencing that will be based entirely on recent advances in nanotechnology and semiconductor fabrication.

However, to realize the potential of the new nanopore based DNA Transistor idea, significant technological challenges have to be overcome. Those challenges can be categoirized as follows:

• 1) Electrochemistry, • 2) Translocation Control of DNA moving through the nanopore, • 3) Sensing of nucleotides

The presentation will describe the basic DNA Transistor idea, the ongoing struggle to make it work and the potential benefits derived from single molecule nanopore based Sequencing.

THE IMPLICATION OF ETHICS IN NANOMEDICINE

GÖRAN HERMEREN

There are several nano-technologiess and many applications of these technologies; and they raise partly different problems, also in medicine. As this conference demonstrates, spectacular progress is made in many areas in the pharmaceutical industry, in medicine and biotechnology, in information and communication technologies, as well as in other industries.

There will be two interconnected subthemes in this talk: (1) The implications of ethically sensitive issues in nanomedicine, in areas such as diagnostics, imaging, testing, and treatment and follow up, for patient-doctor relationship, for training of research ethics committee members etc, and (2) the implications of ethics, that is, the role of ethics in these developments. Ethics not just saying no, of setting limits; it can also point to important issues needing further research.

The spectacular progress does not mean that all ethical issues are solved. Safety issues are obviously important. There are indications that certain types of carbon nano tubes give rise to asbestos-like pathogenic effects related to their structure and length. Studies on rats have shown that nanoparticles can be absorbed via the nose and then transported to the brain where they accumulate. But the extent to which this gives rise to health hazards remains to be studied in more detail. Little is also known about the health impact of cosmetics containing nanoparticles.

However, there is also in danger in focussing only on standards of safety. An ethical analysis of any emerging technology needs to go beyond risk assessment. In fact, excessive focus on risk assessment can be a way of preventing other ethical issues from getting the attention they deserve. These issues range from anthropological issues to consumer freedom, patenting, access and global justice.

There are several well-established ethical points of departure for the discussion of such issues, including utilitarian ones, as well as those based on human rights and human dignity. Fortunately, we do not have to invent the wheel. For the policy debates on problems raised by new and emerging technologies, like synthetic biology, nanotechnologies, information and communication technologies, a starting point are the values enshrined in a number of international documents from the UN, the EU and the Council of Europe.

These documents have the advantage that they have been discussed publicly and have mangaged to get political support. The disadvantage is that they are somewhat vague, and are open to several interpretations. But the positive side of this vagueness is that it facilitates a living debate on the meaning and implications of the articles in these documents. Communication, not just information, is needed.

EXTRACORPOREAL BLOOD PURIFICATION IN INTOXICATED RATS USING FUNCTIONALIZED NANOMAGNETS

INGE K. HERRMANN, Andrea Schlegel, Rolf Graf, Melanie Hasler, Christoph M. Schumacher, Martin Urner, Pierre-Alain Clavien, Wendelin J. Stark, Beatrice Beck-Schimmer*
University and University Hospital Zurich and ETH Zurich, CH

Background: Nanomagnets with metal core are promising agents for both magnetic drug delivery and blood purification due to superior magnetic properties compared to commonly used beads. This presentation will discuss the direct removal of harmful substances from blood by the use of functionalized magnetic metal nanoparticles. We show a first technical implementation of magnetic separation-based blood purification in a rat model of drug intoxication.

Methods: In male intoxicated Wistar rats, the right carotid artery was catheterized and connected through an extracorporeal loop to the right external jugular vein. Blood was pumped through the extracorporeal circuit. Magnetic nanoparticles carrying capturing moieties were continuously injected into the circulating blood through an injection port. Before the blood re-entered the jugular vein, toxin-loaded nanomagnets were separated by a magnetic separator. Toxin plasma concentrations were monitored over time.

Results: We present the first successful technical implementation of a nanomagnet-based blood purification process into an in vivo model. A rat's blood was successfully detoxified in a dialysis-like extracorporeal blood purification circuit within less than one hour. Overdosed drugs and metal ions can be rapidly and efficiently removed from whole blood using functionalized nanomagnets as selective capturing moieties.

Conclusions: Magnetic separation-based blood purification was successfully implemented in a rat model of intoxication and is a potent new method to selectively remove noxious compounds from blood not limited to size-selective criteria. The use of an exteriorized artificial circuit allows safe operation outside the body once high magnetic separation efficiency has been confirmed.

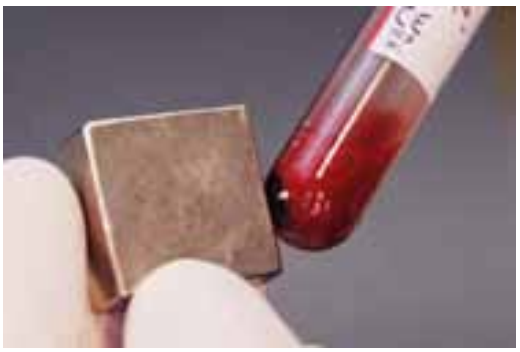

Figure: Magnetic separation of carbon-enapsuled nanoparticles from whole blood.

ANGIOSCAFF - REGENERATIVE MEDICINE

JEFFREY HUBBELL

The AngioScaff Large Scale Integrative Project of FP7 is dedicated to development and characterization of angiogenesis-inducing bioactive and biofunctional scaffolds for tissue repair and regeneration. The AngioScaff consortium innovates both in the scaffold per se and in the biofunctional ligands that are incorporated into the scaffolds, for example developing novel variant forms of growth factors and novel biomolecules to modulate the activity of growth factors. Both biological and synthetic scaffold materials are being developed. Clinical targets in skin, bone, muscle and nerve repair are being pursued, along with basic models of angiogenesis.

BRINGING NANOMEDICININE FROM THE BENCH TO THE BED: THE QUEST FOR CURATIVE PARADIGMS

PATRICK HUNZIKER
Prof. Dr. med.; University Hospital Basel, Switzerland

For important diseases of today's world, medicine offers only partial solutions that postpone critical events rather than fundamentally curing a disease process. Examples are
• cancer, where typical therapies postpone death by months to a few years;
• atherosclerosis, where therapy and secondary prophylaxis reduce acute risk, but patients almost inevitably return with recurrent atherosclerotic manifestations;
• infectious disease, where improved rapid diagnostic testing and antimalarial therapy and protective measures may initially reduce the patients risk but may also increase the severity of future infection due to loss of partial immunity.

Nanomedicine is an ideal approach to render therapy more focussed, more effective, and less toxic. The challenge now is to find comprehensive management schemes that integrate personalized diagnosis based on array diagnostics, genomic and proteomic analysis with nano-based materials and drugs and find clinical scenarios that achieve persistent cures.
• What are the critical parameters predicting disease course and how can this information flow into the design of personalizable nanomedicines that are highly active, minimally toxic, and economically reasonable ?
• How to treat a cancer such that it does not recur ?
• How to treat atherosclerosis such that the risk for myocardial infarction or stroke within a time window of ten years is close to zero ?
• How to design a nano-based malaria vaccine such that it confers long-lasting immunity ?

Nanomedicine has already shown that it can confer gradual benefits to patients with severe disease. This talk discusses frontiers that need to be crossed to progress beyond gradual benefits and achieve breakthroughs with a major impact in our quest to spend our limited lifetime in better health, thanks to curative nanomedical approaches.

CARDIOVASCULAR APPLICATIONS IN NANOMEDICINE - OVERVIEW

PATRICK HUNZIKER
Prof. Dr. med.; Cardiologist, Deputy Head of the Clinic for Intensive Care of the University Hospital Basel (CH)

Fascinating advances have been achieved by nanomedicine developments in the fields of cancer drug delivery, and have led to important insights into nanomaterial design strategies and have elucidated many aspects of the interaction of nanomaterials with life at the cellular level, at the organ level, and at the level of the whole organism. These insights form an important basis of knowledge to application of the principles of nanomedicine to widespread diseases not yet in the full focus of nanomedicine development: Inflammatory disease and infection, cardiovascular disease, inborn errors of metabolism, and brain diseases. Here we use the example of atherosclerotic disease and inborn error of metabolism to delineate the challenges of applying nanomedicine in diseases where the goal of a therapy is not to destroy target cells, and present the intelligent nano-objects with increasing complexity beyond drug delivery as new therapeutic paradigm. Cardiovascular disease, and in particular atherosclerosis, is the leading killer disease in the western world. Atherosclerosis was long thought to be a «degenerative» disease, but this notion was abolished when it became clear that some individuals may achieve a very old age but still may be remain essentially free of atherosclerosis. Clinical events caused by atherosclerosis include stroke, angina pectoris, heart attack, arrhythmias, heart failure, and

many other manifestations, which are a heavy burden not only to the affected patients but also to the healthcare systems as such. Atherosclerosis is currently understood as a complex disease with important genetic, nutritional, metabolic, biomechanical, and in particular inflammatory causes and cofactors. Very early atherosclerotic lesions show clear evidence of endothelial activation, e.g. of the selectine family, which go together with alterations of vascular permeability. Then, cellular invasion of the arterial wall by various immune cell types is observed. Once these immune cell types have left the blood pool, a complex maturation process into different phenotypes e.g. of macrophages occurs; these different phenotypes have differential biologic and clinical effects, some of which may be beneficial, may lead to slow disease progression or to sudden complications. While simple target cell killing of atherosclerotic-related cells has important downsides, we will show several possible biological targets for nanomedicine and use experimental data to discuss current achievements and limitations of nanomedical targeting strategies in atherosclerosis. As therapeutic activities beyond target cell killing is a requirement for many diseases beyond cancer and atherosclerosis, we will then discuss the concept of complex, intelligent nanomaterial like artificial polymeric/organic nanosize organelles. Biological organelles are typically characterized by an own physicochemical environment, e.g. through an enclosing membrane, by an own enzymatic equipment, and in particular by a specific biological task within the host cell. Inspired by these principles of life, we will introduce the concept of synthetic nanoscale organelles built from polymeric-biological hybrid materials based on our experimental results (e.g., Benhaim, Hunziker et al, NanoLetters 2008). This evolution of complex functional nanosystems goes well beyond current drug delivery strategies. Its potential, but also the limitations of current knowledge render this field not only an important area of development but also a fascinating new challenge to scientific exploration. The question, how much «intelligence» or complexity will fit in a nanoscale object lead to the theoretical and practical question of ultimate limits in terms of «intelligence per size» ratio, which may have reached in living structures, but where technological development is still far from the optimum. Medical applications of such artificial organelles in hereditary disorders of metabolism will be added to show the genericity of the approach.

OSTEOARTICULAR AND DENTAL REGENERATIVE NANOMEDICINE

NADIA BENKIRANE-JESSEL
INSERM, UMR977, Strasbourg, France

We report here, a «Smart Hybrid Materials Equipped by Nanoreservoirs of Therapeutics» a unique nanotechnology strategy used to entrap, protect, and stabilize therapeutic agents into polymer coatings acting as nanoreservoirs enrobing nanofibers of implantable membranes. Upon contact with cells, therapeutic agents become available through enzymatic degradation of the nanoreservoirs. As cells grow, divide, and infiltrate deeper into the porous membrane, they trigger slow and progressive release of therapeutic agents that, in turn, stimulate further cell proliferation. This constitutes the first instance of a smart living nanostructured hybrid membrane for regenerative medicine. The cell contact-dependent bioerodable nanoreservoirs described here will permit sustained release of drugs, genes, growth factors, etc., opening a general route to the design of sophisticated cell-therapy implants capable of robust and durable regeneration of a broad variety of tissues.
ARTiOS NanoMedicine Start-up Project

1. F. Fioretti, C. Mendoza-Palomares, M. Helms, D.A. Alam, L. Richert, Y. Arntz, S. Rinckenbach, F. Garnier, Y. Hakel, S.C. Gangloff, **N. Benkirane-Jessel**, ACS Nano. 2010, 22, 3277.
2. S. Facca, C. Cortez, C. Mendoza-Palomares, N. Messadeq, A. Dierich, A.P.R. Johnston, D. Mainard, J.C. Voegel, F. Caruso, **N. Benkirane-Jessel**, Proc. Natl. Acad. Sci. USA. 2010, 107, 3406.
3. C. Mendoza, A. Ferrand, S. Facca, F. Fioretti, D. Mainard, **N. Benkirane-Jessel**, ACS Nano. 2012, 6, 483.

NANOPARTICLE-BASED CANCER THERAPY FOR PATIENTS WITH RECURRENT GLIOBLASTOMA

ANDREAS JORDAN
MagForce Nanotechnologies AG, Max-Dohrn-Str. 8-10, 10589 Berlin, Germany, Charité Universitaetsmedizin Berlin, Klinik für Strahlentherapie, Augustenburger Platz 1, 13353 Berlin, Germany

Thermotherapy using magnetic nanoparticles (NanoTherm® therapy) is a new approach for the local treatment of solid tumors and one of the first clinical applications of nanotechnology in cancer therapy. The principle of the method is the direct introduction of a dispersion of magnetic nanoparticles (NanoTherm®) into a tumour and their subsequent activation by an 100 kHz alternating magnetic field (NanoActivatorTM) to produce heat. The magnetic fluid consists of superparamagnetic iron-oxide nanoparticles (iron concentration 112 mg/ml) with a mean diameter of 12 nm and an aminosilane type shell. MagForce received European regulatory approval for its medical products NanoTherm and NanoActivator for the treatment of brain tumours in 2010. Further multicentre randomized clinical trials for GBM and prostate carcinoma patients will start this year. Currently a new generation of nanoparticles is being developed, which can offer even greater therapeutic potential to the NanoTherm therapy. Through modification of the nanoparticle surface with functional drug delivery systems, it is possible to establish a temperature dependent release of drugs. Examples of those new constructs and their functionality are described. Through the enhancement of the antitumor effect of those so-called conjugates in comparison with the heat application alone (non-conjugated particles), the number of particles required to inactivate a certain number of cancer cells might be decreased. With further optimization of the particle performance, targeted approaches with systemic delivery of tumor-specific nanoparticles will become more and more feasible.
Another new product development are the so-called surgical pads, which allow for the first time a laminar heat delivery, e.g. to heat bone surfaces as well as the laminar heating of a resection wall after complete resection of a solid tumor. The heat performance of those pads can be adjusted to the required power deposition through the number of polymer matrix layers and the nanoparticle concentration under AC magnetic field excitation. Further indications for NanoTherm® will be possible through these new developments, which might be difficult to access by direct injection of the nanoparticles into the tumor as it is the conventional route of delivery so far.

NANOPARTICULATE ORAL DOSAGE FORMS – IMPLEMENTATION OF INDUSTRIAL REQUIREMENTS

MICHAEL JUHNKE
Novartis Pharma AG, Technical R&D, P.O. Box, CH-4002 Basel, CH

Nanoparticulate drug delivery approaches are expected to address challenges in the delivery of modern and conventional drugs for targeting tissues, accessing deep molecular targets and controlling drug release [1]. Orally administered nanoparticulate drugs have shown increased dissolution rates and bioavailability as well as allow to reduce doses, limit pharmacokinetic variability and eliminate food effects [2, 3]. Marketed nanoparticulate drug products are available for oral administration as solid and liquid dosage forms [2, 3]. Basically, bottom-up and top-down approaches can be considered for the production of nanoparticulate drugs [2, 3]. Drug compounds targeted for oral administration are mostly produced with about 100 – 200 nm drug particle size as nanoparticulate drug suspensions [2, 4]. Those drug nanosuspensions are versatile intermediates, which can be converted into standard oral dosage forms, like capsules, tablets and orally administered suspensions [3, 4].

Pharmaceutical industry developing new molecular entities (NMEs) is facing unprecedented challenges to its business model, due to patent expirations of marketed products, increased duration and costs

for R&D per product launch, and, limited output of truly innovative 'first-in-class' medicines [5, 6]. In addition, there is ample evidence that more complex NMEs are generated during discovery with limited solubility and bioavailability, decreasing chances for successful development and commercialization [7, 8]. Hence, the classical R&D business in pharmaceutical industry is currently in a state of upheaval to optimize productivity and flow of truly innovative new medicines [5, 9]. In order to optimize R&D approaches, shifting attrition of NMEs to the early stage of development, and, reducing cycle times during development are typically pursued [5, 10]. In consequence thereof, the decision-making assessment at the early stage of development, regarding efficacy, toxicity and formulation principle has to be performed for an increased number of NMEs with minimal cycle times, and, with minute amount of NME available [10]. For clinical development, especially at late stage, reduced cycle times will provide an optimized R&D productivity with pivotal clinical trials as rate limiting step and technical deliveries of the supply chain in advance [5].

Today's demands for an optimized productivity in R&D can be translated to the development of dissolution rate limited and less bioavailable NMEs as nanoparticulate drugs with technical requirements, like, available and predictable at the pre-clinical development stage, suitable for high dose toxicity studies, short cycle times especially at pre-clinical stage and first-in-human study, flexible regarding complex properties of NMEs, applicable for a wide dose range including high doses, easy manufacturability of the desired market form, reliable up-scaling and finally, accepted by health authorities. The production of drug nanosuspensions by wet media milling is universally applicable with respect to properties of drug, available from pre-clinical to production scale, providing reliable up-scaling and, is regulatory accepted by several marketed products [2-4]. Drug nanosuspensions are directly utilized at pre-clinical development stage for efficacy and toxicity assessments [2, 10]. Further clinical development is mostly aiming for solid oral dosage forms. Therefore, drug nanosuspensions are produced as intermediates by wet media milling, which will be converted by established drying technologies, like spray-granulation or spray-drying, into redispersable drug nanoparticulate powders suitable for capsules and tablets [4, 11, 12]. Advantageously, the formulation principle is preserved from pre-clinical development stage to commercialization.

Here, today's requirements in pharmaceutical R&D for the development of NMEs in relation to dissolution rate limited and less bioavailable drugs targeted for oral administration will be presented. The technology related implementation for developing nanoparticulate drugs by wet media milling, including the process and analytical toolbox, especially at the pre-clinical and first-in-human development stage will be addressed in the presentation.

[1] Petkar KC, Chahvan SS, Agatonovik-Kustrin S, Sawant KK, Crit. Rev. Ther. Drug Carr. Syst. 28 (2011) 101-164.
[2] Kesisoglou F, Panmai S, Wu Y, Adv. Drug Delivery Rev. 59 (2007) 631-644.
[3] Singh A, Worku ZA, van den Mooter G, Expert Opin. Drug Delivery 8 (2011) 1361-1378.
[4] Merisko-Liversidge E, Liversidge GG, Adv. Drug Delivery Rev. 63 (2011) 427-440.
[5] Paul SM, Mytelka DS, Dunwiddie CT, Persinger CC, Munos BH, Lindborg SR, Schacht AL, Nat. Rev. Drug Discovery 9 (2010) 203-214.
[6] Scannell JW, Blanckley A, Boldon H, Warrington B, Nat. Rev. Drug Discovery 11 (2012) 191-200.
[7] Thomas VH, Bhattachar S, Hitchingham L, Zocharski P, Naath M, Surendran N, Stoner CL, El-Kattan A, Expert Opin. Drug Metab. Toxicol. 2 (2006) 591-608.
[8] Leeson PD, Springthorpe B, Nat. Rev. Drug Discovery 6 (2007) 881-890.
[9] PricewaterhouseCoopers, Pharma 2020: Virtual R&D – Which path will you take?, 2008. Available at http://www.pwc.com/pharma2020, e-accessed 23-March 2012.
[10] Saxena V, Panicucci R, Joshi Y, Garad S, J. Pharm. Sci. 98 (2009) 1962-1979.
[11] Van Eerdenbrugh B, Van den Mooter G, Augustijns P, J. Pharm. Sci. 364 (2008) 64-75.
[12] Kayaert P, Anné M, Van den Mooter G, J. Pharm. Pharmacol. 63 (2011) 1446-1453.

NANOMEDICINE LINKS WITH BIOELECTRONICS FOR CARDIOVASCULAR APPLICATIONS

VARVARA KARAGKIOZAKI
Dr. Med., MSc; 1. Nanomedicine Group, Lab for "Thin Films -Nanosystems & Nanometrology", Department of Physics, Aristotle University of Thessaloniki, Greece
2. AHEPA Hospital, AUTh, Thessaloniki, Greece

Organic bioelectronics holds promise to make major contribution to nanomedicine. Especially, how living cells respond to conductive polymers is of paramount importance for tissue regeneration activities. In the case of drug eluting stents (DES), the delayed endothelialization caused by the elution of anti-proliferative drugs may cause late stent thrombosis and subsequent clinical manifestations of unstable angina, heart attack or even cardiac arrest.
In this study, the efficacy of diverse formulations of Poly (3, 4-ethylenedioxythiophene) poly (styrenesulfonate) (PEDOT: PSS) nanocoatings to promote the proliferation of L929 fibroblasts on the samples was studied. The PEDOT: PSS dispersions with the addition of 6% (% v/v) dimethyl sulfoxide to increase its conductivity were filtered and spin-coated. Nanoscale techniques such as Atomic Force Microscopy were implemented for surface characterization of the nanomaterials. Their biological behavior was assessed by MTT cell proliferation/cytotoxicity assay combined with SEM studies.
The cellular response towards the electronic nanomaterials was analyzed and the results were discussed in terms of surface nanotopography and conductivity. AFM studies shown that the engineered biomaterials are atomically smooth. It was revealed that the PEDOT nanocoatings (having thickness below 100nm) with higher conductivity promote cellular adhesion and showed higher cytocompability compared to the ones with lower conductivity and controls. SEM images verify the above findings. This study highlights the potential application of conductive polymers as stent nanocoatings to enhance tissue regeneration onto stent surface for avoidance of late stent thrombosis.

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PLASMONIC PHOTOTHERMIC ANGIOPLASTY WITH MULTIFUNCTIONAL NANOPARTICLES AND STEM CELLS AS THE NEW TOOL FOR INTERVENTIONAL CARDIOLOGY

ALEXANDER N. KHARLAMOV, Perrish A.N., Gabinsky J.L., Matveev S.A.
Ural Institute of Cardiology, Yekaterinburg, Russia
Ural State Medical Academy, Yekaterinburg, Russia

Background. Some modern angioplasty techniques generally just affect the geometry of the plaque and have some inherent clinical and technical limitations. Our previous bench-to-bedside studies confirmed high efficacy and safety of nanomedicine-based approach for the management of atherosclerosis.
Methods. A total of 120 patients 45-65 years old with PCI (percutaneous intervention) and CABG (coronary artery bypass surgery) indications were assigned to the three groups (40 patients into the group with PCI indications and nanointervention but without stenting, 40 – cardiac surgery group with nanointervention, and 40 – with PCI indications to sirolimus stenting control). Patients with PCI indications underwent delivery of nanoparticles (NPs) inside of induced pluripotent stem cells (iPS) or CD73+CD105+ mesenchymal stem cells (MSCs) in medium via catheter-based percutaneous intra- and transmural injection into the plaque and artery. CABG patients run the delivery with bioengineered on-artery patch on the basis of bovine scaffold and iPS or MSCs (with NPs) by MICS (mini-invasive) cardiac surgery. We have used a modified method for the preparation

of 90-100 nm versatile NPs with iron-silica core and gold-polymeric shell as described by Lee (2008) and Deng H (2005). Studied 10 mm pull-back of proximal left anterior descending arteries was observed by 40-45 MHz near-infrared spectroscopy (NIRS) and virtual histology intravascular ultrasound (IVUS-VH).

Results. A change of the total vessel volume - TVV (mm3) immediately after the laser irradiation/ in 24 weeks in groups were -18.9/ -46.2%, -10.8/ -33.6% and -1.1/ -2.2% (p<0.01) respectively, total plaque volume (TPV) was changed from 233 to 229/209, 236 to 230/221, and 238 to 222/219 mm3 (p<0.01), total lumen volume (TLV) – 304 to 305/318, 303 to 305/ 311, 305 to 317/ 315 mm3 (p<0.05) from baseline to immediately/ at week 24 in groups respectively. Restenosis confirmed in 3 (7.5%) patients of stenting group only. An impact over mineral deposits and calcium necrotic core was predominated in PCI group (-33.4% vs -22.1% and +3.7% respectively, p<0.005). Anti-inflammative and anti-apoptotic effects, signs of neovascularization and restoration of artery function were predominated in subsets with progenitor cells (p<0.01). Coronary flow-mediated vasodilation was observed after hyperemia and injection of nitroglycerine (+10.2 and +16.6%, +8.2 and +9.6%, +8.1 and +9.8% in groups respectively, p<0.05). Mean hazard ratio between PCI group and stenting control if compare with CABG and stenting control achieved 1.05 (CI 95%: 0.95-1.16, p<0.05) and 1.03 (CI 95%: 0.93-1.09, p<0.05) with favor of nanomedicine-related approaches.

Conclusion. Plasmonics using multifunctional (imaging and therapy) nanoparticles is being the high-effective and safe alternative to stenting and CABG for angioplasty especially in combination with stem cells promising the rejuvenation of arteries and revolutionizing current strategy in patients with coronary artery disease.

Clinical development. On the basis of our previous bench and bedside experience, we have proposed a new conception of the optimal composition for nanoparticles and relevant clinical strategy. The potential progress of the technology includes developing of two different approaches for delivery with the use of induced pluripotent stem cells (iPS) and MSCs as the main carriers for NPs. The first approach has more indications for PCI patients and implicates micro-infusion catheter-based [Mercator] trans- or intramural injection of stem cells (in gel or medium) into the artery or perivascular tissues (on the same admission day under the control of IVUS). The second delivery route has a clinical potential for patients with CABG indications, and includes growing of the bioengineered patch (with bovine decellularized or polymeric scaffold and autologous or allogenious recovered stem cells) with the subsequent mini-invasive on-artery open heart transplantation (3-6 weeks procedure under the imaging control).

The main strategy is focused on the use of composite NPs. A core/ shell ratio should be achieved until with a total radius less than 50 nm as this is an optimal nanodetonation parameters for the transcutaneous activation with a microwatt near-infrared (NIR) laser (developing Lipiscan-based IVUS/NIR spectroscopy + NIR high-energy laser, InfraReDx) verified on the level of 821 nm, 35-44 W/cm2 for 7 minutes exposure with estimated 7-10 cm penetration of tissue, for percutaneous catheter-based activation (with Mercator injector) - irradiation with focused laser pulses such as 420-820 nm, 12 ns, 0.1-5 J/cm2, 100 pulses. The team expects an influence of the following tissue-destructive factors: high-heat plasmonic detonation of nanoshells with irreparable burning/ melting of targeted tissues, vapor bubbling of cellular cytoplasm and extracellular matrix with subsequent degradation/ melting of tissues, and destructive effects of acoustic and shock waves. Novel composite multifunctional nanoparticle with both diagnostics (IVUS, OCT, new acousto-optics development) and treatment (drug delivery, plasmonic photothermic destruction, antibodies-mediated against macrophages, polymeric-related cleaning of cholesterol) potentials with support of stem cells as carriers and tissue keeper (metabolic and repair properties) (see table 1). New clinical strategy (see table 2) and innovative nanotechnology system potentially may become an alternative to stenting and CABG.

Synergistically combining PPTA and NPs-related plaque imaging, these composite NPs offer the potential to revolutionize the detection, diagnostics, follow-up and multi-component treatment of CVD.

Keywords: nanoparticles, plasmonics, angioplasty, atherosclerosis, stem cells, IVUS, stenting, CABG.

Table 1: Novel proposed composite nanoparticle with optimal structure for optimal clinical development.

Composition	Structure	Meaning and properties
Core (50-70%)	Silicon	• Good dielectric, metalloid and energy absorber with optimal potential for PPTT (melting point - 1414ºC)
1st Inner Layer (15-30%)	Gold Alloy, White Gold, or Composition of Platinum and Silver	• Good PPTT booster with effect of the energy mirror and higher level of the energy accumulation. Gold and silicon are the best composition for PPTT due to optimal thermal conductivity and cross-enhancement properties. The main task for colloidal chemistry now is to generate a composition with melting point higher than 1100°C in order to synthesize graphene on the surface of nanospheroid (melting points for transition metals: silver - 961ºC, gold - 1064ºC, white gold with palladium - 1097ºC, platinum - 1769ºC; metal "flow" is slightly above the melting point ±100ºC).
2nd Inner Layer (10-15%)	Silicon Carbide	• Energy absorber with optimal potential for PPTT. Ideal basis for an epitaxial growth of graphene on the surface with heating process (1100 degrees of centigrade or higher, melting point for silicon - 1414ºC).
Outer Layer (molecular mono- or few layered sheet)	Graphene Oxide	**Unique properties** • non- or low-toxic (oxidative stress, needle-like impact), paramagnetic, high thermal conductivity with low interfacial thermal resistance • high optical transparency, high mechanical strength and flexibility • antibacterial properties, good visibility with X-ray, OCT, IVUS and optico-acoustics approaches (great, or even ideal diagnostics potentials)
Corona (out of metal spheroid, but bound to outer layer via collagen-like domain)	Polymers (poly (ethylen) glycol – PEGylated lipids, PEG-COOH, and poly-lactic acid-based) + Specific Antibodies/ Peptides	**Potentials for** • excellent absorption of the NPs by cell • optimal tool for antibodies/ peptides binding – target therapy against macrophages, etc. (some targetable receptors: ICAM-1, VCAM-1, P-selectin, E-selectin, CD36, CD68, SR-A1, LOX-1, $\alpha_v\beta_3$-integrin) + stem cells' binding • drug delivery system (with anti-platelet drugs, particularly ticagrelor) • cleaning/ brushing cholesterol whereas properties of polymers • imaging potentials using Gd-DTPA amphiphile (MRI signal ehancement); MRI contrast agent gadolinium • catalase enzyme: prevention of oxidative stress (incl. graphene-related) • VEGF: management of neovascularization • Anti-toxic properties: PEGylation of grapheme oxide and noble metals dramatically attenuates nanotoxicity

Table 2: Novel proposed clinical strategies in patients with indications for PCI and CABG.

Type of intervention	Patients with indications for PCI	Patients with indications for CABG
Admission	All-comers, early <6 hrs, late-comers >12hrs	All-comers, early <6 hrs, late-comers >12hrs
Prior diagnostics	Grey-scale IVUS with NIR spectroscopy (NIRS) and mapping of vessels (on the same admission day). We can utilize one IVUS-catheter to resolve simultaneously two objectives: to scan a vessel (with NIRS) prior to further activation of our NPs (with catheter-based laser on the platform of Lipiscan) with the use of different energy parameters, but in NIR spectrum	Grey-scale IVUS with NIRS and mapping of vessels (on the same admission day)
Stenting	Stenting with biodegradable or DES stent (admission day). Polymer or magnesium alloy biodegradable stent allow sustain a lumen for a while, and being a carcass for a vessel, preventing collapse and negative remodeling of artery after the nanointervention	Stenting with biodegradable or DES stent (admission day) if necessary
Nanointervention	1) Recover and sorting of stem-progenitor cells from circulation (rear-event FACS with MoFlo of Beckman-Coulter) and/ or fat (Celution systems, Cytori Therapeutics) and/ or bone marrow (MarrowXpress, ThermoGenesis) (admission day). 2) Generation of the composite NPs (with modified aqueous-phase method) and cultivation in the medium with stem-progenitor cells (1-2 days; admission day + extra 1 day in case of culturing) 3) Intervention – PCI-like with catheter (on 2nd day of admission) with (a) InfraRedx IVUS (to map a vessel), MRI control (MRI-angiography-like, or MRI temperature imaging options); (b) Mini-injection of stem cells with NPs in gel with Mercator-injector directly into the plaque (both to atheroma and adventitia, or pervascularly) with subsequent InfraReDx IVUS follow-up, MRI follow-up; (c) InfraReDx catheter NIR laser irradiation on site, or laser NIR transcutaneously (not focused and penetration is 7-10 cm)	1) Recover and sorting of stem-progenitor cells from circulation (rear-event FACS with MoFlo of Beckman-Coulter) and/ or fat (Celution systems, Cytori Therapeutics) and/ or bone marrow (MarrowXpress, ThermoGenesis) (admission day). In that case we have to launch a growing of the 'bioengineered on-artery' patch immediately 2) Growing of bioengineered on-artery patch (3-5 weeks after recover) on the basis of bovine decellularized or polymeric scaffold with allogenious or autolgous iPs and MSCs. 3) Generation of the composite NPs and cultivation in the medium with stem-progenitor cells (1-2 days; admission day + extra 1 day in case of culturing) 4) Intervention – CABG-like with IVUS-catheter follow-up (in 3-5 weeks afterwards) with (a) InfraReDx IVUS (to map a vessel) and MRI follow-up; (b) transplantation of bioengineered patch (grew with autologous and allogenious iPS or MSCs) directly on targeted artery with fixation on free myocardium with subsequent InfraReDx IVUS, MRI follow-up; (c) InfraReDx catheter NIR laser irradiation on site, or laser NIR activation of NPs transcutaneously (not focused and penetration is 7-10 cm)
Total duration of the intervention, days	1-2	30-45
Follow-up	InfraRedX IVUS follow-up, MRI follow-up	InfraRedX IVUS follow-up, MRI follow-up

PET/CT AND PET/MRI FOR MOLECULAR IMAGING

ANDREAS KJAER

Prof. Dr.med. MD, DMSc,; Dept. of Clinical Physiology, Nuclear Medicine & PET, Copenhagen University Hospital, Copenhagen (DK). President of Scandinavian Society for Clinical Physiology and Nuclear Medicine

The change in paradigm towards individualized, tailored therapy has led to a need for diagnosing at the molecular level. Most of the molecular biology methods used today need tissue sampling for in vitro analysis. In contrast, molecular imaging allows for non-invasive studies at the molecular level in living, intact organisms. With PET

it is possible to label bio-molecules with radioactive isotopes. This method can be used for non-invasive visualization of tumor specific receptors and tissue characteristics such as angiogenesis and ability to metastasize. Especially within cancer biology the technique is expected to lead to a break-through in diagnosing and treatment. Among the different techniques for molecular imaging, the nuclear medicine based technologies have the greatest potential for translational use since methods developed in animal models may directly be transferred and used in humans. Furthermore, PET has a high sensitivity and allows for quantification. The lecture will present some recent examples from our institution on how PET/CT and PET/MRI may be used for tailoring and monitoring cancer therapy.

NANOSTRUCTURED POLYMER CONTAINERS FOR MULTIPLE STIMULI DRUG CONTROLLED RELEASE

GEORGE KORDAS[*a], P. Bilalis[a], E. K. Efthimiadou[a], L.-A. Tziveleka[a], A. Chatzipavlidis[a, b], C. Tapeinos, A.– F. Metaxa[a] and G. S. Pappas[a]

[a] NCSR "Demokritos", Institute of Materials Science, Aghia Paraskevi Attikis, Athens, GR-15310, Greece.
[b] National Technical University of Athens, School of Chemical Engineering, 9 Heroon Polytechniou St, Zografos, Athens GR-15780, Greece.

Recent research at the intersection of physical and life sciences has revealed the utilization and applicability of various smart nanocontainers, of sophisticated architectures, composed of different materials and structures,1-3 in the field of nanomedicine. Modern drug delivery systems exhibit sufficient chemical stability, biocompatibility, and controllable size over the range of nanoscale, large surface areas, while combining interior networks and cavities that could play the role of drug reservoir.
Herein we present on the one hand, the synthesis of novel magnetic, pH and redox sensitive nanocontainers using a sacrificial template-directed synthesis procedure4 followed by chemical deposition of magnetic nanocrystals via co-precipitation (Fig. 1a). Our objective is to engineer a new nanoscopic device that combines stability, high drug loading along with pH and redox response. The key features of our fabricated nanocontainers are their gradual and controlled collapse once met with highly reducing environment in combination with operative pH responsiveness (Fig. 1b). Furthermore, their magnetic response could be further utilized for efficient magnetic hyperthermia performance. In this way, a diverse set of triggers is combined in one nano-platform.

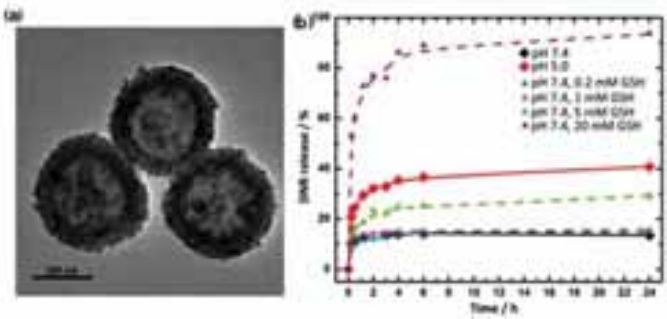
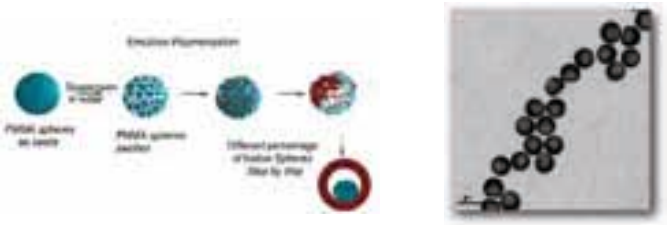


Fig. 1: (a) TEM image and (b) pH and redox responsiveness of the synthesized magnetic nanocontainers

On the other hand, we present the synthesis of hollow microspheres via seed emulsion polymerization. The shell of these nanospheres can be formed using different monomers that respond to different stimuli such as temperature, pH and redox potential. In Figure 2a a schematic illustration of the formation of hollow spheres by seed emulsion polymerization methodology5,6 is presented. In Figure 2b, the Strawberry-like surface is depicted in which a cavity is formed.7 The loading and triggered release behavior has been investigated employing Doxorubicin hydrochloride (DOX) as a model drug.
We consider that the polymer nanocontainers described in this report represent a new generation of drug release nano-platforms for biomedical applications. Based on their distinct response to multiple stimuli, combined in some cases with good magnetic response and magnetic hyperthermia applicability, these nanocontainers can serve as multi-responsive drug carriers.
Currently, the above described empty nanocontainers are being tested in vivo using mice. Preliminary results of this study will be also reported.



a) **b)**

Figure 2. a) Synthetic scheme, b)TEM images of Strawberry-like microspheres.

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REFERENCES

1 F. Caruso, R. A. Caruso and H. Möhwald, Science,1998, 1111.
2 S. Ganta, H. Devalapally, A. Shahiwala and M. Amiji, J. Controlled Release, 2008, 126, 187.
3 M. A. Cohen Stuart, W. T. S. Huck, J. Genzer, M. Müller, C. Ober, M. Stamm, G. B. Sukhorukov, I. Szleifer, V. V. Tsukruk, M. Urban, F. Winnik, S. Zauscher, I. Luzinov and S. Minko, Nature Mater., 2010,9, 101A.
4 A. Chatzipavlidis, P. Bilalis, E. K. Efthimiadou, N. Boukos and G. Kordas, Langmuir,2011, 27, 8478.
5 Yang et al, Langmuir 24 (2008) 13736-13741.
6 Kataoka K. et al, Angew. Chem. Int. Ed. 42 (2003) 4640-4643.
7 N. Oku, Y. Namba, S. Okada, Biochim. Biophys. Acta 1126 (1992) 255– 260.

FLUORESCENT POLYCATIONS AS FAST STAINING AGENT FOR CHARGE DISTRIBUTION ON TUMOR CELLS

SILKE KROL, Subhra Mandal, Giacinto Scoles

Since the beginning of chemotherapy it is known that cancer cells have somehow different membrane properties than normal cells and in consequence polycations were one of the first chemotherapeutics. However due to the high toxicity to normal body cells this approach was abandoned. Nowadays other chemotherapeutics are available and the polycation-tumor cell interaction was long forgotten.
Recently when we started a study about the charge distribution on growing fungi and dividing cells we rediscovered the special membrane properties of cancer cells. The findings may have an impact in the detection of circulating cancer cells (CTC) in peripheral blood of cancer patients. Early detection as well as therapeutic follow-up holds a huge potential for the prediction of the development of the disease in terms of relapse and/or metastatic spreading and the prognosis for the patient. One of the major obstacles in the exploration of the useful tool and its translation in clinical routine is the rarity of the event to find single CTCs in the huge number of blood cells and its significance for the outcome of the disease. Several techniques are used in order to extract CTCs from the patient's blood like gradient centrifugation or more specifically immunomagnetic isolation, an antibody guided magnetic bead binding and magnet aided removal of the marked cells from the whole pool of blood cells (e.g. Cristofanilli et al. 2004 and for a review: Allan and Keeney 2010). Some results from previous clinical studies indicate that an accuracy of 1 to 1.000.000 cells is required for predicting the prognosis of patients with metastatic cancers as the threshold between good and

poor prognosis is as low as 3-5 cells per mL blood volume (de Bono et al. 2008).
In the blood stream the CTCs can be recognized by these features, such as membrane elasticity, surface charge, or for arrest required surface proteins if selective markers can be identified. We expect that with this technique we may be able in the future to provide a fast and reliable technique to identify the tumor cells in blood even those which can escape recognition by antibody because it has a different match of surface receptor.

REFERENCES

• Agatha G, Häfer R, Zintl F (2001) Fatty acid composition of lymphocyte membrane phospholipids in children with acute leukemia. Cancer Lett 173:139-144
• Allan A. L. & M. Keeney "Circulating Tumor Cell Analysis: Technical and Statistical Considerations for Application to the Clinic," Journal of Oncology, 1-10, 2010.
• Boussif O, Delair T, Brua C, Veron L, Pavirani A, Kolbe HV (1999) Synthesis of Polyallylamine Derivatives and Their Use as Gene Transfer Vectors in Vitro. Bioconjugate Chem 10:877-883
• Cristofanilli M., G. T. Budd, M. J. Ellis, et al., "Circulating tumor cells, disease progression, and survival in metastatic breast cancer," The New England Journal of Medicine, 351, 781–791, 2004.
• Cross SE, Jin Y-S, Tondre J, Wong R, Rao J, Gimzewski JK (2008) AFM-based analysis of human metastatic cancer cells. Nanotech 19:384003-384011
• de Bono J. S., H. I. Scher, R. B. Montgomery, et al., "Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer," Clinical Cancer Research, 14, 6302–6309, 2008.
• Dobrzynska I, Szachowicz-Petelska B, Sulkowski S, Figaszewski Z (2005) Changes in electric charge and phospholipids composition in human colorectal cancer cells. Mol Cell Biochem 276:113–119
• Gottfried EL (1971) Lipid patterns in human leukocytes maintained in long-term culture. J Lipid Res 12:531-537
• Krol S, Diaspro A, Magrassi R, Ballario P, Grimaldi B, Filetici P, Ornaghi P, Ramonio P, Gliozzi A (2004) Nanocapsules: coating for living cells. IEEE TRANSACTIONS ON NANOBIOSCIENCE 3:32-38
• Liebes LF, Pelle E, Zucker-Franklin D, Silber R (1981) Comparison of Lipid Composition and 1,6-Diphenyl-1,3,5-hexatriene Fluorescence Polarization Measurements of Hairy Cells with Monocytes and Lymphocytes from Normal Subjects and Patients with Chronic Lymphocytic Leukemia. Cancer Research 41:4050-4056
• Moroson H (1971) Polycation-treated Tumor Cells in Vivo and in Vitro. Cancer Res 31:373-380
• Regelson W, Kuhar S, Tunis M, Fields J, Johnson J, Gluesenkamp E (1960) Synthetic Polyelectrolytes as Tumour Inhibitors. Nature 186:778-780
• Rosenbluth MJ, Lam WA, Fletcher DA (2006) Force microscopy of nonadherent cells: a comparison of leukemia cell deformability. Biophys J 90:2994–3003
• Suresh S (2007) Biomechanics and biophysics of cancer cells. Acta Mater 55:3989–4014
• Suresh S (2007) Elastic clues in cancer detection Nat Nanotechnol 2:748–749

PERSONALIZED NANOMEDICINE: COMBINING NON-INVASIVE IMAGING WITH TUMOR-TARGETED DRUG DELIVERY TO INDIVIDUALIZE (CHEMO-) THERAPEUTIC INTERVENTIONS

TWAN LAMMERS

Personalized medicine aims to individualize therapeutic interventions on the basis of ex vivo and in vivo information on patient- and disease-specific characteristics. By non-invasively visualizing how well image-guided nanomedicines - i.e. submicrometer-sized drug delivery systems containing both drugs and imaging agents within a single formulation, and designed to more specifically deliver drug molecules to pathological sites - accumulate at the target site, patients likely to respond to nanomedicine-based therapeutic interventions can be preselected. In addition, by longitudinally monitoring how well patients respond to nanomedicine-based therapeutic interventions, drug doses and treatment protocols can be individualized and optimized during follow-up. Furthermore, non-invasive imaging information on the accumulation of nanomedicine formulations in potentially endangered healthy tissues can be used to exclude patients from further treatment. Consequently, combining non-invasive imaging with tumor-targeted drug delivery seems to hold significant potential for personalizing nanomedicine-based (chemo-) therapeutic interventions, to achieve delivery of the right drug to the right location in the right patient at the right time.

TARGETING BY NANOMEDICINES IN INFLAMMATORY DISEASE

CLAUS-MICHAEL LEHR[1,2], Eva-Maria Collnot[1], Fransisca Leonard[2], Efrat Harel1,[3], Nico Mell[1], Hussain Ali[2]
[1]Department of Drug Delivery (DDEL), Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz Centre for Infectious Research (HZI), Saarland University, 66123 Saarbruecken, Germany; [2]Biopharmacy and Pharmaceutical Technology, Saarland University, 66123 Saarbruecken, Germany; [3]Hebrew University of Jerusalem, Faculty of Medicine, School of Pharmacy, Institute for Drug Research, P.O.Box 12065, Jerusalem 91120, Israel

Inflammatory bowel diseases (IBD) such as Crohn's disease or Ulcerative colitis are autoimmune, chronic, episodic, inflammatory conditions of the gastrointestinal tract. Classical therapy of IBD with steroids or immunomodulators delivered via pellets, capsules, or tablets is often inefficient mainly due to limited drug release time and enhanced elimination of drug carriers as the result of diarrhoea. The emergence of biologicals such as TNF-α antibodies, has significantly increased the treatment options for IBD in recent years. Still, these new therapeutics have to be applied systemically resulting in sometimes severe adverse effects. Nanomedicine may enhance the efficacy of conventional IBD therapeutics and open up new routes of application for next generation drugs: Passively targeting the inflamed intestinal areas, nanoparticles of ~100 nm size have been shown to accumulate in the inflamed tissue, while bigger particles of 1 or 10 μm showed no specificity for diseased intestinal areas [1]. Moreover, polylactide-co-glycolide (PLGA) nanoparticles loaded with the anti-inflammatory phosphodiesterase IV inhibitor rolipram showed higher and prolonged anti-inflammatory activity and reduced central nervous adverse effects in a TNBS rat model [2].

Further investigating this EPR (enhanced permeability and retention) – like effect at the inflamed colonic mucosa we developed a co-culture model of the inflamed intestine based on Caco-2 enterocytes and blood derived macrophages and dendritic cells [3]. Via addition of the pro-inflammatory cytokine interleukin-1β a reversible inflammation can be induced in this in vitro model. The system responds to the pro-inflammatory stimulus with a decrease in barrier function, re-organization of tight junctions, release of pro-inflammatory markers and increased mucus production, thus mimicking pathophysiological phenomena observed in IBD in vivo. Different nanoformulations of the glucocorticoid budesonide were applied in the in vitro system and evaluated for their mechanism of accumulation and anti-inflammatory activity [4].
Both budesonide loaded PLGA nanoparticles and the free drug solution were able to recover epithelial barrier function (quantified via measurement of transepithelial electrical resistance) and reduce release of pro-inflammatory marker IL-8. However, treatment with free budesonide solution was only effective for up to 24 h after application as after 48 h a rebound of IL-8 release to the level of the non-treated inflamed control was observed. In contrast PLGA budesonide treated cells retained low IL-8 levels. The prolonged activity of the nanoparticulate formulation can be ascribed to the formation of local drug depots, as particles accumulate between the cells in the area of the tight junctions. A liposomal budesonide formulation

seemed to be preferentially taken up and processed by the immune cells in the co-culture model. The resulting dose dump in the immune cells negatively affected the barrier function and resulted in increased IL-8 levels.
Aiming to further enhance the efficacy of nanocarrier accumulation, active targeting ligands for the inflamed intestinal mucosa were investigated. In the inflamed colonic mucosa of colitis patients an increased expression of transferrin receptor has been reported both in the basolateral aspects of crypts and on the apical surface [5]. Indeed anti-TfR immunoliposomes applied the luminal side showed greatly enhanced adherence in vivo compared to non-non-specific immunoliposomes.
As a first topical application of a biological in IBD therapy a nanoformulations of the potent, anti-inflammatory cytokine IL-10 was developed. IL-10 is a promising IBD therapeutic, but failed in clinical trials upon subcutaneous injection, due to its short serum half-life and severe adverse effects at higher doses. Drug loaded albumin nanoparticles were prepared by spray drying and embedded in Eudragit S-100 microparticles to ensure stability during gastrointestinal passage. The nanoparticle in microparticle oral system succeeded in protecting IL-10 integrity during the preparation process and releasing bioactive cytokine at the desired pH of 7.4. In vivo testing of the oral cytokine delivery system is ongoing.

1. Lamprecht, A., U. Schafer, and C.M. Lehr, Size-dependent bioadhesion of micro- and nanoparticulate carriers to the inflamed colonic mucosa. Pharm Res, 2001. 18(6): p. 788-793.
2. Lamprecht, A., et al., Biodegradable nanoparticles for targeted drug delivery in treatment of inflammatory bowel disease. J Pharmacol Exp Ther, 2001. 299(2): p. 775-781.
3. Leonard, F., E.M. Collnot, and C.M. Lehr, A three-dimensional coculture of enterocytes, monocytes and dendritic cells to model inflamed intestinal mucosa in vitro. Mol Pharm, 2010. 7(6): p. 2103-2119.
4. Leonard, F., et al., In vitro screening of budesonide nanoformulations for treatment of inflammatory bowel disease in a novel inflamed 3D cell culture model. ALTEX, 2012. in press.
5. Harel, E., et al., Enhanced Transferrin Receptor Expression by Pro-inflammatory Cytokines in Enterocytes as a Means for Local Delivery of Drugs to Inflamed Gut Mucosa. PLoS ONE, 2011. 6(9): p. e24202.

DPC TECHNOLOGY FOR SAFE AND EFFECTIVE SIRNA DELIVERY

DAVID L. LEWIS

Achieving safe and effective in vivo delivery of siRNA to the appropriate tissue and cell type is key for development of RNAi as a therapeutic modality. We have developed a platform technology named Dynamic PolyConjugate (DPC) that enables targeted delivery of siRNA. Key features of the DPC technology include: new classes of membrane-active and biodegradable polymers, reversible chemical masking of the polymers so that membrane-lytic activity is revealed only in the acidic environment of endosomes, and the ability to attach ligands to guide the polymer and the siRNA cargo to specific cell types in vivo. We have demonstrated the utility of this technology by ligand-mediated delivery of siRNA to liver hepatocytes in mice, rats, and non-human primates resulting in high-level knockdown of the targeted gene. Importantly, DPCs display a low toxicity profile enabling siRNA redosing and long-term target gene knockdown.
We are utilizing DPC technology to develop an RNAi-based therapeutic for chronic Hepatitis B virus infection. This disease is a global health threat that results in 1,000,000 deaths annually from hepatocellular carcinoma, liver cirrhosis or liver failure. None of the currently available HBV therapeutics significantly reduces the viral antigen expression that hinders the immune system's ability to eradicate the virus. RNAi has the potential to be much more effective in this regard by knocking down expression of viral mRNAs, as well as the pregenomic RNA and the replicative intermediates that arise from it. Over 140 siRNAs that target conserved sequences in HBV were designed and then screened for efficacy in cultured cells. The most potent of these were formulated as hepatocyte-targeted DPCs and injected intravenously into mouse models of HBV infection. Single-dose DPC injections in a replication-competent, transiently transgenic HBV mouse model resulted in a 3-4 log reduction of serum HBsAg, a 2-3 log reduction in serum HBV DNA, and dramatically decreased HBV RNA and DNA in liver. Similar results were achieved in a transgenic mouse model of chronic HBV infection. In multi-dose studies, four biweekly injections of anti-HBV siRNA DPCs in mice carrying a hepatocyte-specific reporter gene fused to HBV sequences resulted in a 3-4 log reduction in gene expression over 2 months without changes in toxicity markers. These pre-clinical studies will form the basis of our clinical program for the treatment of patients with chronic HBV infection.

THE NANOMECHANICAL SIGNATURE OF BREAST CANCER

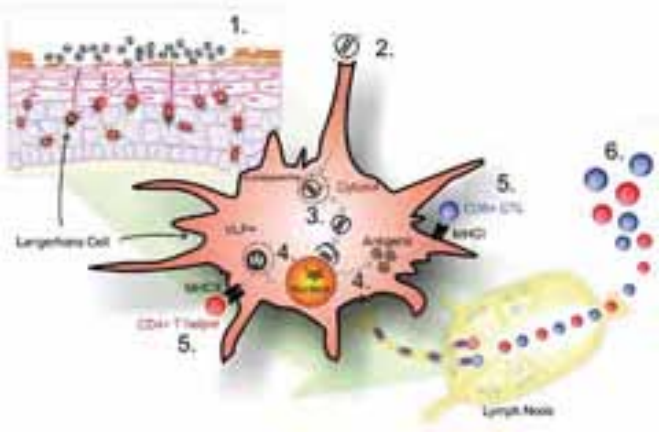
RODERICK Y.H. LIM

Tumor mechanobiology is an important yet unresolved aspect of cancer progression. How the mechanical properties of cells evolve from a healthy stage to malignancy and manifest themselves in tissues is poorly understood. Here, correlative stiffness maps obtained by indentation-type atomic force microscopy (IT-AFM) are used to identify distinct tumor stages of native human breast biopsies by resolving their local mechanical stiffness at the nanoscale. Healthy ductal epithelium and benign lesions are characterized by a uniform stiffness distribution. In comparison, primary cancer lesions owing to tissue heterogeneity exhibit a broad background stiffness distribution that is accompanied by a characteristic compliance representing cancer cells. Importantly, the stiffness profiles from each specific stage of tumor progression are validated in MMTV-PyMT transgenic mice. We remark further that hypoxia is apparent in areas of soft cancer phenotype that occur from early to late stages of tumor progression. Detecting the soft phenotype also in lung metastases suggests that the compliance of malignant cells in the primary cancer is correlated to aggressiveness and thus promotes migration and metastasis. Overall, our study unveils the clinical translational significance of nanomechanical signatures in the diagnosis of breast cancer.

LANGERHANS CELL-TARGETING IMMUNOTHERAPEUTIC NANOMEDICINE (DERMAVIR) TOWARDS THE CURE OF HIV

JULIANNA LISZIEWICZ
Genetic Immunity Budapest, Hungary & McLean VA

HIV/AIDS is successfully managed with the combination of antiretroviral drugs (cARV). However, cARV can neither eliminate HIV-infected cells from the reservoirs nor reconstitute HIV-specific immunity, thus these drugs must be taken daily and lifelong. The concerns about adverse effects over decades of therapy, the evolution of resistance, and the financial burden of treatment call for a cure of HIV. Towards the cure novel disease-modifying therapies are investigated for either eradication or immune control of HIV. DermaVir, the clinically most advanced immunotherapeutic nanomedicine, boosts HIV-specific memory T cells to kill the infected cells. Such T cell responses are associated with control of plasma viremia and delayed disease progression in HIV-infected patients who naturally control virus replication.
DermaVir is the first clinical product employing our novel Langerhans cell(LC)-targeting plasmid DNA (pDNA) delivery system developed for the induction of potent memory T cell responses. This delivery system consists of two components: a pDNA[1] formulated with a polymer (PEIm) to synthetic "pathogen-like" nanomedicine and the DermaPrep transdermal CE-marked medical device. We showed that the DermaPrep device delivers the 1.2 x 1013 nanoparticles into the epidermis 50-110 mm deep in proximity to activated LCs. Then the nanomedicine orchestrate controlled processes to enter the cells via endocytosis, to escape from the endosome/lysosome, to traffic the pDNA into the nucleus for the expression of antigens[2].



DermaVir consistently demonstrated safety, immunogenicity and preliminary efficacy in preclinical and clinical trials summarized in the next Table:

Main Studies	Safety	Immunogenicity	Efficacy
SIV$_{mac}$-infected Macaques	Safe	Memory T cells	Viral load reduction Survival benefit
GLP Rabbit Safety	Safe as placebo		
GLP Rat Fertility	Safe as placebo		
Phase I (EU) 9 pts + HAART	Safe	Memory T cells (Optimal dose: 0.4mg)	
Phase I/II (USA) 24 pts + HAART	Safe as placebo	Memory T cells (Optimal dose: 0.4mg)	
Phase II (EU) 36 pts, no ART	Safe as placebo	Memory T cells (Optimal dose: 0.4mg)	Viral load reduction (Optimal dose: 0.4mg)

Based on the potent induction of HIV-specific central memory T cells3 we speculate that DermaVir could reduce HIV latent reservoirs and maintain undetectable viral load after treatment simplification in HIV-infected patient population. For durable efficacy repeated DermaVir immunization will be required. LC-targeted synthetic delivery of DNA-encoded antigens achieved the clinical proof in HIV disease and holds great promises in development of novel vaccines for the treatment of cancer and other infectious diseases.

[1] Somogyi et al. Vaccine 2010, [2] Lorincz et al. Nanomedicine 2011, [3]Lisziewicz et al. Plos One 2012 (in press)

DEVELOPMENT OF DRUG DELIVERY NANOPLATFORM FOR MEDICAL IMPLANTS

STERGIOS LOGOTHETIDIS
Lab for "Thin Films -Nanosystems & Nanometrology", Department of Physics, Aristotle University of Thessaloniki, Greece

There are clinical needs for the development of biomaterials for implants with diverse functionalities such as the controllable and multiplex drug release from their surface to serve different goals in the fight against diseases. Biodegradable polymers can be applied on a variety of implants for the controlled and local drug delivery.
Herein, a biodegradable and nanoporous polymeric platform for drug delivery was developed and thoroughly characterized. It was synthesized by two types of poly (DL-lactide-co-glycolide) (PLGA 65:35, PLGA 75:25) and polycaprolactone in a multilayer configuration by the spin coating technique. The polymer degradation rates and properties were taken into account in order to achieve sustained and controlled drug release. The antiplatelet drug, dypiridamole was loaded into the surface nanopores of the platform. Surface characterization was made by Atomic Force Microscopy and Spectroscopic Ellipsometry. Platelet adhesion and drug release kinetic studies follow.
The study revealed that the engineered multilayer films are highly nanoporous. Their nanoporosity can be tailored by tuning the growth parameters, essential for drug loading and release. Ellipsometry studies revealed the structural characteristics, film thickness and optical properties even of the single layers in the triple layer construct, providing essential information for the drug loading.
Platelet adhesion studies shown that the dipyridamole-loaded coatings inhibit platelets aggregation that is prerequisite for clotting. The films exhibited sustained release profiles of dipyridamole over 70 days. Thus, the development of this novel drug delivery platform with tailored characteristics has evolved as a new perspective for controlled drug release in a wide spectrum of drug eluting implants.

"LIGAND-TARGETED NANOMEDICINES: FROM BENCH TOP TO CLINIC"

PHILIP S. LOW
Department of Chemistry, Purdue University West Lafayette, IN 47907 USA

We have developed methods to target drugs specifically to pathologic cells, thereby avoiding collateral toxicity to healthy cells. To achieve this specificity, we have searched for ligands that bind selectively to diseased cells and have linked these ligands to therapeutic or imaging agents via a spacer designed to optimize drug PK/PD. In the case of cancer, we have exploited the up-regulation of the folate receptor on malignant cells to target the following pharmaceuticals to cancer cells in vivo: i) cytotoxic drugs, ii) protein toxins, iii) gene therapy vectors and siRNA constructs, iv) fluorescent dyes for fluorescence-guided surgery, v) radioimaging agents, vi) MRI contrast agents, vii) liposomes with entrapped drugs, viii) radiotherapeutic agents, ix) immunotherapeutic agents, and x) enzyme constructs for prodrug therapy. Current clinical trials of six folate-linked drugs demonstrate that the folate receptor-targeting strategy holds great promise for increasing drug potency while reducing unwanted toxicity. Data from the design, synthesis, development and human testing of several of these drugs will be presented.
Recently, we have also developed a ligand that selectively targets attached drugs to prostate cancer cells with ~10 nM affinity. Imaging and therapeutic studies suggest that the new prostate cancer-specific ligand can not only improve diagnosis of the disease, but also enhance treatment of the cancer with little toxicity to normal cells. Other ligands that target pancreatic, stomach and esophageal cancers are also undergoing preclinical development and will be described.
Outside of the cancer field, we are developing drug targeting strategies for the imaging and therapy of rheumatoid arthritis, psoriasis, Crohn's disease, atherosclerosis, lupus, osteoarthritis, diabetes, and multiple sclerosis. We are also developing targeted drugs for treatment of several viral infections. Results from preclinical and clinical studies with these latter targeted drugs will also be described.

AMYOTROPHIC LATERAL SCLEROSIS: THE LATEST INNOVATIONS IN INVESTIGATING NEURODEGENERATIVE DISORDERS BASED ON SECARS MICROSCOPY & MR IMAGING

LINA H. MACHTOUB
Universitätsklinik für Radiologie, Innsbruck Medical University, Innsbruck, Austria

The explosive growth of biocompatible nanotechnologies has set the stage for an evolutionary leap in diagnostic imaging and therapy. The scope of this work highlights the novel application of recently developed emerging imaging modality based on surface-enhanced coherent anti-Stokes Raman scattering (SECARS) microscopy in investigating the molecular mechanisms associated with neurodegenerative disorder systems, using established experimental models of amyotrophic lateral sclerosis (ALS) (1,2). The experiments were performed on transgenic rat model expressing multiple copies of mutated (G93A) human SOD-1 gene, after CD4+ lymphocytes were magnetically labeled with i.v.i. CLUSPIO antibodies. Marked intensity enhancements have been observed in CLUSPIO treated ALS brain using SECARS microscopy. The observed enhancement has been correlated to lipid peroxidation and degeneration observed in these regions, based on selective association of lipids to up-taken USPIO, which shows high accumulation in the brainstem and midbrain region. The obtained results were compared with MR imaging, which shows marked hyperintensities with prominent lateral ventricle and cerebral aqueduct enlargements in these regions. The

involvement of perturbed sphingolipid metabolism resulting in ceramide and cholesterol ester accumulation in motor neurons in ALS would suggest novel approaches for future therapeutic intervention. Moreover, the potential contribution of SECARS microscopy in conjunction with the optical properties of the MR contrast agents can be promising for the designing of future clinical magnetic and optical probes for live cell imaging and noninvasive characterization of molecular events associated with CNS disorders.

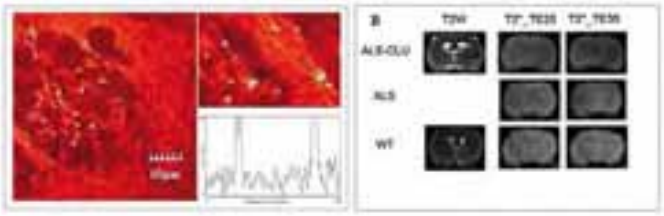


Figure 1.(A) SECARS image of brain tissue from CLUSPIO treated ALS rats, taken at 2850 cm-1. The corresponding cross section profile shows the intensity enhancement along the indicated line. (B) MRI of the brain of the ALS rat treated with CLUSPIO ("ALS-CLU") as compared to the MRI of the ALS rat without CLUSPIO injection ("ALS") and of the wild type rat ("WT"). T2W protocol reveals the dilated ventricles in the ALS model (images in the left column). T2* protocol reveals the hypointensities (note regions delimited by ellipsoids) allegedly caused by CLUSPIO seen with time to echo (TE) 25 ms (middle column) and further augmented with TE 35 ms (right column).

(1) **Machtoub, LH**., Investigating neurodegenerative disorder systems using USPIO- nanoparticles with (SECARS) microscopy, Journal of Neurology 2010; 257:S65

(2) **Machtoub L**, Bataveljić D, Andjus PR., Molecular imaging of brain lipid environment of lymphocytes in amyotrophic lateral sclerosis using magnetic resonance imaging and SECARS microscopy. Physiol Res. 2011;60 Suppl 1:S121-7

TARGETED IMAGING AND RADIONUCLIDE THERAPY OF NEUROENDOCRINE MALIGNANCIES

HELMUT MAECKE

Somatostatin receptors (belonging to the large family of G-protein coupled receptors) are overexpressed on a variety of human tumors, in particular on neuroendocrine tumors of the pancreas and the gut. The high expression of these receptors is the molecular basis to develop vectors for imaging (Single Photon Emission Computed Tomography, SPECT and Positron Emission Tomography, PET) and Targeted Radionuclide Therapy, TRT). Ideally these two vectors have identical or at least very similiar biological properties and pharmacokinetics; then they can be considered a theranostic pair. They may combine a powerful diagnostic vector which is being used as a predictive imaging agent with a targeted radiotherapeutic. Several critical parameters determine the suitability of imaging probes for somatostatin receptors; chemically they are usually peptides.
First, receptor binding affinity should be in the low nanomolar range. This may be difficult to achieve as these peptides need to be conjugated with chelators and labeled with metallic radionuclides.
Second, internalisation, uptake into the tumor cell, appears to be important as it constitutes an active uptake mechanism.
Third, peptides are often metabolically unstable because of in vivo proteolysis.
Fourth, the peptides have to be labeled stably to avoid premature release of the radionuclide which may cause bone uptake (most radiometals are bone seekers) and consequently bone marrow toxicity.
To cope with these prerequisites we synthesised [DOTA,Tyr3]octreotide (DOTATOC). DOTA (1,4,7,10-tetraaza-cyclododecane-1,4,7,10-tetraacetic acid) is a cage-type macrocyclic chelator which allows stable encapsulation of radiometals for SPECT, PET and TRT and therefore safe clinical use.
The conjugation of the radiometal complex resulted in only a slight decrease of the somatostatin receptor binding affinity and the metabolic stability was high in human serum.
In particular the Ga-68 labeled DOTATOC proved to be a highly sensitive probe for PET and allows to predict the potential for targeted radionuclide therapy. Several clinical studies showed the superiority of this probe compared to a commercially available SPECT probe.
The same DOTA-conjugated peptide which is used in diagnostic imaging now was used in targeted radionuclide therapy. It was labelled with the beta-particle emitting radionuclides 90Y (high energy beta-emitter with a range of a few mm in tissue) and/or with 177Lu (low energy beta-emitter with an average range of approximately 1 mm). At the university hospital Basel, department of nuclear medicine about 1700 patients were treated with the two radiotherapeutic vectors (Walter et al, JCO, 2011) and analysed retrospectively. Multivariate regression of 1109 patients showed that tumoral uptake in the pretherapeutic imaging study was predictive for overall survival. Kidney toxicity of this treatment modality is the dose limiting side effect. Again it was shown that kidney dose determined in the imaging study predicted kidney toxicity.
In a second cohort study comparing a single radiopharmaceutical (90Y-DOTATOC) with a combination of 90Y-DOTATOC) in one step followed by 177Lu-DOTATOC authors showed that the combination was more efficacious(Walter et al, JCO, 2012) .
Overall the patients treated with 90Y-DOTATOC and 177Lu-DOTATOC targeted radionuclide therapy showed a longer survival compared to historical controls but kidney toxicity is a concern. New strategies to overcome this are being developed and will be discussed.

NANOPARTICLE CHARACTERIZATION FOR CANCER THERAPEUTICS AND DIAGNOSTICS: LESSONS LEARNED FROM NCI'S NANOTECHNOLOGY CHARACTERIZATION LAB (NCL)

SCOTT MCNEIL

NCI's Nanotechnology Characterization Laboratory (NCL) conducts preclinical efficacy and toxicity testing of nanoparticles intended for cancer therapeutics and diagnostics. The NCL is a collaborating partnership between NCI, the U.S. Food and Drug Administration (FDA) and the National Institute of Standards and Technology (NIST). The NCL characterizes nanoparticles' physical attributes, their in vitro biological properties, and their in vivo compatibility in animal models. The Laboratory accelerates the transition of basic nanoscale particles and devices into clinical applications by providing critical infrastructure and characterization services to nanomaterial providers, and is a national resource available to investigators from academia, industry and government. The NCL is also partnering with the National Institute of Environmental Health Sciences (NIEHS) to characterize engineered nanomaterials. Engineered nanomaterials are becoming increasingly prevalent in wide range of commercial products, prompting government organizations to conduct proactive studies into the overall safety of these materials. Through a formal collaboration with the NIEHS, NCL will thoroughly characterize the physicochemical properties of various commercial nanomaterials as part of an NIEHS consortium established to help investigate the role nanomaterial physicochemical characteristics have in influencing molecular interactions and biological responses. NCL's many collaborations with nanotech investigators and expertise with a variety of nanoparticle drug delivery platforms have allowed us to elucidate trends relating physicochemical properties such as size and surface chemistry to nanoparticle behavior in biological systems, biodistribution, safety, and efficacy. This presentation will include some of the NCL's recent findings regarding nanoparticle biocompatibility and toxicity. Funded by NCI contract No: HHSN261200800001E.

QUANTUM DOT TECHNOLOGIES FOR IMPROVED UNDERSTANDING OF CELLULAR INFORMATION LEADING TO IMPROVED TISSUE BASED CANCER DIAGNOSTICS

PHIL MILLER

The need for improved diagnostic and prognostic information in oncology is critical to achieve the goal of personalized healthcare for cancer patients. It is often over looked that cancer is a disease of cells in tissue and that each tumor has information unique to a specific patient. Thus cellular based tissue information is critical for understanding patients, their disease, disease progression and management. The most accurate tissue based information demands that the tissue remain intact and with complete morphological context for the tumor and its microenvironment. Unfortunately virtually all advanced molecular and proteomic technologies do not preserve the morphological context and those that have been applied to tissue often lack high sensitivity and high spatial resolution. Our objective in developing direct quantum dot (QD) multiplexing assays for tissue is to provide high content information with high sensitivity, high spatial resolution and morphological context. Understanding cellular information in this context will lead to unique personalized diagnostic and prognostic information for oncologist and cancer patients. In addition Quantum dot (QD) technology is somewhat universal as it can be applied for in situ multiplexed detection of DNA, RNA and proteins in tissue.

The unique physical properties of QDs give them the capability to provide high content and context information from tissue. These properties include single wavelength excitation, multiple tunable emission peaks, photo stability and quantification. We have exploited these properties by developing a unique bioconjugate hapten system in conjunction with a spectral imaging system. We have successfully integrated these technologies into a complete system for the multiplexed analysis of human tissue. We have applied this system to the detection of genomic DNA, mRNA, protein and protein/protein interaction. We have developed multiplexing assays for each of these molecular entities. Data will be presented demonstrating a variety of multiplexed applications up to nine discrete fluorescent signals in human tissue. The clinical importance of these applications in oncology will be discussed.

Technologies that can generate multiplexed signals that are highly sensitive provide excellent spatial resolution, quantitation and maintain morphological context are needed for advanced cellular/tissue based analysis. Quantum dots are a unique nanotechnology which can meet these demands.

THE MECHANISTIC ASPECTS OF COMPLEMENT ACTIVATION BY NANOPARTICLES

MOIEN MOGHIMI

Centre for Pharmaceutical Nanotechnology and Nanotoxicology, University of Copenhagen, DK-2100 Copenhagen Ø, Denmark

The complement system is a network of over thirty different soluble and membrane-bound proteins that can be activated via three different initiation pathways (classical, alternative and lectin pathways) that all converge at the step where the central complement protein C3 is cleaved. The key function of complement is pattern recognition of danger signals, such as pathogen associated molecular patterns and foreign surface of biomedical materials including nanoparticles. Uncontrolled complement activation can induce many inflammatory and life threatening conditions. Indeed, a large body of experimental and clinical evidence strongly attests that the infusion of nanoparticulate systems, including regulatory-approved stealth nanomedicines, in some individuals is associated with cutaneous, respiratory and circulatory disturbances where complement is believed to play a contributing role. Furthermore, inflammatory reactions are encountered in extracorporeal circulation procedures and these also have routes in complement activation. Consequently, complement testing is among one of the parameters currently included in the criteria for assessment of haemocompatibility of biomaterials defined in ISO 10993-4.

A detailed mechanistic understanding of material/nanomaterial properties that triggers complement is necessary as this could lead towards innovations for design and surface engineering of immunologically safer nanomedicines and biomedical devices. The interaction between materials/nanomaterials and the complement system is complex and regulated by inter-related factors that include morphology, chemical composition, nanoscale size, and surface characteristics. Each of these parameters may affect complement activation differently and through different sensing molecules and initiation pathways. Recent 'structure-function' and high-throughput approaches are beginning to thrive and are providing better understanding of material features that incite complement. For instance, changing the conformation of adsorbed block copolymer on nanosphere surfaces with the aim of circumventing the body's defence system unexpectedly trigger complement through a different initiation pathway. This underlines the limitations in surface engineering with certain block copolymers in generating immunocompatible nanoparticles. On the other hand, high-throughput approaches in polymer design are helping to map out the responsible structural motifs that induce complement activation for safer biomaterial design. Efforts are also being made in understanding naturally evolved microbial strategies that evade complement and for their translation to nanoparticle and biomaterial design. There are still many technical difficulties and limitations in material design and surface engineering approaches for generating immunologically safe nanomedicines and biomaterials, but parallel immunogenomic approaches should be initiated to establish relationships between material properties and adverse population-based immunological responses where complement may exert a contributing role. Such initiatives may eventually provide laboratory tests for predicting individuals at risk of advanced medical interventions requiring nanomedicines and functionalized particulate systems and biomaterials.

• **Moghimi, S. M.**, T. L. Andresen and Hunter, A. C. (2012) Factors controlling nanoparticle pharmacokinetics: an integrated analysis and perspective. Annual Rev. Pharmacol. Toxicol. 52: 481–503; • **Moghimi, S. M.**, Peer, D. and Langer, R. (2011) Re-shaping the future of nanopharmaceuticals: ad iudicium. ACS Nano 5: 8454–8458; • **Moghimi, S. M.**, Andersen, A. J., Ahmadvand, D., Wibroe, P. P., Hunter, A. C. and T. L. Andresen (2011) Material properties in complement activation. Adv. Drug Deliv. Rev. 63: 1000–1007; • **Moghimi, S. M.**, Andersen, A. J., Hashemi, S. H., Lettiero, B., Ahmadvand, D., Hunter, A. C., Andresen, T. L., Hamad, I. and Szebeni, J. (2010) Complement activation cascade triggered by PEG-PL engineered nanomedicines and carbon nanotubes: the challenges ahead. J. Control. Rel. 146: 175–181; • **Moghimi, S. M.** and Hunter, A. C. (2010) Complement monitoring of carbon nanotubes. Nature Nanotechnol. 5: 382; • Hamad, I., Al- Hanbali, O., Hunter, A. C., Rutt, K. J., Andresen, T. L. and **Moghimi, S. M**. (2010) Distinct polymer architecture mediates switching of complement activation pathways at nanosphere-serum interface: implications for stealth nanoparticles engineering. ACS Nano 4: 6629–6638; • **Moghimi, S. M.** and Andresen, T. L. (2009) Complement-mediated tumour growth: implications for cancer nanotechnology and nanomedicine. Mol. Immunol. 46: 1571–1572; • Hamad, I., Hunter, A. C., Rutt, K. J., Liu, Z., Dai, H. and **Moghimi, S. M.** (2008) Complement activation by PEGylated single-walled carbon nanotubes is independent of C1q and alternative pathway turnover. Mol. Immunol. 45: 3797–3803.; • Hamad, I., Hunter, A. C., Szebeni, J. and **Moghimi, S. M.** (2008) Poly(ethylene glycol)s generate complement activation products in human serum through increased alternative pathway turnover and a MASP-2-dependent process. Mol. Immunol. 46: 225–232. **Moghimi, S. M.**, Hamad, I., Andresen, T. L., Jørgensen, K. and Szebeni, J. (2006) Methylation of the phosphate oxygen moiety of phospholipid-methoxypoly(ethylene glycol) conjugate prevents PEGylated liposome-mediated complement activation and anaphylatoxin production. FASEB J. 20: 2591–2593 (Full text: doi: 10.1096/fj.06-6186fje, electronic pages E2057–E2067).

HIGH THROUGHPUT GENOMICS FOR NOVEL STARTING POINTS IN CANCER THERAPY

JAN MOLLENHAUER[1,2]
[1]Lundbeckfonden Center of Excellence NanoCAN
[2]Molecular Oncology, University of Southern Denmark, Odense, DK

The next generation of anti-cancer therapies will likely build up on so-called synthetic lethal nanodrugs, which cause selective elimination of cells with cancer-specific molecular fingerprints and thus providing large therapeutic windows. High resolution molecular profiling of a patient`s individual tumor can be achieved by novel next generation sequencing methods, providing comprehensive maps of gene mutations, activity patterns of genes and non-coding RNAs, as well as epigenetic changes. This information can in principle be used to identify sites of vulnerability in the individual patient`s cancer cells and, consecutively to select appropriate synthetic lethal nanodrugs with match to these profiles. Comprehensive efforts are now undertaken within the International Cancer Genome Consortium to provide these landscapes and first molecular maps have been derived for some of the major cancer types, including breast cancer.

Our strategy is to systematically analyze molecular alterations that have been derived from molecular profiling of breast cancer in order to use this for the design of synthetic lethal nanodrugs. To this end, we have been analyzing ~150 molecular alterations in breast cancer and identified numerous novel breast cancer growth and bona fide metastasis modulators. In parallel, we set up high-throughput robotic drug screens, allowing e.g. for genome-wide siRNA scans, and sensitive chip technologies, which allow for profiling protein quantities using minimal amounts of cells. These two strategies will be employed to analyze the newly recovered genes for their suitability to serve as starting point for synthetic lethal nanodrugs. In these approaches, we specifically focus on the eradication of cancer stem cells, which are thought to represent the tumor-replenishing population, being responsible for drug resistance and recurrence of metastatic cancer.

COMBINING MULTIFUNCTIONAL NANOPARTICLES AND ULTRASOUND IN CANCER THERANOSTICS – FIRST OUTLOOK TOWARDS IN VIVO RESULTS

ÝRR A. MØRCH[1,2], Per Stenstad[1], Ruth B. Schmid[1], Rune Hansen[3], Siv Eggen[2], Yngve Hansen[2], Mercy Afadzi[2], Catharina deLange Davies[2]

[1] SINTEF Materials and Chemistry, Trondheim, Norway
[2] Dept. of Physics, Norwegian University of Science and Technology, Trondheim, Norway
[3] SINTEF Technology and Society, Trondheim, Norway

INTRODUCTION

The ability to create nanoparticles (NPs) incorporating multiple functionalities, including imaging and therapy as well as cell targeting, opens new possibilities in the combination of diagnosis and therapy into so called theranostic NPs. Drug-carrying NPs make it possible to

- 1) Improve early detection of cancer
- 2) Improve cancer-specific therapy and limit side effects by targeting the drug-carrying NPs to cancer cells
- 3) Visualize the site of drug delivery and the distribution of the drug

4 •) Monitor the efficacy of the therapy.

Combining multifunctional NPs with focused ultrasound treatment further enables therapeutic molecules to reach their targets while limiting the exposure to normal tissue. Several aspects of ultrasound waves may be useful for improved delivery of drugs encapsulated in nanoparticles including local heating of tissue, increased diffusion of NPs, and generation of radiation force [1]. In addition, microbubbles injected into the blood stream can be used both for ultrasound imaging of the tumor vasculature, and to facilitate sonoporation further improving cellular uptake of NPs and drugs. In the present study we present a novel multimodal, multifunctional drug delivery system consisting of microbubbles stabilized by polymeric nanoparticles.

EXPERIMENTAL METHODS

Nanoparticle synthesis and characterization: Miniemulsion polymerization was used to prepare nanocapsules of the biocompatible and biodegradable polymer poly(butyl-2-cyanoacrylate) (PBCA) [2]. Oil-in-water emulsions were prepared by emulsifying a monomer phase, consisting of butyl-2-cyanoacrylate (BCA), co-stabilizer, model drug (retinyl palmitate) and the fluorescent dye Nile Red, in an acidic aqueous medium containing surfactant. The initiation of the anionic polymerization was carried out by adding Jeffamine or Tween80 to the emulsion, resulting in PEGylated NPs. Particle size and morphology was determined using Zetasizer and scanning electron microscopy (SEM), respectively.

Microbubble preparation and characterization: Microbubbles stabilized by PBCA NPs were prepared by mixing the nanoparticle dispersion with proteins and air using an ultra-turrax. The size and number of microbubbles was determined using Coulter Counter and their morphology was analyzed by confocal laser scanning microscopy (CLSM).

Animals: Balb/c nude mice bearing a subcutaneous tumor (xenograft of human PC3 prostate adenocarcinoma) in the leg.

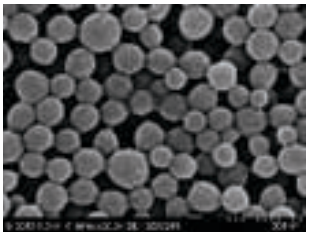
Distribution of NPs in tumor: Mice were injected intravenously with nanoparticles and the tumor treated with focused ultrasound (10 min, 300 kHz, 5% duty cycle, MI 2.4). One group was treated immediately after injection, whereas another group was treated 24 hrs after NP injection. Tumors were frozen and microtomed, and sections analyzed by confocal laser scanning microscopy (CLSM).

In vivo visualization of microbubbles: Mice were injected with PBCA NP-stabilized microbubbles while image acquisition using Vevo 2100 high frequency ultrasound imaging system from VisualSonics.

RESULTS AND DISCUSSION

Multifunctional nanoparticles

Stable PEGylated PBCA submicron particles encapsulating hydrophobic model drugs were produced in one step using miniemulsion polymerization (Fig 1A). The particle diameter could be varied from 100 to 250 nm (PDI < 0.2).



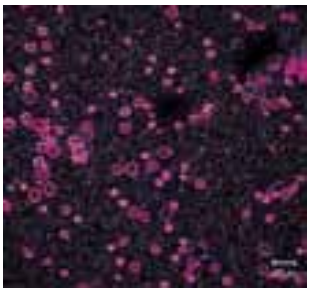


Figure 1: A: SEM image of PEGylated poly(butyl cyanoacrylate) particles prepared in one step using the miniemulsion polymerization method. **B**: CLSM image of microbubbles stabilized by PBCA nanoparticles. The particles (fluorescently labeled) form a shell around the bubbles.

Preliminary analysis of CLSM images of mouse tumor slices showed that PBCA NPs had diffused into the tumor center upon ultrasound (US) exposure both when US was applied immediately after injection and when treated with US 24h hours after NP injection. However, the concentration of PBCA particles in mouse tumors was higher in mice where particles were allowed to circulate before ultrasound treatment. The higher concentration of accumulated particles is probably a result of the EPR effect due to leaky tumor vasculature [3]. The direct effect of ultrasound on particle uptake and distribution in tumors is currently under investigation by our group.

Figure 2: CLSM image through an equatorial section of a mouse

tumor. Blood vessels are seen in red and PBCA particles in green. The three images are of the same slice, where the two upper images are split images of the bottom image.

Nanoparticle-stabilized microbubbles

A method for producing air-filled microbubbles (diameter of 1-6 μm) stabilized by a shell of PBCA nanoparticles (Fig 1B) was developed. The bubbles were stable for a long period of time (> months) in water. The nanoparticle surface properties, especially the hydrophobicity/hydrophilicity balance, were found to be the most important factors determining the successful assembly of particles on the bubble surface.

Nanoparticle-stabilized microbubbles were intravenously injected into mice bearing tumors and visualized using an ultrasound imaging system for small animals. The microbubbles were found to provide good contrast in the tumor blood vessels, and could easily be destroyed by increasing the ultrasound power output.

CONCLUSION

We have shown that stable, PEGylated biodegradable polymeric nanoparticles can be formed in a single step, which is highly beneficial from a regulatory and industrial point of view. The nanoparticles developed can further be successfully utilized for stabilization of microbubbles with ideal acoustic properties. Hence, improved diagnosis can be combined with therapy by incorporating nanoparticles containing the appropriate drugs into the microbubble surface. A method for synthesizing PEGylated particles with targeting molecules in one step is currently being developed in our lab.

REFERENCES

1. Frenkel, V. Adv. Drug Deliv. Rev. 60, 1193-1208, 2008.
2. Landfester, K. Ann. Rev. Mat. Res. 36, 231-279, 2006
3. Yuan, F. Seminars Radiat. Oncol. 8, 164-175, 1998

NANOMEDICINE: IS THERE A HOPE FOR CHAGAS'S DISEASE PATIENTS?

MARIA JOSE MORILLA and Eder Lilia Romero
Programa de Nanomedicinas. Universidad Nacional de Quilmes, Buenos Aires, Argentina, www.nanomedicinas.unq.edu.ar

With 15-20 millions infected (2-3 % the population of South and Central America plus south California), an incidence of 200.000 new cases per year, an estimated annual mortality of 50.000 and more than 6,5 billons USD annual lost due to mortality and morbidity (WHO), the Chagas disease remains as the main parasitic disease of the Americas. Nowadays, there is no cure for this orphan disease. Because of the lack of interest of pharmaceutical companies and the absent intervention of the governments, there are only two drugs approved for treatment: the hydrophobic nitroimidazole benznidazole and in certain cases the nitrofurane nifurtimox, both administered by the oral route. These have been the only available drugs during the last half century, in spite of being effective only in the acute phase of paediatric patients, after two or three months of heavy daily doses. These drugs are poorly tolerated by the adults, whom frequently discontinue the treatments. In spite of their high bioavailability, the trypanocidal activity of these drugs is against trypomastigotes, the extracellular forms, present in blood during the short periods of parasitemia of the frequently asymptomatic acute phase. They are however, inefficient to target intracellular amastigotes nests. Because of these reasons, there is a strong need of developing new treatments capable of eliminating the intracellular forms, within short periods of time while remaining economically affor able for low income population. In this context nanotechnology could build strategic therapeutics, alternative to the search for new therapeutic targets that proposes the medicinal chemistry. In the acute phase, when the reticule endothelial system cells are colonized, a nano-objects based treatment must rely on the intracellular delivery of trypanocidal drugs. Our laboratory has successfully faced this approach on preclinical models. Balb-c mice infected with lethal doses of RA strain trypomastigotes, received nine intravenous doses of 100 nm diameter pH-sensitive liposomes loaded with the hydrosoluble nitroimidazole etanidazole, a drug with a very low trypanocidal activity in its free form. After 3 weeks (at a 200 fold lower dose of etanidazole than in the free form that resulted innocuous) the parasitemia was eliminated. The effect was achieved by pH sensitive liposomes that were taken up by infected cells by a degradative pynocitic pathway; this led to a massive delivery of etanidazole to the cell cytoplasm where the amastigotes reside. Remarkably, the pH-sensitive liposomes are not active against trypomastigotes. In other words, the parasitemia was reduced after modifying the intracellular traffic of the etanidazole to favour cytoplasm targeting. The ultimate challenge of an antichagasic treatment is however, impairing or reducing the cardiac damage caused by the perpetuation of the amastigotes within cardiomiocytes. Here anatomic pathological changes in the envelope of cardiac cells could be used to achieve a passive targeting of nano-objets against targets other than phagocytic cells. This should be achieved during the initial stadium of the chronic chagasic cardiomiopathy or even better during the indeterminate form. The thickening and loss of permeability barrier of the basement membrane enveloping cardiac cells is also found in diabetes and metabolic syndrome. Therefore, different to the occurred with amphotericin B and its nanotechnological formulation, the targeted to cardiomiocytes based-antichagasic treatments, could led to a technical platform useful against diseases affecting developed countries.

VIRUS-LIKE PARTICLES IN VACCINOLOGY

CHRISTIAN MOSER
Pevion Biotech AG, Worblentalstrasse 32, CH-3063 Ittigen, CH

Viruses are natural nanoparticles and used in various forms as vaccines. The spectrum ranges from crude preparations of live virus to fully synthetic viruslike particles (VLP). Notably, particle size, structure, and composition can strongly influence the resulting immune response.
The presentation will provide an overview of the established VLP vaccines (HBV, HPV, Influenza, HAV) and give examples of the next generation of VLPbased vaccines in clinical development.

NANOSTRUCTURING OF POLYETHERETHERKETONE (PEEK) FOR LOAD BEARING POLYMER IMPLANTS

BERT MÜLLER

Polymer implants are promising alternatives to the contemporary load-bearing metal implants. Polyetheretherketone (PEEK) is not only isoelastic to bone but also allows investigating the surrounding soft tissues using imaging modalities including magnetic resonance imaging or computed tomography, which is particularly important for cancer patients. In order to reach osseointegration, the commercially available PEEK implants require costly coatings, which restricts their usage. As an alternative to titanium or hydroxyapatite coatings, plasma treatments can be applied. The talk shows how plasma-induced preparation of nanostructures on polymer films and on injection-molded micro-cantilever arrays as well as the associated chemical modifications of the surface promote osseointegration. In vitro cell experiments indicate the suitability of the activation procedure. In addition, we show that microstructures such as microgrooves 1 μm deep and 20 μm wide cause cell alignment. The combination of micro-injection molding, simultaneous microstructuring using inserts/bioreplica and plasma treatments permits the fabrication of PEEK and other polymer implants with nature-analogue, anisotropic micro- and nanostructures.

GENOTOXICITY AND APOPTOSIS AS MECHANISMS OF CELLULAR TOXICITY BY NANOMEDICINES

FABRICE NESSLANY
Dr; Institut Pasteur de Lille, FRANCE

Nanoparticles (NPs) are usually defined as particles at dimensions between approximately 1 and 100 nanometers. They have considerable general industrial applications and although only a few nanoparticle medicinal products are available at the present time, a spectacular development of these products can be expected over the years to come. In the short and medium term, the main use of nanoparticle medicinal products (NMP) is for vectorization of drug substances and in medical imaging.

However, NPs (e.g. nanovehicles, nanovectors alone) may be genotoxic by various mechanisms of action among which:

- primary direct mechanism (NPs interact directly with DNA or produce free radicals inducing DNA damage or chromosome segregation disruption during mitosis),
- primary indirect mechanism (depletion of antioxidants and/or increase in oxidative damage of DNA via mitochondrial activity and/or inhibition of DNA repair),
- secondary effects mainly driven by inflammation

It is now recognized that the strict application of standard genotoxicity testing methods to nanomaterials is probably not relevant. For example, bacterial mutagenicity based assays may not be suitable because these prokaryotes lack the ability to perform endocytosis, the bacterial cell wall possibly being a barrier for many nanomaterials (Singh N et al., 2009 ; Landsiedel et al., 2009). In addition, some genotoxic mechanisms of action of NPs are due to interaction with mitochondria, whereas such a mechanism can not be demonstrated in bacteria. Such impairments may make it difficult to interpret the results of these cellular models.

Therefore, in order to gain a complete picture of how nanoparticles can interact with cellular systems, the use of a battery of tests that measure different endpoint (DNA fragmentation with the "Comet assay" and micronucleus assay for both clastogenic and aneugenic effects), is necessary. These recommendations are pragmatic and provide an acceptable, immediately feasible programme for the safety evaluation of NMPs and are published in the book « International Pharmaceutical Product Registration » or available on the Web site of Afssaps (http://www.afssaps.fr/).

On the other hand, Nanoparticles may also induce apoptotic cell death by different mechanisms, e.g. ROS dependent mitochondrial pathway and through lysosomal membrane destabilization and lipid peroxidation. Although the final outcome is similar (apoptosis), the molecular pathways activated by NPs differ depending upon the chemical nature of the NPs.

Overall, the complexicity of the (geno)toxicity assessment confirms the need for developing specific models, more adapted, more specific and sensitive.

TARGETED DELIVERY OF CISPLATIN-LOADED NANOGELS FOR THE BRAIN TUMOR TREATMENT

NATALIYA NUKOLOVA[1,2], Baklaushev V.P.[1,2], Khalansky A.S.[3], Grinenko N.F.[2], Yusubalieva G.M.[1,2], Bychkov D.A.4, Gubskiy I.L.[1], Kabanov A.V.[5,6], Chekhonin V.P.[1,2]

• [1]Russian State Medical University, Department of Medical Nanobiotechnology, Moscow, Russia, • [2]Serbsky National Research Center for Social and Forensic Psychiatry, Department of Fundamental and Applied Neurobiology, Moscow, Russia, • [3]Research Institute of Human Morphology of the Russian Academy of Medical Sciences, Moscow, Russia, • [4]Lomonosov Moscow State University, Department of Geology, Moscow, Russia, • [5]Center for Drug Delivery and Nanomedicine, Medical Center of University of Nebraska, Nebraska, Omaha, USA, • [6]Lomonosov Moscow State University, Department of Chemistry, Moscow, Russia

INTRODUCTION

Targeted drug delivery for brain tumor treatment is one of the important objectives in nanomedicine. Human glioblastoma multiforme is the most frequent and aggressive type of brain tumors. The most rapidly migrating glioma cells are positive for membrane protein connexin 43 (Cx43) [1]. The purpose of this study was to design cisplatin-loaded nanogels conjugated with monoclonal antibodies to Cx43 for treatment of glioma 101/8 and glioma C6 in vivo. Cx43-conjugated nanogels could enhance in vitro and in vivo anticancer effect of cisplatin formulations on Cx43-overexpressed tumor cells.

METHODS

Nanogels were synthesized using poly(ethylene oxide)-b-poly(methacrylic acid) and loaded with anticancer drug cisplatin (CDDP) [2]. Specific targeting ligand, monoclonal antibodies to Cx43 (mAb Cx43), was coupled to surface of nanogels via flexible PEG linker. Unbound mAb Cx43 and free CDDP were removed by gel filtration chromatography (Sepharose CL-6B, PBS, 0.5 ml/min). Particle size, loading capacity, stability of nanogels and activity of conjugated vectors were evaluated. Glioma C6 cells were used to evaluate the cellular uptake of cisplatin-loaded nanogels as well as their cytotoxicity. Free CDDP, CDDP-loaded nanogels and 5% dextrose (control) were injected 3 times via tail vein at a 5-day interval at 5 mg/kg CDDP equivalents (5 groups, 6 rats/group). Antitumor effect of formulations (CDDP, nanogel/CDDP, IgG-nanogel/CDDP, mAbCx43-nanogels/CDDP and 5% dextrose) on glioma 101/8 and glioma C6 was evaluated by measurement of tumor volume using 7T MR-tomograph (ClinScan, Bruker).

RESULTS AND DISCUSSION

The mAbCx43-conjugated nanogels represented stable negatively charged particles about 120 nm in diameter. These nanogels can be efficiently loaded with cisplatin (up to 35 % w/w) and exhibit sustained drug release. ELISA assay indicated the activity of mAb Cx43 after conjugation. The FITC-labeled mAbCx43-conjugated nanogels showed enhanced internalization in comparison with untargeted nanogels in glioma C6 cells. This led to a considerable increase of cytotoxicity of CDDP-loaded targeted nanogels compared to the untargeted nanogels in Cx43-overexpressed cells. In vivo analysis in tumor-bearing rats (high grade glioma 101/8 and C6) indicated significantly reduced tumor burden and increased lifespan with cisplatin loaded mAbCx43-nanogel treatment compared to other formulations. Free CDDP showed a similar antitumor activity in comparison with mAbCx43-nanogel/CDDP, however revealed profound loss of body weight (systemic toxicity) and neurotoxicity (dilatation of the subarachnoid space and ventricle of brain), Figure 1.

CONCLUSION

The drug-loaded mAbCx43-conjugated nanogels exhibited enhanced tumor growth inhibition (glioma 101/8 and glioma C6), increase in the lifespan and no loss of the body weight in comparison with other investigated formulations. These nanogels could be used as nano-carriers for targeted delivery of chemotherapeutic agents in Cx43-positive high grade gliomas.

LITERATURE

[1] Targeted delivery of liposomal nanocontainers to the peritumoral zone of glioma by means of monoclonal antibodies against GFAP and the extracellular loop of Cx43, V.P. Chekhonin et al / Nanomedicine: Nanotechnology, Biology, and Medicine 8 (2012) 63–70
[2] Folate-decorated nanogels for targeted therapy of ovarian cancer. N.V.Nukolova et al/ Biomaterials. 32(23), (2011) 5417-26

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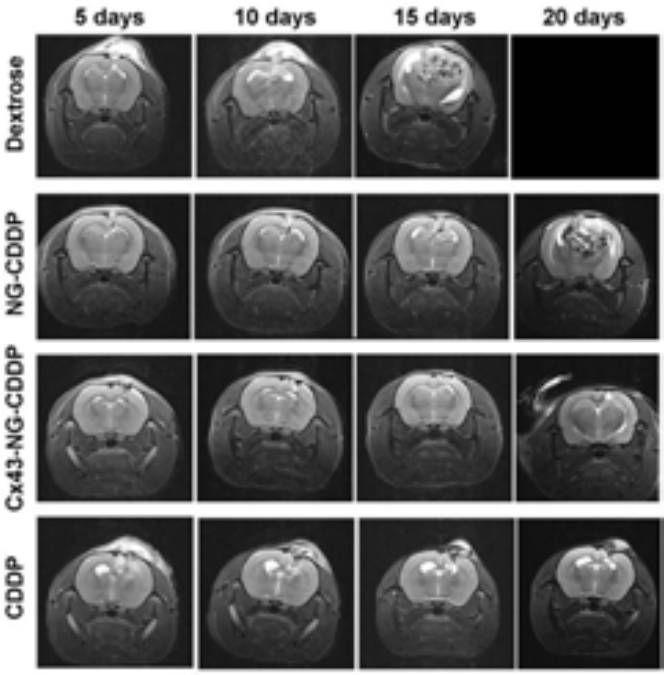


Figure 1. MRI images of the glioma 101/8 in rats after treatment by dextrose, CDDP-loaded nanogels (NG-CDDP), CDDP-loaded specific targeted Cx43-nanogel (Cx43-NG-CDDP) and free CDDP.

AN OVERVIEW OF FDA REGULATION, ADVANCES IN REGULATORY SCIENCE, AND A PERSPECTIVE ON APPROACHES TO NANOTECHNOLOGY

CARLOS PEÑA

Nanotechnology allows scientists to create, explore, and manipulate materials measured in nanometers (billionths of a meter). The U.S. Food and Drug Administration (FDA) regulates a wide range of products, including foods, cosmetics, drugs, devices, veterinary products, and tobacco products, some of which may contain nanomaterials or involve the application of nanotechnology. Such materials can have chemical, physical, and biological properties that differ from those of their larger scale counterparts. The FDA has developed a regulatory science program to develop the tools, methods, and expertise necessary to evaluate products that contain nanomaterials or otherwise involve the use of nanotechnology. The 'Overview of FDA Regulation, Advances in Regulatory Science, and a Perspective on Approaches to Nanotechnology' presentation will provide interested parties timely information about current perspectives and advances in this emerging technology area.

THE METASTASIS CASCADE OF SOLID TUMOURS – UNRESOLVED SECRET

JEAN YVES PIERGA

Metastasis is the leading cause of cancer mortality. The metastatic cascade represents a multi-step process which includes local tumor cell invasion, entry into the vasculature followed by the exit of carcinoma cells from the circulation and colonization at the distal sites. During tumor cell invasion, cells demonstrate diverse and evolving physical phenotypes that cannot typically be defined by any single molecular mechanism, and mechanobiology has been used to study the physical cell behaviors that comprise the "invasive phenotype". A necessary step in metastasis is the dissemination of malignant cells into the bloodstream, where cancer cells travel throughout the body as circulating tumor cells (CTC) in search of an opportunity to seed a secondary tumor. The primary cancer adapts the secondary site of tumor colonization involving the tumor-stroma crosstalk. The migration and plasticity of cancer cells as well as the surrounding environment such as stromal and endothelial cells are mandatory. CTC represent a valuable diagnostic tool: evidence indicates that the quantity of CTC in the blood has been shown to relate to the prognosis of the disease, and samples are readily obtained through routine blood draws. As such, there has been a push toward developing technologies to reliably detect CTC using a variety of molecular and immunocytochemical techniques. In addition to their use in diagnostics, CTC detection systems that isolate CTC to be characterized and to facilitate the development of personalized cancer therapies. The emerging insight into physical interactions between tumor cell and stroma may help to understand disease progression and may lead to new approaches to developing cancer diagnostics and therapies. Moreover, the development of techniques for the direct manipulation of CTC in circulation would be a major progress. New physical approaches may ultimately help to better predict, identify tumor metastasis and prevent their development.

NAMDIATREAM

ADRIELE PRINA-MELLO

Dr.; NAMDIATREAM Deputy Coordinator, PhD, School of Medicine and CRANN, Trinity College Dublin (IRL)

NAMDIATREAM project is focused on the developing of multimodal nanotechnology-based toolkits for the detection of biomarkers of most common cancer types, rare and single malignant cells and cancer metastases. These toolkits will enable identification of cells indicative of early disease onset in a high specificity and throughput format in clinical, laboratory and point-of-care devices, providing a breakthrough in the current diagnostic standards of sensitivity and selectivity. The pan-European consortium NAMDIATREAM consolidates the efforts of 22 leading academic, research, clinical and industrial partners.

The aim of the NAMDIATREAM project is to develop breakthrough multimodal nanotechnological tools for early detection of rare tumour cells and cancer biomarkers. Current clinical cancer diagnostic systems suffer from insufficient specificity and sensitivity. Recent advances in nanotechnology enable to establish innovative approaches to cancer detection in non-invasive and miniaturised volume formats. Here we report the latest achievements from the interdisciplinary group of European scientists focusing their efforts towards the development of complementary in vitro diagnostic and imaging techniques applicable for early stages of malignant disease onset and progression. These novel techniques are based on supersensitive "lab-on-a-bead", "lab-on-a-chip" and "lab-on-a-wire" nanodevices exploiting magnetic, optical, plasmonic and non-linear optical advanced nanoscale material properties. They allow for identification of true "molecular signatures" of specific biomarkers and cancer cells in clinical samples. Diagnostic devices developed by NAMDIATREAM are based on cost-effective technological solutions enabling to reach a qualitatively new level of cell and molecular detection specificity and sensitivity. NAMDIATREAM develops nanotechnology-enabled toolkits to address three of the most frequent cancer types: breast, prostate and lung. These toolkits are validated both at pre-clinical and proof-of-principle stages as novel probes and devices for detection of cancer molecular targets in compliance with the OECD regulatory policies in nanomaterials, implementing a tight safety regulatory and quality assurance control for the development of novel diagnostic, prognostic and monitoring technologies. Through close interaction with the project industry partners the innovative technology concepts proposed in NAMDIATREAM based on super-sensitive nanocarriers will be adapted for high-throughput readout systems using advanced flow cytometry, protein microarray and high content screening approaches, operating at the molecular, cellular and tissue levels. The involvement of several strong industrial partners into the project ensures a significant potential for efficient translation of the experimental results into clinical and point of care diagnostic and imaging applications.

NAMDIATREAM project is a large-scale integrating project funded by the European Commission FP-7 Programme, (EU NMP4-LA-2010-246479), www.namdiatream.eu. NAMDIATREAM is a member of the "Targeted NanoPharmaceuticals and Early Diagnostics" EU projects cluster.

THE USE OF POLYCOATED NANOPERTICLES FOR VASCULAR BYPASS SURGERY

MICHAEL REINERT
Prof Dr. med.; Department of Neurosurgery, Inselspital Bern, University of Bern (CH)

Laser tissue soldering (LTS) is a promising technique for tissue fusion especially for minimal invasive vascular soldering, but is limited by the lack of reproducibility particularly when the amount of indocyanine green (ICG) applied as energy absorber cannot be controlled during the soldering procedure. Nanotechnology enables the control over the quantitative binding of the ICG. The aim is to establish a highly reproducible and strong tissue fusion using ICG packed polycoated nanoparticles for vascular bypass surgery. Developments from classical tissue soldering over nanoshell to polycoated nanopartilces polymer embeded is demonstrated.

CLINICAL PHASE I TRIAL WITH IMMUNOLIPOSOMES TARGETING THE EPIDERMAL GROWTH FACTOR RECEPTOR

CHRISTOPH ROCHLITZ, Reto Ritschard, Thomas Dieterle, Lukas Bubendorf, Stefanie Deuster, Andreas Wicki, Richard Herrmann and Christoph Mamot

BACKGROUND

Immunoliposomes (ILs) combine antibody-mediated tumor recognition with liposomal delivery and, when designed for target cell internalization, provide intracellular drug release in order to increase specificity and efficacy of the encapsulated drug. In animal studies we have shown the immunoliposomal approach to be active and promising when targeting the epidermal growth factor receptor (EGFR).

METHODS

ILs were modularly manufactured under GMP conditions with Fab' fragments from MAb C225 (cetuximab), covalently linked to pegylated liposomes containing doxorubicin (PLD). This first in man single-center phase I clinical trial of anti-EGFR ILs-dox was designed for patients (pts) with various solid tumors, overexpressing EGFR (DAKO EGFR pharmDx-test). ILs-dox was administered i.v. q 4 weeks at a doxorubicin (dox) dose of 5, 10, 20, 30, 40, 50 and 60 mg/m2, 3 pts per dose level, for a maximum of 6 cycles. In addition to weekly safety monitoring, echocardiography was performed q 2 cycles, and pharmacokinetic assessments during cycle 1. The primary objective of this study was the establishment of MTD; secondary objectives included PK, tumor response, and time-to-progression.

RESULTS

After failure to standard treatments 26 pts were included between January 2007 and May 2010. Median age 62 years, WHO PS-0 in 3, PS-1 in 19 and PS-2 in 4 pts. Most common histologies included pancreatic, H&N, colorectal and urothelial cancer. Two cases of neutropenia, defined as a dose limiting toxicity, occurred on dose level 7 (= 60 mg dox/m2). On all lower doses the compound was very well tolerated, e.g. skin toxicity grade 1 in 2 pts, no hand-foot-syndrome, no alopecia, no cumulative toxicity. Therefore, 50 mg dox/m2 was defined as the maximum recommended dose for further phase II development. Best response to treatment included 1 CR, 1 PR and 8 SD lasting 2-12 mo (median 5.75 mo). Mean total dox half-life was calculated to be 31.0 hrs (+/- 7.6 hrs) and for the attached monoclonal antibody fragment of C225 17.7 hrs (+/- 4.3 hrs), respectively. Conclusions: Anti-EGFR DOX loaded Ils are safe and well tolerated up to 50 mg dox/m2. Clear evidence of clinical activity was observed warranting further evaluation in phase II trials.

NANOMEDICINE: IS THERE A HOPE FOR CHAGAS'S DISEASE PATIENTS?

EDER LILIA ROMERO and Maria Jose Morilla
Programa de Nanomedicinas. Universidad Nacional de Quilmes, Buenos Aires, Argentina, www.nanomedicinas.unq.edu.ar

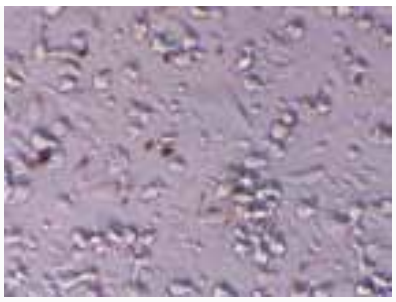
With 15-20 millions infected (2-3 % the population of South and Central America plus south California), an incidence of 200.000 new cases per year, an estimated annual mortality of 50.000 and more than 6,5 billons USD annual lost due to mortality and morbidity (WHO), the Chagas disease remains as the main parasitic disease of the Americas. Nowadays, there is no cure for this orphan disease. Because of the lack of interest of pharmaceutical companies and the absent intervention of the governments, there are only two drugs approved for treatment: the hydrophobic nitroimidazole benznidazole and in certain cases the nitrofurane nifurtimox, both administered by the oral route. These have been the only available drugs during the last half century, in spite of being effective only in the acute phase of paediatric patients, after two or three months of heavy daily doses. These drugs are poorly tolerated by the adults, whom frequently discontinue the treatments. In spite of their high bioavailability, the trypanocidal activity of these drugs is against trypomastigotes, the extracellular forms, present in blood during the short periods of parasitemia of the frequently asymptomatic acute phase. They are however, inefficient to target intracellular amastigotes nests. Because of these reasons, there is a strong need of developing new treatments capable of eliminating the intracellular forms, within short periods of time while remaining economically affor able for low income population. In this context nanotechnology could build strategic therapeutics, alternative to the search for new therapeutic targets that proposes the medicinal chemistry. In the acute phase, when the reticule endothelial system cells are colonized, a nano-objects based treatment must rely on the intracellular delivery of trypanocidal drugs. Our laboratory has successfully faced this approach on preclinical models. Balb-c mice infected with lethal doses of RA strain trypomastigotes, received nine intravenous doses of 100 nm diameter pH-sensitive liposomes loaded with the hydrosoluble nitroimidazole etanidazole, a drug with a very low trypanocidal activity in its free form. After 3 weeks (at a 200 fold lower dose of etanidazole than in the free form that resulted innocuous) the parasitemia was eliminated. The effect was achieved by pH sensitive liposomes that were taken up by infected cells by a degradative pynocitic pathway; this led to a massive delivery of etanidazole to the cell cytoplasm where the amastigotes reside. Remarkably, the pH-sensitive liposomes are not active against trypomastigotes. In other words, the parasitemia was reduced after modifying the intracellular traffic of the etanidazole to favour cytoplasm targeting. The ultimate challenge of an antichagasic treatment is however, impairing or reducing the cardiac damage caused by the perpetuation of the amastigotes within cardiomiocytes. Here anatomic pathological changes in the envelope of cardiac cells could be used to achieve a passive targeting of nano-objets against targets other than phagocytic cells. This should be achieved during the initial stadium of the chronic chagasic cardiomiopathy or even better during the indeterminate form. The thickening and loss of permeability barrier of the basement membrane enveloping cardiac cells is also found in diabetes and metabolic syndrome. Therefore, different to the occurred with amphotericin B and its nanotechnological formulation, the targeted to cardiomiocytes based-antichagasic treatments, could led to a technical platform useful against diseases affecting developed countries.

NANOPARTICLES AND THE PULMONARY IMMUNE SYSTEM

BARBARA ROTHEN-RUTISHAUSER[1,2], F. Blank[2], E. Seydoux[2], K. Fytianos[1], A. Fink[1] and Ch. von Garnier[2]
[1]Adolphe Merkle Institute, University of Fribourg, Marly, Switzerland
[2]Respiratory Medicine, Berne University Hospital, Berne, Switzerland

Nanoparticles (NP) synthesized by nanotechnology may have many different shapes and chemical compositions; therefore their properties can be specifically designed for a particular clinical diagnostic or therapeutic application. Studies have shown that NP can either stimulate or suppress various immune response pathways [1]; however the effects of NP on the immune system in relation to particle properties (size, surface modification, solubility and shape) remain largely unknown to date.

Working in the emerging field of "nano-immunology", our aim is to understand the immunological properties of various NP. In order to harness the unique properties of NP for novel clinical applications in the treatment of allergic respiratory disease, we plan to develop and utilize specifically designed NP to investigate immune-modulatory effects in the lung with various experimental approaches. Of special interest are dendritic cells that are key antigen-presenting cells, orchestrating both innate and adaptive immune functions [2]. A first study has shown that the model biomedical poly(vinylalcohol)-coated super-paramagnetic iron oxide NP (PVA-SPIONS) can impact dendritic cell phenotype, function, and downstream immune responses by reverting the dendritic cells to a more immature-like state (high capacity for antigen uptake, low capacity for T cell stimulation) that may be important for inducing tolerance [3].



Phase contrast image of dendritic cells incubated for 24h with 20nm gold-NPs.

Further investigations are ongoing in our lab to provide a library of reference NP with different sizes, shapes and surface modifications as well as different cores in order to study possible immune-modulatory effects of such particles. An important focus lies on the reproducible synthesis and in-depth characterization of the NP-suspensions by various methods such as zeta potential, size distribution and colloidal stability. With a well characterized set of particles immune-modulatory effects in dendritic cells are analyzed by studying NP uptake, antigen-processing, surface marker modulation as well as antigen presentation to T cells.

Our current understanding of immune responses to a specific type and size of NP remains incomplete because physico-chemical properties of NP (size, shape, surface charge), shell characteristics (density, thickness, colloidal stability), and thorough in vitro and in vivo immunological characterization have not been systematically performed to date. By combining the expertise from specialists in different fields, we foresee that findings from this multidisciplinary project will ultimately enable to address several of these critical issues.

[1] Dobrovolskaia et al. Evaluation of nanoparticle immunotoxicity. Nat Nanotechnol (2009). [2] Holt et al. Characterization of dendritic cell populations in the respiratory tract. J Aerosol Med (2000). [3] Blank et al. Polymer-coated nanoparticles alter dendritic cell-dependent T cell stimulation. Nanotoxicology (2011).

This project is supported by the National Research Programm 64 "Opportunities and Risks of Nanomaterials" and by the Swiss National Foundation.

GI TARGETING OF BIOMARKERS FOR REAL-TIME DIAGNOSTICS OF MALIGNANCY

ABRAHAM RUBINSTEIN
The Hebrew University of Jerusalem School of Pharmacy Institute for Drug Research, P.O.Box 12065 Jerusalem 91120

Early detection is imperative for the prevention and efficient treatment of gastrointestinal (GI) malignancy. This could be attained by real time diagnosis, joining improved imaging and pharmaceutical approaches for the detection of biomarker molecules either in the lumen or the mucosa of the alimentary canal. We have exploited a variety of strategies to tackle this challenge.

Employing a1- antitrypsin precursor (A1AT) as a secreted biomarker model of gastric carcinoma, we developed a platform with immunoassay capabilities, comprising a sensing and detecting compartments. It was made of a microarray-type support grafted with trypsin as a capturing moiety and a detecting compartment made of near infrared (NIR) fluorescently labeled nanoparticles conjugated to A1AT-specific antibodies. The specific recognition reaction between the captured A1AT and the immuno-nanoparticles generated a profound fluorescence with a signal to noise ratio (SNR) of 12-32, in a biomarker-concentration dependent manner. The optical recognition signal was intense enough to be detected by a video capsule simulator with a SNR of 6-20 (1).

For the early detection of colon polyps we prepared a fluorescently labeled (NIR range) cationized polyacrylamide (CPAA) aimed at targeting the overexpressed sialic acid in colonic malignant cells and tissues. The specific attachment of the polymer was tested in SW-620, SW-480, HT-29 and LS-147T cancer cells and found to be CRC staging dependent. The optimal polymeric product was tested, successfully, in gut sac preparations of the dimethylhydrazine induced rat model. To increase the polymer's targeting capabilities, a FITC labeled recognition peptide (EPPT1) that targets the transmembrane glycoprotein underglycosylated MUC-1 was conjugated to the polymeric backbone and tested in HT-29 and LS-147T cells, followed by an in vivo examination in an orthotopic mouse model. Only the lowest EPPT1 molar ratio in the dually recognition polymer increased the polymer binding to the cells, probably due to quenching phenomena and steric hindrance, requiring further optimization studies (2).

Detection of mRNA alterations is another approach for identifying biomarkers as means of differentiating benign from malignant lesions. By choosing the K-ras oncogene as a target gene, two types of molecular beacons (MBs) based on either phosphothioated DNA (PS-DNA-MB) or peptide nucleic acid (TO-PNA-MB, where TO = thiazole orange) were synthesized and compared in vitro and in cell lines. Their specificity was examined in wild-type K-Ras (HT29) or codon 12 point mutations (Panc-1, SW480) cells. Incubation of both beacons with total RNA extracted from the Panc-1 cell line (fully complement sequence) showed a fluorescent signal for both beacons. Major differences were observed, however, for single mismatch mRNA transcripts in the HT29 and SW480 cells. PS-DNA-MB weakly discriminated such single mismatches in comparison to TO-PNA-MB that was profoundly more sensitive. Cell transfection of TO-PNA-MB with the aid of PEI resulted in fluorescence in cells expressing the fully complementary RNA transcript (Panc-1) but undetectable fluorescence in cells expressing the k-RAS mRNA that has a single mismatch to the designed TO-PNA-MB (HT29). A weaker fluorescent signal was also detected in SW480 cells; however, these cells express approximately one fifth of the target mRNA of the designed TO-PNA-MB. In contrast, PS-DNA-MB showed no fluorescence in all cell lines tested post PEI transfection. Based on the fast hybridization kinetics and on the single mismatch discrimination found for TO-PNA-MB we believe that such molecular beacons are promising real-time imaging of endogenous mRNA with single nucleotide polymorphism (SNP) resolution (3).

REFERENCES

• Khazanov E., Yavin E., Pascal A., Nissan A., Kohl Y., Reimann-Zawadzki M., and Rubinstein A., Detecting a secreted gastric cancer biomarker molecule by targeted nanoparticles for real-time diagnostics, Phram. Res. 29: 983-993, 2012.

• Bloch M., Kam Y., Yavin E., Moradov D., Nissan A., Ariel I. and Rubinstein A., The relative role of charge and a recognition peptide in luminal targeting of colorectal cancer by fluorescent polyacrylamide, Submitted.
• Kam Y., Rubinstein A., Nissan A., Halle D. and Yavin E., Detection of endogenous k-RAS mRNA in living cells at a single base resolution by a PNA molecular beacon. Molecular Pharm, 9: 685-693, 2012.

ACKNOWLEDGMENTS

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CANCER GENETICS-GUIDED DISCOVERY OF SERUM BIOMARKERS FOR CANCER DIAGNOSIS AND TREATMENT RESPONSE

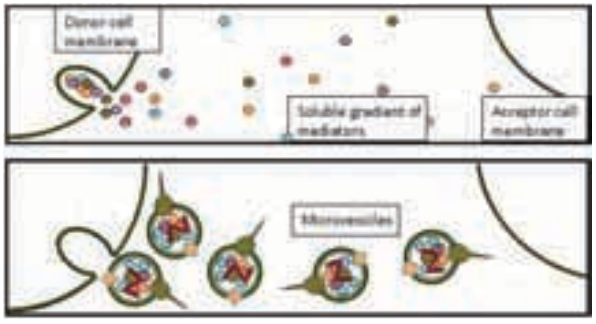
RALPH SCHIESS

A key barrier to the realization of personalized medicine for cancer is the identification of biomarkers. A two-stage strategy for the discovery of serum biomarker signatures corresponding to specific cancer-causing mutations and its application to prostate and ovarian cancer in the context of the commonly occurring phosphatase and tensin homolog (PTEN) tumor-suppressor gene inactivation will be presented. PTEN is one of the most commonly inactivated genes in human cancer and has been identified as lost or mutated in several sporadic cancers, including endometrial carcinoma, glioblastoma, breast, and prostate cancer. It is expected that signaling-pathway-activating mutations such as PTEN loss produce changes in the proteomes of affected tissue, and these changes should be detectable as discrete biomarker signatures in the serum.

NATURAL MICROVESICLES: A NOVEL SOURCE FOR ARRAY DIAGNOSTICS

RAYMOND M. SCHIFFELERS, Pieter Vader, Grietje Andringa, Susan M. van Dommelen, Sander A.A. Kooijmans, Brigitte van Oirschot, Richard van Wijk, Wouter W. van Soline
Laboratory Clinical Chemistry & Hematology, University Medical Center Utrecht, Utrecht , The Netherlands

Recent research points out an endogenous carrier system transporting biologicals between cells. The biologicals delivered by these microvesicles (a broad term to include various vesicular structures like exosomes, shedding vesicles, ectosomes etc.) play an important role in many disease processes, most notably inflammation and cancer. These microvesicles have created excitement in the research community. Until the role of microvesicles was appreciated, the main line of transport of biologicals between cells was thought to occur via gradients of soluble mediators (e.g. growth factors) and their cognate receptors. Local production of a mediator results in high regional levels and strong responses of neighboring cells, whereas cells that are further away receive a less intense signal and respond moderately, if at all (Figure, top panel). Only recently it has become apparent that mediators can also travel as quantum pieces of complex mixtures of biologicals (including nucleic acids) over longer distances, via microvesicles (Figure lower panel).



By analyzing the physical characteristics and contents of the microvesicles information can be obtained on the status of the donor cell. Intriguing findings reported in literature include observations that certain RNA species are specifically packaged in microvesicles to affect acceptor cell phenotype and that membrane receptors on the vesicles' surface can be functionally transferred to acceptor cell membranes leading to changes in cell activity.

In this presentation, the challenges and opportunities for analysis of microvesicles for diagnostic purposes will be discussed.

ENZYME REPLACEMENT THERAPY IN LYSOSOMAL DISEASES

SIMO SCHWARTZ JR.

Prof. Dr. med., PhD; Nanomedicine Coordinator Molecular Biology and Biochemistry, Research Center for Nanomedicine (CIBBIM-Nanomedicine) Vall d'Hebron Univ. Hospital Research Institute, Barcelona (SP)

It is worth noting that because rare diseases have a low incidence in the overall population (defined as lower than 1:10.000 people) the development of specific treatments rises questions related to the potential exploitation benefits for the pharma market. Even though several actions have been taken to improve the benefits/risks ratios for marketed treatments, there is still scarcity on available effective clinical treatments for many rare diseases. These facts reinforces the need for further research and development of new therapeutic strategies for rare diseases, among them Fabry.
Regarding the social interest, Fabry disease (FD) is the second most prevalent lipid lisosomal storage diseases. Although considered a rare disease, the improvement of the current therapy used in FD will have a great impact at social and clinical levels, due to several reasons. First, and more importantly, current enzyme replacement therapy (ERT) does not work in patients with advanced renal, cardiac and cerebrovascular symptoms, so there is an urgent need to improve its efficacy. Second, the real incidence of FD is though to be underestimated because many late-onset patients are currently underdiagnosed. Thus, the development of a better treatment for FD would benefit a wider population that that initially estimated. Third, in diagnosed Fabry patients, once the treatment starts, it requires life-long intravenous injections every other week with annual cost per patient exceeding 150.000€. If nanotechnology-based systems are able to improve the stability, internalization and efficacy of the current recombinant enzyme, it would open the window to cheaper and more stable medicines, facilitating it access to many Fabry patients worldwide. Finally, if nanotechnology-based DDS confirmed to be effective in FD, it would pave the way to design similar strategies to other lisosomal storage diseases.

A NEW APPARATUS FOR THE LOW COST, HIGH THROUGHPUT, DETECTION OF CIRCULATING TUMOR CELLS

GIACINTO SCOLES

Prof. Dr.; University Hospital of the University of Udine, Udine (I) and Biology Department, Temple University, Philadelphia, PA (USA)

In this paper I will summarize first the current consensus on the detection of Circulating Tumor Cells (CTCs) as prognostic and diagnostic tool.
While it appears to be known that the absence of CTCs does not imply the certain absence of tumors there is widespread agreement on the fact that the presence of CTCs has to imply the presence of one or more tumors.
From there to placing the emphasis on the time behavior of the number of CTCs, as opposed to the determination of their absolute number, the step is short and rather logic. It follows that if the analysis of CTCs could be carried out with high throughput and at low cost the

usefulness of such measurements to verify the working of any given chemo-therapic cocktail could be rapidly established and would have a rather large impact on the statistics resulting from the treatment of these type of tumors.
We will then review the only established method to analyze the number of CTCs
and describe briefly the working principles of a fairly large number of apparata that have been recently proposed to solve this problem. After having pointed out the shortcomings of most of these techniques we will outline our proposed solution and the progress done so far to realize it.
This work was carried out in collaboration with my colleagues L.Casalis, D. Cesselli D. Cojoc and M. Lazzarino. and with our students l. Ianeselli and L. Venturelli in the MONALISA consortium of labs.

NEW FRONTIERS IN INTERVENTIONAL CARDIOLOGY: FROM METALLIC STENTS AND BIORESORBABLE SCAFFOLDS TO NANOPLATFORMS

PATRICK W. SERRUYS
Prof. Dr. M.D., Ph.D., FESC, FACC; Department of Interventional Cardiology, Thorax Center, Erasmus University Medical Center, Rotterdam, The Netherlands

Effective prevention of atherosclerosis and treatment of its complications remain a major clinical challenge. Nanomedicine allows for the creation of agents specifically designed to enhance the diagnosis (imaging), targeting and treatment of atherosclerosis. Given the rapid advances in nanoagent synthesis and utility, clinical application of these technologies can be anticipated in the near term.
The HMGCoA reductase inhibitors have a remarkable track record of lowering cholesterol and improving survival. Unfortunately, a 6.39 mm2 regression of atheroma was documented only with 30% reduction in events. It is likely however that fibrous tissue and mineral deposits may prove impervious to metabolic manipulation. Current percutaneous coronary intervention (PCI) with drug-eluting (DES) stents is associated with delayed healing, late stent thrombosis, abnormal vasomotor function and neoatherosclerosis. New devices such as the fully bioresorbable scaffolds (BRS) treat the diseased coronary vessels and elutes an anti-proliferative drug that counteracts constrictive remodeling, excessive neointimal hyperplasia and delays re-endothelization. The first two publications on the first generation of everolimus-eluting bioresorbable scaffold (BRS, Abbott Vascular, Santa Clara, USA) have reported a few seminal observations made at 6-month and 2-year follow-up using multiple imaging modalities: a) complete bioresorption has been documented by optical coherence tomography (OCT), intravascular ultrasound (IVUS) greyscale and IVUS radiofrequency backscattering; b) physiologically or pharmacologically induced vasomotion has been fully restored in the scaffolded area; c) between 6 and 24 months, late luminal enlargement with plaque media regression without apparent remodeling has been established.
The serial analysis of the second generation of everolimus-eluting BRS (Abbott Vascular) confirmed at medium term the safety and efficacy of this new device. Non-serial observations have shown no signs of shrinkage of the BRS at 6 months and recovery of vasomotion at one year, indicating the disappearance of the mechanical integrity of the scaffold. The ABSORB Cohort B trial is a multicentre single-arm trial assessing the safety and performance of the ABSORB BVS (Rev.1.1, Abbott Vascular, Santa Clara, USA) in the treatment of patients with a maximum of two de novo native coronary artery lesions. The first 45 patients (Cohort B1) underwent serial invasive imaging follow-up at 6 and 24 months, such as angiography, IVUS and OCT, whereas the other 56 patients (Cohort B2) with initial follow-up at 12 months will undergo invasive follow-up at 36 months. There were one periprocedural myocardial infarction and two proximal edge target lesion revascularizations, which resulted in a 2-year major adverse cardiac event rate of 6.8%. No possible, probable or definite scaffold thrombosis has been documented. From 6 to 24 months, angiographic late luminal loss increased from 0.17±1.19 mm to 0.27±0.19 mm with an increase in neointima of 0.68±0.43 mm2 on OCT, and 0.17±0.26 mm2 on IVUS. As consequence, diameter stenosis increased from 15±6% at baseline to 19±7% at 6 months and 21±8% at 2 years without intra-scaffold binary restenosis. On OCT, struts are still recognizable at 2 years and showed almost complete coverage (99%). Nevertheless, significant optical and ultrasonic signs of bioresorption are demonstrated together with a significant increase in scaffold area (0.54±1.09 mm2 on IVUS, p=0.003 and 0.77±1.33 mm2 on OCT, p=0.016) and the total plaque/ media area (0.41±0.63 mm2 on IVUS from baseline to two years, p<0.001: yielding process of the scaffold may herald the observed late lumen enlargement seen at 3 years and 4 years in a porcine model.
Nanotechnologies, utilizing nanoparticles and nanocoatings for stents and scaffolds, may open a new era in interventional cardiology, and possibly help to settle majority of current obstacles. We focus on progressive development of technologies mostly in interventional cardiology with the main goal to achieve atheroregression below 40% Glagovian threshold.
Among the state-of-the-art devices we analyze the evolution of approaches from metallic stents of different generations and bioresorbable scaffolds to nanoplatforms with special attention to some impressive examples of nanodevices, the classification of nanomaterials and working mechanisms, underlying atheroprotective effects and establishing perspectives for the consequent clinical development. The next generations of stents and bioresorbable scaffolds, which utilizing nanotechnologies, become an intermediate step between previous generations of devices and new stage of the technical evolution. As examples (see fig. 1), we have some stents with new polymer coatings with better drug-releasing features and circulating cell-capturing system, ultra-thin struts, polymer-free cages with micro- and nanopores drug-loading systems.
We have an ultimate dream about design of the optimal BRS with excellent mechanic properties, appropriate duration of resorption and releasing of drug. Novel optimal BRS platform has to have thinner struts (40-80 μm) and better mechanical properties (radial strength at least 900-990 mmHg) with extra benefits such as slow drug releasing system for mTOR inhibitor or other drugs, such as regulators of lipid (for instance, rosuvastatin, lipoprotein-associated phospholipase A2 inhibitor, new substances such as PCSK9 inhibitors, ApoA1-Milano, monoclonal antibodies against oxLDL), immune-inflammatory (new generations of limus in stable formulation – micro-crystalline or in lipid envelope) and repair responses (including vasomotion and artery remodeling), agents against spontaneous and necrotic-core-mediated calcification (calcitriol, paricalcitol), anti-platelet drugs with minimal local toxicity, atheroprotective potentials and optimal restoration of tissue. The abluminal nanocoating (thickness no more than 100-150 nm, but anyway less than 1-2 μm) with incorporation of lipid-based nanoparticles, which are able to carry any drug, acting acutely, could be helpful for the management of the vessel wall immediately after implantation and prevention of neoatherosclerosis, atherothrombosis or detrimental and perverted biological feedback to the intervention. Local (in lipid-based drug-carrying nanoparticles inside backbone) and systemic administration (for example, low-dose chronic prescription of everolimus up to 2 mg each 2 days, or pulse therapy by 7.5 mg x 3 days, 5 mg x 2 days) of mTOR inhibitor ensures regulation of the re-endothelization and local cellular milieu's response as well as grants general atheroprotective effects. Utilization of the drug-coated balloon for pre-dilatation is another solution for the local anti-proliferative therapy. Calcium-phosphate or magnesium bioresorbable nanoparticles in backbone and sophisticated management of the polymer structure with alterations of carbon binds are able to substantially radically alter mechanical properties of the scaffold and allow reducing the thickness of struts. PDLLA-luminal layer with circulating progenitor cells capturing system using antibodies against CD73, CD105 (preferably with pro-mesenchymal phenotype) or CD34 (bone marrow-derived) and CD133 (endothelial cells) antigenes promotes re-endothelialization during first 48-72 hours, preventing atherothrombosis, and at the same time under control of slow-releasing local and low-dose systemic treatment with mTOR inhibitor adjusts repair and immune responses, disabling neoatherosclerosis.
Nanotechnologies are currently developing typically as drug delivery and diagnostic tools. A new application recently emerged, where NPs were used to treat atherosclerotic plaque (laser technique, elec-

trosurgical removing, plasmonics, or using a radio frequency sparking). Nanomedicine has become the most advanced breakthrough of the device evolution in interventional cardiology and an important tool in the imaging, targeting and treatment of atherosclerosis. This is due, in large part, to the ability to generate multifunctional nanoagents bearing combinations of targeting, diagnostic and therapeutics moieties, allowing for the tailoring of the properties of the synthesized nanomaterials, and heralding to embody our dream about ultimate atheroregression below 40% Glagovian threshold and restoration of vessel.

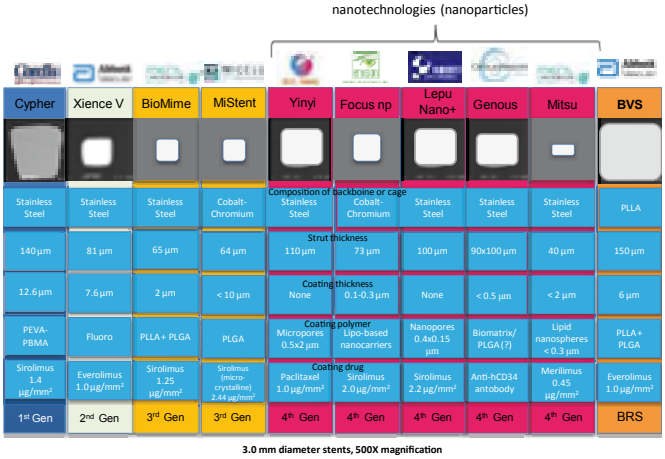


Fig. 1. Characteristics of new generation of metallic stents and bioresorbable scaffolds developed with nanotechnologies.

SAVEME: A MODULAR ACTIVE NANO-PLATFORM FOR ADVANCED CANCER MANAGEMENT: CORE NANOSYSTEMS, TUMOR TARGETING AND PENETRATION, MOLECULAR IMAGING & DEGRADOME BASED THERAPY

LOUIS SHENKMAN

An estimated 3.2 million new cancer cases and 1.7 million deaths per year in Europe define cancer as a crucial public health problem. SaveMe is developing a novel modular nanosystems platform integrating advanced functionalized nano-core particles and active agents. The modular platform will enable the design of diverse active nanosystems for diagnostic or therapeutic applications as defined by their active agent compositions: ligating only crucial active agents, at the optimized composition per application, while excluding other agents to minimize toxicity risks. As a model system, SaveMe will develop and validate the new platform for pancreatic cancer. Pancreatic cancer has the highest one-year mortality rate of any cancer and is Europe's sixth deadliest cancer. The overall five-year survival rate is 4%, and has not improved during the last 25 years. Most pancreatic tumors are detected late, at metastatic stage and 85% are unresectable at the time of detection. This is due, in part, to the limitation of current imaging systems in diagnostic accuracy, particularly in determining resectability and evaluation of sub-cm nodal and metastatic disease.

For early diagnosis, active nanosystem will be developed for molecular MR, PET and gamma camera imaging, enabling efficient diagnosis and guided surgery. For that purpose, novel functionalized nano-core systems will be conjugated with semi-confluent active shell layer. Three types of shell layers will be designed (1) iron oxide nanoparticles as advanced MRI contrast agents; (2) DOTA complexes for MRI (with Gd3+); or PET (with Ga-68); or gamma camera (with Ga-67); (3) both iron oxide nanoparticles and DOTA-Ga-68 complexes for a sequential or simultaneous MR/PET imaging, as well as a novel hybrid PET/MRI prototype.

For therapeutics, active nanosystems will be developed to deliver antibodies or nucleic acids as therapeutic agents, including: (1) anti-matrix metalloproteases (MMP)-inhibitory-scFv, based on recently developed mAbs, and (2) therapeutic siRNAs. These non-classic anti-tumor drugs will be developed based on tumor degradome/protease web studies, since tumor cell growth, invasion and metastasis are essentially dependent on selective protease activity. In addition, the targeted nanosystems can be loaded with conventional anti-neoplastic drugs.

The nanosystems will be designed for intravenous (IV) administration. Targeting moieties employed will consists of tumor cell targeting peptides, including novel somatostatin receptor analogues (SSTRs) subtypes and targeting moieties based on newly emerging selective biomarkers, e.g. the receptor/ligand system Gal-1/tPA. The later may allow selective targeting of pre-malignant pancreatic cancer. To enable specific tumor tissue penetration, a PEG–MMP-substrate-PEG agent for optimal tissue diffusion will also be employed. To date, we have designed and screened novel generic core polymeric nanosystems for optimal nanomaterial properties (NC average size and low size dispersivity, functionality level, water compatibility, minimal aggregation level) enabling the formation of corresponding delivery vehicles of highly potent non-classic drugs for cancer diagnosis, guided surgery and therapy. Polymers used for that fabrication purpose are non-toxic biodegradable and biocompatible polymers classified as GRAS (Generally Recognized As Safe) like PLGA (poly[lactic-co-glycolic acid] co-polymer), PEG (polyethyleneglycol) derivatives including PLGA hybrids, human serum albumin (HSA), and polyacrylates (PAs, PAs, PLGAs/PEG-PLGAs, PEGs, HSA). Various nanofabrication methodologies have been explored. They include polyacrylate (PAs) Huisgen "Click" cycloadditions (Intramolecular polymer single chain cross-linking/collapse), NC nanoprecipitation (solvent deposition) with oil/water emulsion and desolvation methods (for the fabrication of mainly PLGA-COOH/PLGA-PEG-COOH polymeric particulate systems.

Regarding selective targeting for pancreatic cancer, two avenues have been explored. The first utilizes somatostatin analoges as a targeting moiety, and several novel SST analogs peptides (termed PTRs) have been developed. Binding affinity of all PTRs was studied using 9 human pancreatic cell lines and all PTRs demonstrated rapid intracellular internalization in several models. The second group of targeting moieties includes Galectin 1 and mesothelin. The expression of these receptors was proved in mRNA and protein levels by QRT-PCR and western blot, respectively, in human PaCa cells, followed by positive IHC stain of ex-vivo tumors implanted into mice, as well as human excised samples. Four novel tPA-like peptides, which is the Gal-1 ligand, were developed in order to serve as potential targeting moieties.

Regarding novel non-classic drugs based on cancer degradomics, identification of key extracellular MMPs associated with pancreatic tumor development and progression that may be candidates for therapeutic intervention and for functional activation of PEG-coated nanoparticles in vivo has been investigated. A suite of genes that are consistently over-expressed in pancreatic cancer, including S100P, FN1, THBS2, PNLIPRP1, CLPS, CPA2 and AMY1A has been identified. In addition, generation of novel, highly specific inhibitory antibodies using agents that mimic structural features of the active sites of MMPs has been accomplished.

In-vitro analysis of toxicity and biocompatibility of the obtained NPs and related components (PLGA, PLGA with Gallium and alizarin; HSA-based particles CAN and LP; Gallium, Alizarin; PTR86 – somatostatin peptide; anti-MMP9 antibodies) fabricated thus far was performed. All tested preparations had no toxic or apoptogenic effects on liver, kidney and immune cells. The preparations did not display major side effects in other tests (red blood cells hemolysis, platelet aggregation, complement activation, serum protein coagulation) used for the evaluation of their biocompatibility. The obtained results demonstrated, on the in vitro level, no essential restrictions for application of the tested preparations in anticancer medications.

THE CHALLENGE OF GLOBAL INTERRUPTION OF MALARIA - CURRENT TOOLS

ROBERT SINDEN

The talk will review the recent adjustment in the objectives of current research programmes to reengage with the concepts of local elimination and global eradication of malaria parasites. The potential contributions of nanotechnologies to meet these new objectives will be highlighted.

CHALLENGES FOR CLINICAL USE OF INTRAVENOUSLY INJECTED NANOPARTICLES

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 in order to bring NPs into common clinical use as fast as possible. With a long experience from pharmaceutical R&D, I will discuss improvements that should be made in biological studies with NPs. The issue of deciding whether NPs are taken up or just adsorbed to cells will be discussed, as well as different cellular uptake mechanisms and where the NPs end up within the cells. Moreover, the experimental setup related to issues such as degradation and toxicity of the NPs studied will be reviewed.
The design of animal studies, including which time points to take samples and which parameters to analyze, is very important when aiming at developing drugs for clinical use. 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 be documented in a way required to receive market approval. The design of such studies and interpretation of the data obtained will be discussed, as well as the use of theranostic NPs versus NPs specific for therapy or imaging.

REFERENCES

• **Skotland T**, Iversen TG, Sandvig K: New metal-based nanoparticles for intravenous use: requirements for clinical success with focus on medical imaging. Nanomedicine 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.

REMINERALISATION OF HARD TISSUES BY NANOPARTICLES

WENDELIN J. STARK
Institute for Chemical- and Bioengineering, Department of Chemistry and applied Biosciences, ETH Zurich, Switzerland

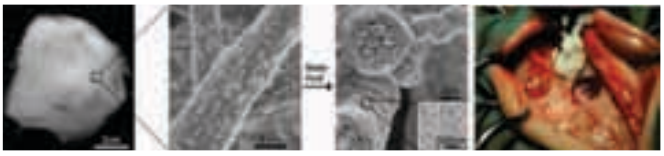


Figure 1. The cotton wool-like PLGA/a-CaP scaffolds enable fast mineralization when coming in contact with body fluid and are easy-to-apply by surgeons.

Bone repair and regeneration of defects with bioresorbable implant materials are in great demand in reconstructive surgery. At present, biomaterials are made using solid state reactions and sol-gel processes but often limited in terms of composition, high remaining solvent content and reactivity. Here, the direct synthesis of bioactive glass [1] and tricalcium phosphate (TCP) nanoparticles by flame spray synthesis [2] is shown. These nanoparticles can be applied as a direct biomedical product or as part of a composite material for implant applications. As prepared TCP nanoparticles enable the formation of smoothly injectable cements and pastes which can be used in minimal invasive surgery with considerable fast setting time due to their high specific surface area [3]. It is further demonstrated how these particles can be combined with a biodegradable polymer, poly(lactide-co-glycolide) to produce a highly bioactive bone fixation device [4]. The preparation of a highly porous fibre network with an open structure by electrospinning results in a cotton wool like material suitable for the filling of bone defects [5]. The formation of nano-sized hydroxyapatite confirms the high bioactivity of the compressible composite material suggesting applications for non-load bearing bone defects. Flame derived bioactive glass shows an increased remineralisation of human dentin [6] and advanced antimicrobial properties for root canal disinfection [7]. In order to reinforce biodegradable polymer matrices, the particles were further introduced into poly(3-hydroxybutyrate) inducing a nano-structured surface topography, increased stiffness and enhanced protein adsorption [8] aiming for hard tissue engineering.

REFERENCES

[1] Brunner, T.J., Grass, R.N. & Stark, W.J., Chem. Commun., 13, 1384-86, (2006).
[2] Loher, S., Stark, W. J., Maciejewski, M. et al., Chem. Mater. 17, 36-42, (2005).
[3] Brunner, T.J., Bohner, M. et al., J. Biomed. Mater. Res., 83B, 400-7, (2007).
[4] Mohn, D., Ege, D., Feldman, K. et al., Polym. Eng. Sci., 50(5), 952-60, (2010).
[5] Schneider, O.D., Loher, S. et al., J. Biomed. Mater. Res., 84B, 350-62, (2007).
[6] Vollenweider, M., Brunner, T.J. et al., Acta Biomater., 3, 936-43, (2007).
[7] Gubler M., Brunner, T.J., Zehnder, M. et al., Int. Endod. J., 41, 670-8, (2008).
[8] Misra, S.K., Mohn, D. et al., Biomaterials, 29, 1750-61, (2008).

NEW NON-ORAL DRUG DELIVERY SYSTEMS FOR PARKINSON'S DISEASE TREATMENT

FABRIZIO STOCCHI
Prof. Dr. med.; Director of the Parkinson's Disease and Movement Disorders Research Centre at the IRCCS San Raffaele, Rome (I)

The management of parkinsonian patients is frequently complicated by sometimes unpredictable or drug-resistant akinetic periods and abnormal involuntary movements. These alterations in l-dopa responsiveness manifested by motor fluctuations and dyskinesias is one of the most limiting factor in the long term treatment of Parkinson's disease. Evidence suggests that many of these phenomena directly reflect oscillations in l-dopa plasma levels and the rate of l-dopa transport to the brain.
The use of standard oral formulations of l-dopa does not allow maintaining adequate plasma levels throughout the day, and also slow release preparations have not been successful in severely complicated patients. Many studies have shown that fluctuations in motor performance occur when l-dopa is administered intermittently but not when it is administered continuously.
Long lasting and dramatic reductions in motor complications have also been observed in advanced PD patients, where treatment with continuous infusion of levodopa or a dopamine agonist (apomorphine, lisuride) is associated with reduced "off" periods and dyskinesias. For example, patients randomized to receive a continuous subcutaneous infusion of lisuride have marked reductions in both "off" periods and dyskinesias in comparison to those randomized to treatment with standard oral formulations of levodopa.
CALIPSO study was a 6-week prospective randomised double-dummy, double-blind comparison between an high-dose of oral do-

pamine agonist (pramipexole) with a continuous subcutaneous infusion of lisuride as add-on to levodopa in complicated parkinsonian patients. In this study lisuride showed to be superior to ral agonists in improving motor complications.
Apomorphine is a potent dopamine receptor agonist water-soluble that have been shown to successfully control motor fluctuation when subcutaneously infused in complicated parkinsonian patients.
Today is also possible to infuse levodopa continuously in the duodenum with good control of motro fluctuations and dyskinesia.

PUBLIC-PRIVATE PARTNERSHIPS IN TARGETED NANOMEDICINE DEVELOPMENT

GERT STORM
Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, The Netherland
MIRA Institute for Biomedical Technology & Technical Medicine, Twente University, The Netherlands

The application of nanotechnology is having a major impact across many fields of medicine with novel nano-based materials appearing in diagnostics, imaging, drug delivery systems, biosensing, and medical materials and devices. This lecture will particularly focus on the (pre)clinical impact of public-private partnerships on the development of targeted nanomedicines. In addition to a condensed overview of the current clinical status of the field of targeted nanomedicines, two examples of public-private partnerships will be given to illustrate how close collaborations between the major stakeholders can contribute to help overcoming major innovation challenges.

The first example will deal with the EC-sponsored (FP6) MEDITRANS project (www.meditrans-ip.net) on targeted nanomedicines. Platform technologies have been developed with broad applicability to disease treatment, as exemplified by the choice for cancer and chronic inflammatory disorders (rheumatoid arthritis, Crohn's disease, multiple sclerosis) as target pathologies. Nanomedicines (based on carrier materials like polymeric and lipidic nanoparticles, nanotubes, and fullerenes) are endowed with superior targeting and (triggerable) drug release properties. In parallel, MRI imaging probes will be designed that report on the in vivo localization of the targeted nanomedicines, specific biomarkers, the drug release process and therapeutic outcome (imaging-guided drug delivery). The consortium consisted of 30 partners from 9 EU member states (including 1 new member state) and 3 associated states, and includes 13 industrial companies, 11 universities and 6 research institutes. The total budget was around €16 M.

The second example will focus on the dutch government-sponsored (CTMM) HIFU-CHEM project (www.ctmm.nl) based on a consortium of 6 partners, 5 from The Netherlands and 1 from the USA, with a budget of around €7 M. Magnetic Resonance imaging-guided High Intensity Focused Ultrasound (MR-HIFU) is an emerging technology that allows non-invasive thermal therapy with real-time monitoring. MR-HIFU is casted for a significant role in image-guided interventional oncology. It can be used for controlled thermal ablation of tumor tissue, but also holds great potential for improving the efficacy of chemotherapy through the triggered local release of anticancer agents from thermosensitive targeted nanomedicines. In HIFU-CHEM, the clinical application of MR-HIFU for the treatment of liver metastases and bone metastases is addressed.

Both examples illustrate the positive effects on clinical translation of targeted nanomedicine research conferred by multidisciplinary partnerships where academia and industry define synergistic ways of in-depth collaboration.

HEMOCOMPATIBILITY TESTS OF NANOPARTICLES AND BIOLOGICALS WITH FOCUS ON COMPLEMENT ASSAYS

JANOS SZEBENI
Nanomedicine Research and Education Center, Semmelweis University and Bay Zoltan Nonprofit Ltd, Budapest, and Faculty of Health Care, Department of Nanobiotechnology and Regenerative Medicine, Miskolc University, Miskolc, Hungary

Infusion, or hypersensitivity reactions (HSRs) are frequent side effects of intravenously administered nanomedicines and biologicals. These, mostly mild and well tolerated, but occasionally severe or fatal allergic reactions represent a hemo-incompatibility due to activation of the complement (C) system. The presentation will outline the HSRs caused by marketed nanomedicines (liposomal drugs, micellar systems, protein-polymer-conjugates, imaging agents, drug carrier nanosystems) and antibody therapeutics (mAbs), pointing out the remarkable similarity of clinical symptoms and difference from true (IgE-mediated) allergy. Beside information on the prevalence, risk factors and molecular and cellular mechanism of C activation-related pseudoallergy (CARPA) caused by the above agents, the biological background of C activation by nanoparticle and mAb-based medicines will be highlighted. Regulatory agencies are increasingly aware of the adverse immune effects of drugs in the above categories, emphasizing the need for appropriate immune toxicity tests in the preclinical stage. In fact, the close correlation between C activation and HSRs provide rationale for using in vitro C activation and in vivo CARPA assays as predictors of infusion hypersenstivty and other adverse immune reactions. The talk will briefly touch on the available methods for C activation and CARPA testing, namely ELISA of C cleavage (C3a, C5a, C4d, Bb, SC5b-9) products, the hemolytic (CH50) assay, FACS measurement of basophil leukocyte activation, a fluorescent bead assay for SC5b-9 and multiplex C byproduct analysis, the porcine assay of cardiopulmonary distress and CARPA tests in other animal species. Finally, a decision tree will be suggested for the application of the above immune toxicity tests.

DETERMINANTS FOR ACCESS TO EFFECTIVE MALARIA TREATMENT

THOMAS TEUSCHER, Eric Mouzin
Roll Back Malaria Partnership Secretariat, Geneva, Switzerland

Malaria control is one of the most effective public health interventions available to reduce child mortality. Over the last 10 years, 1.1 million lives of children under the age of 5 have been saved by scaling up malaria prevention and control measures. The efficacy of new tools, including long lasting insecticide-treated bednets (LLINs), rapid diagnostic tests (RDTs) and artemisinin-combination therapies (ACTs) is largely credited for the recent successes achieved. Field effectiveness however can be more challenging to obtain than clinical efficacy alone. Global health professionals need to take into account determinants for access to effective malaria preventive and therapeutic interventions. Nanomedicine could be of great help to solve some of the current effectiveness challenges like finding a single-dose treatment regimen, simplifying age-related therapeutic dosage or drug administration in severe malaria. Rapid diagnostic testing challenges could also benefit from recent nanotechnology advances.

PREDICTIVE NANO-PERIODIC PROPERTY PATTERNS FOR DESIGNING OPTIMUM NANO-THERAPIES

DONALD A. TOMALIA
PH.D.; NanoSynthons LLC, The National Dendrimer & Nanotechnology Center, 1200 N. Fancher Avenue, Mt. Pleasant, MI 48858 USA

A concept and framework that describes first steps toward defining a "central paradigm" for the unification of nanoscience was introduced in 2009 [1-3]. The concept was based on traditional first principles of chemistry and physics. It proposed that at least six hierarchically dependent, design parameters were involved in the important transfer of structural information and physico-chemical property patterns from the picoscale→subnanoscale→nanoscale levels. This transferred structural information and these physico-chemical patterns literally define important new emerging properties of interest to the pharmaceutical industry that result from hierarchical symmetry breaking as proposed by physics Nobel Laureate, P.W. Anderson [4].

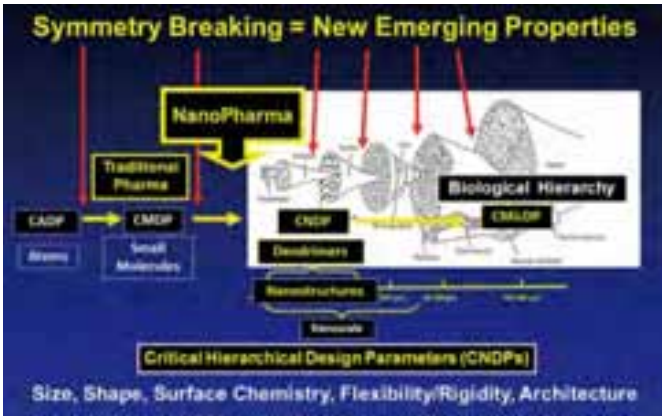


Figure 1. Hierarchical transfer of structural information from the atomic→small molecule→nanoscale→micron scale based on critical hierarchical design parameters [1-3].

These critical design parameters referred to as: CADP, CMDP and CNDP (i.e., atomic, molecular, nano-level, respectively) include: size, shape, surface chemistry, flexibility/rigidity, architecture and elemental composition. Although this concept has been supported by innumerable anecdotal examples in the literature, until present, it lacked important x-ray confirmed structural proof for unequivocal verification. Recent advances by both chemists (i.e., V. Percec, et al. [5] and C. Mirkin, et al. [6]), as well as physicists (i.e., A. Castleman, et al. and S. Khanna, et al. [7]) have now begun to fulfill and validate these concept principles. "As such, one must ask, what do these developments have to do with nano-pharma and nanomedicine?"

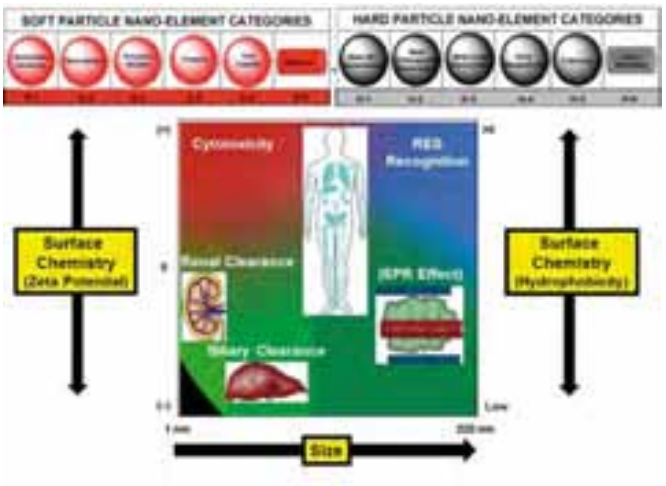


Figure 2. Nano-periodic property patterns for Soft/Hard Nano-Element Categories [1,13] as a function of size and surface chemistry.

This lecture will focus on predictive, CNDP dependent nano-periodic property patterns based on nanoscale size, shape, surface chemistry and architecture that portend significant implications in the nano-pharma and nanomedicine fields. These nano-periodic patterns, which are applicable to both Soft (i.e., dendrimers, proteins, etc.) and Hard (i.e., metal chalcogenide-quantum dots, metal oxide clusters, silica nanoparticles and carbon nanotube) type nano-element categories, provide powerful options for optimizing in vivo excretion modes [8-10] for many diverse nanoscale devices (i.e., structures, conjugates, assemblies) presently under consideration for a wide variety of nano-therapies [11-12].

REFERENCES

• D. A. Tomalia, J. Nanopart. Res. (2009), 11, 1251.
• D.A. Tomalia, Soft Matter, (2010), 6, 456.
• D.A. Tomalia, New. J. Chem. (2012), 36, 264.
• P.W. Anderson, Science, (1972), 177, 393.
• B. Rosen, D.A. Wilson, C. J. Wilson, V. Percec, et al., J. Am. Chem. Soc. (2009), 131, 17500.
• R.J. Macfarlane, B. Lee, C. Mirkin, et al., Science, (2011) 334, 204.
• A. Castleman Jr., S.N. Khanna, J. Phys. Chem. (2009), 113, 2664.
• H. S. Choi, W. Liu, P. Misra, E. Tanaka, J. P. Zimmer, B. I. Ipe, M. G. Bawendi, J. V. Frangioni, Nat. Biotech., (2007).
• K. Kostarelos, (2010), PNAS, 107, 12369.
• A.A. Burns, J. Vider, U. Wiesner, et al., Nano Letters, (2009), 9, 442.
• B.Y.S. Kim, J. T. Rutka, W.C.W. Chan, New England Journal of Med., 363, 2434 (2010).
• A. Menjoge, R.M. Kannan, D.A. Tomalia, Drug Discovery Today, (2010), 15, 171.
• S. McNeil, (2009) Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology, 1, 264.

IMMUNOTOXICITY TESTING OF NANODRUGS, NANOCARRIERS & NEW METRHOD PREVENTING PSEUDOALLERGIC REACTION

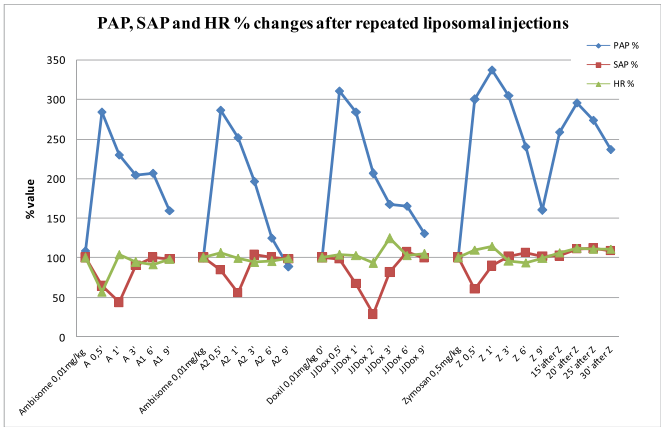
RUDOLF URBANICS, [1,2], Rosivall, L.[3,4], Skrol, S.[5], Stellacci, F [5], Venturelli, E.[6], Kostaleros, K.7, Szebeni, J. [1,2,3]
Semmelweis University - Nanomedicine Center[1]; SeroScience Ltd.[2]; Semmelwis University, Dept of Pathophysiology[3]; HAS-SU Pediatrics and Nephrol Research Group[4]; Instituto Neurologico Carlo Besta[5]; CNRS Immunologie et Chimie Thérapeutiques[6]; The School of Pharmacy, University of London[7].

Nanotechnology is producing new therapeutic and diagnostic tools with improved kinetic, efficacy and diminished toxicity properties. Nanocarriers may transport active agents to a specific target site, providing maximal therapeutic activity with enhanced safety, while preventing the degradation or inactivation of the active agent. The improved efficacy, diminished toxicity and better pharmacokinetic properties may provide the required complexity of drugs to address the needs of modern medicine, but at the same time these advantageous characteristics have been accompanied by new adverse reactions. Nanoproducts, especially if applied intravenously, often evoke pseudoallergic reactions.
The size of these new formulations is in the 50-200 nm range, and the human immune system readily recognizes them as foreign materials, and triggers an immune response. The triggered response can be an immediate hypersensitivity reaction (HSR) and/or an antigen-specific immune response, which may cause antibody production against the drug thereby altering its pharmacokinetic properties.
This presentation deals with the acute immunotoxic properties of nanodrugs/nanocarriers, presenting a sensitive in vivo animal model, to detect this potentially life-threatening side-effect, and describing a potential preventing method1. The pseudoallergic reaction (also called anaphylactoid, idiosyncratic or infusion reaction) correlates with the activation of the complement (C) system, called C activation-related pseudoallergy (CARPA)2,3. CARPA resembles the symptoms of Ig-E mediated anaphylactoid reactions, but without the presence of immunoglobuline Ig-E. These reactions arise at first application of nanomaterials and are less severe or completely absent upon repeated exposures. The HSR occurs rapidly, it is dose dependent and usually shows spontaneous resolution. The occurance rate is

high (2-40%) and the reaction occasionally fatal. Fatal reactions are caused by cardiac and/or respiratory arrest, shock, or multi-organ failure.

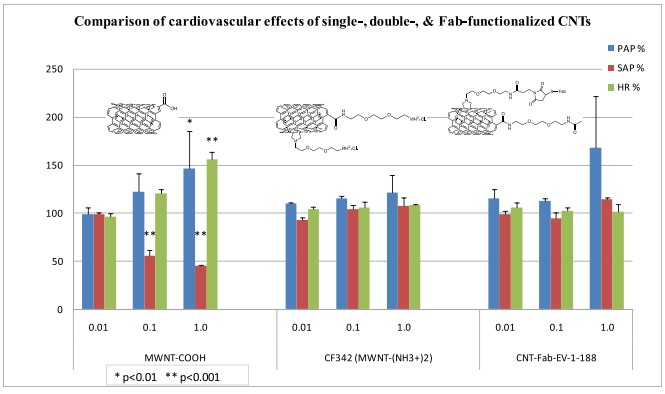
Nanoparticle forming medicines causing CARPA include liposomal and micellar drugs (Doxil, Ambisome, Taxol), but monoclonal antibody therapies also elicit similar reactions, as well as the different nano-carriers like carbon nanotubes, polyethylenimine block copolymers4, or gold nanoparticles.

The reaction can be tested in an animal model, the pig CARPA model. Pigs provide a sensitive model for human pseudoallergic reactions. In case of intravenous application of liposomal drugs like Doxil or AmBisome, CARPA reactions occur in > 90% of pigs. In humans moderate to severe HSRs occur in 45% of patients following Doxil4 treatment and in about 36% according to an MTD study5 with AmBisome. Pigs show all characteristics of severe human CARPA reaction, it is characterized by pulmonary pressure change, usually reaching a 250-300% increase in pressure, followed by systemic pressure increase/decrease, and with tachycardia and ECG alterations, dyspnoe and flush. Occasionally, respiratory or cardiac arrest leads to a fatal outcome. In most cases the reaction spontaneously ceases and the physiological parameters recover. As an example, major physiological parameter changes (pulmonary arterial pressure – PAP, systemic arterial pressiure – SAP and heart rate – HR) are presented after repeated injection of AmBisome and Doxil, both liposomal drugs, and the positive reference material Zymosan.



Different polymers and other gene and drug delivery systems, similarly to liposomes may also evoke immune side effects. These nano-carriers may deliver poorly soluble pharmaceuticals or deliver drugs to earlier unreachable sites, intracellular targets. As as an example, reactions of carbon nanotubes (CNTs) are demonstrated in the figure below. Some of the in vivo safety issues for CNTs can be addressed by surface functionalisation, which also provides chemistries for antibody attachment. Importantly, such surface changes leading to functionalised nanotubes appear to enhance their biocompatibility, mobility around the body and cellular uptake and at the same time lead to non-toxic clearance profiles[6].

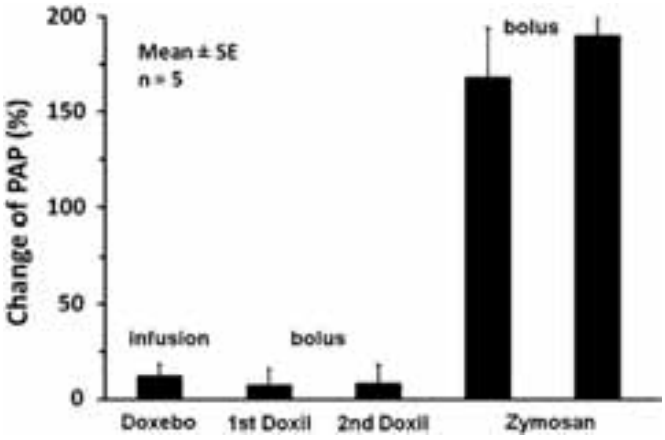
In the next figure cardiovascular effects of f-CNTs are demonstrated in the pig model. The expriment clearly demonstrates that the functionalization leads to diminished reactogenicity.



Gold nanoparticles by functionalization, or PEGylated polycations with higher molecular weight PEG are also less reactogenic.

Doxil, a PEGylated liposome (but not the PEG-free liposome AmBisome!, see the first figure) shows tachyphylaxis after repeated application. This observation raises the possibility of using this effect as a preventive measure for anaphylactic reactions.

Not only the liposomal drug Doxil, but the emty, doxorubicin–free liposome (Doxebo) is able to evoke the tachyphylaxis.



We applied Doxebo by low dose, slow infusion, which caused no CARPA reaction itself, and tolerized pigs to consecutive given Doxil doses1, see figure. Note, that the positive control direct C activator Zymosan reaction was not prevented! The protective effect of Doxebo was specific to Doxil.

Massive C consumption in the tachyphylaxis can be ruled out (see the consecutive Zymosan reaction), and the short time-lag for tolerance development speaks agains immune memory. The mechanism behind this tolerizing effect has to be further investigated. This anaphylaxis preventive method can be a clinically useful tool preventing not-predictable severe HSRs, and diminishing treatment costs. The phenomenon might also be utilized for preventing CARPA reactions for other tachyphylaxis- shoving nanoproducts.

The sensitive pig model, besides safety immunotoxic applications at the end of peclinical development, could be useful tool to detect adverse immunitoxicological reactions of nano-products already early in the development phase, giving clues for successful functionalisation, saving development costs and preventing later clinical reactions.

• J. Szebeni, et al., Prevention of infusion reactions to PEGylated liposomal doxorubicin via tachyphylaxis induction by placebo vesicles: A porcine model, J. Control. Release (2012), doi:10.1016/j.jconrel.2012.02.029

• Szebeni et al., Hemodynamic changes induced by liposomes and liposome-encapsulated hemoglobin inpigs: a model for pseudoallergic cardiopulmonary reactions to liposomes. Role ofcomplement and inhibition by soluble CR1 and anti-C5a antibody. Circulation 1999;(17):2302-2309.

• Szebeni, J. Complement Activation-Related Pseudoallergy Caused by Liposomes, Micellar Carriers of Intravenous Drugs, and Radiocontrast Agents. Crit. Rev. Therap. Drug Carrier Systems, 18, 567-606, 2001.

• Chanan-Khan et al., Complement activation following first exposure to pegylated liposomal doxorubicin (Doxil®): Possible role in hypersensitivity reactions. Annals of Oncology 14:1430-1437, 2003.

• Walsh, et al., Safety, Tolerance, and Pharmacokinetics of High-Dose Liposomal Amphotericin B (AmBisome) in Patients Infected with Aspergillus Species and Other Filamentous Fungi: Maximum Tolerated Dose Study. Antimicrobial Agents and Chemotherapy, 45: 3487-3496, 2001.

• Kostarelos, et al., Cellular uptake of functionalized carbon nanotubes is independent of functional group and cell type. Nature Nanotech., 2, 108-113.

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PREREQUISITES FOR SUCCESSFUL DEVELOPMENT OF PARENTERAL NANOPARTICLE DEPOT PRODUCTS

RENÉ VERLOES, N Niemeijer, D Kurland, E Van Beirendonck, H Crauwels, S Lachau-Durand, R Van Heeswijk, K Marien, G. Ispas, K Simmen, P Williams.

A combination of nanotechnology and the NanoCrystal® Technology (a registered trademark owned by Elan Pharma International Limited) has enabled to manufacture a long-acting formulation for parenteral injection (intramuscular, IM) of an investigational antiretroviral, TMC278 (rilpivirine).
TMC278, a next generation Non-Nucleoside Reverse Transcriptase Inhibitor, has potent activity against HIV-1 infection at a daily oral dose of 25 mg. This dose has been investigated in Phase III trials (ECHO, THRIVE) in treatment-naïve HIV-1 patients, yielding mean trough plasma concentrations in the range of 80 ng/mL of TMC278. The oral formulation has been approved as Edurant© in the US and Europe.
The long-acting formulation of TMC278 was tested in a double blind, randomized, placebo-controlled, Phase I study in healthy volunteers using single IM (gluteal) doses of 300 mg (Panel 1) and 600 mg (Panel 2). Plasma levels of TMC278 were shown to be dose-proportional. In the repeated dose part (Panel 3), a loading dose of 1200 mg was administered IM on day 1 followed on day 29 by a second IM dose of 600 mg and a last 600 mg dose on day 57. For all Panels, tolerability, safety and pharmacokinetics of TMC278 were assessed first daily, then weekly for at least 12 weeks after single or repeated dosing until the plasma concentration fell below a predefined threshold of 20 ng/mL. Plasma levels were sustained for 4 weeks, achieving levels comparable to the trough levels observed with a 25 mg daily oral dose of TMC278 (ca 80 ng/mL). For Panel 3, mean AUC28days were 55, 52 and 53 μg.h/mL after the first, second and third IM injection, respectively. Injection site reactions were mild, only observed in few subjects and of short duration; all doses were generally well tolerated. No delayed reactions were reported. Laboratory findings were normal and besides some mild, transient and expected increases in few inflammatory parameters (CRP, blood sedimentation rate, fibrinogen), there was no evidence of clinically relevant adverse reaction to treatment. There were no effects on vital signs, body temperature or ECG profile. Low frequency (monthly) parenteral dosing may improve adherence during prophylactic use of antiretrovirals in uninfected people at high risk of HIV-1 infection. TMC278 LA is a promising candidate that warrants further clinical investigation for those goals.

MECHANICAL FACTORS THAT REGULATE BACTERIAL ADHESION AND CLEARANCE

VIOLA VOGEL
Department of Health Sciences and Technology, ETH Zürich, CH

Cells and bacteria exploit tensile mechanical forces to explore their environments, to bind to and remodel extracellular matrix, and to interact with each other and finally to clear pathogens. A closer look at bacterial adhesins and their receptors reveals that many of their interactions can be regulated by mechanical forces. The adhesin of E. coli forms a catch bond with surface-exposed mannoses which allows E. coli to colonize surfaces preferentially under fluid flow conditions, in contrast to the general expectation (fluid flow is typically thought to be able to rinse off bacteria from surfaces). It turns out now that macrophages take advantage of these same catch bonds to remove sessile E.coli from surfaces. A completely different mechano-regulated mechanism has evolved by which the adhesins of S. aureus recognize connective tissue, particularly wound sites. By combining steered molecular dynamic simulations with stretch-assays, we could demonstrate that the adhesin of S. aureus can distinguish physically stretched from relaxed fibronectin fibers. After conducting a sequence alignment of all bacterial adhesins that all bind to the same N-terminal domains of fibronectin, we can now predict which bacterial adhesins bind in a mechano-regulated manner.

EARLY DIAGNOSTICS AND TREATMENT MONITORING OF BREAST CANCER USING NANOTECHNOLOGICAL PLATFORMS

YURI VOLKOV[1], Adriele Prina Mello[1], Tatsiana Rakovich[1], Omar Kazem Mahfoud[1], Igor Nabiev[1] and Luigi Bonacina[2]
[1]Department of Clinical Medicine, Trinity College Dublin (IRL) and [2]University of Geneva (CH).

Breast cancer, as one of the most frequent types of cancer in women accounts up to 30% of all newly diagnosed invasive tumours and contributes to over 15% of all cancer-related deaths due to the insufficient sensitivity and accuracy of existing diagnostic procedures. Despite the relative success of contemporary surgical treatment, efficient subsequent monitoring is required to assess the aftermath of primary tumour removal and to evaluate and adjust the course of follow-up adjuvant therapies. Breakthrough advances in the overall breast cancer treatment outcome are dependent on improved diagnostics and tumour progression monitoring, via lowering the thresholds of primary tumour detection down to individual cells and by implementing highly sensitive and informative cancer biomarker identification techniques.
Ultra-small probes based on fluorescent semiconductor nanoparticles (quantum dots) functionalised with single domain antibodies against common breast cancer cell receptors such as HER2 (found to be over-expressed in 15-30% of all invasive breast cancers) are capable of providing a bright photo-stable signal from cells located in deep tissue locations not accessible to conventional immunodiagnostic systems. Coupling of these fluorescent probes with magnetically barcoded nanomaterials opens a prospect to develop miniaturised point of care diagnostic devices for identification of soluble cancer biomarkers in a cost-effective multiparametric format. Another opportunity for acquiring a strong light emitting signal efficiently distinguishing the individual malignant cells from the dense mass of the surrounding tissue is provided by probes based on non-centrosymmetric nanomaterials with second harmonic generating properties. Due to their non-resonant excitation characteristics and low level of photobleaching, a broad range of readout wavelengths can be exploited, providing a superior contrast from the non-cancerous tissue components. Current state of the art in the development of these nanotechnology-enabled toolkits for cancer detection as well their benchmarking against the existing diagnostic standards will be presented and discussed.
Supported by NAMDIATREAM Project (EU FP7 NMP4-LA-2010-246479), www.namdiatream.eu

WHAT DOES IT TAKE TO BRING siRNA TO THE CLINIC

HANS-PETER VORNLOCHER
Axolabs GmbH, Fritz-Hornschuch-Strasse 9, 95326 Kulmbach, D

Over the last decade RNA interference (RNAi) has emerged as a powerful method for efficient and specific gene silencing and has revolutionized functional gene analysis as well as target identification in academic and industrial settings. In addition, RNAi holds tremendous promise as a novel therapeutic modality comparable to therapeutic proteins. The extremely potent and highly specific gene knockdown that can be achieved utilizing RNAi-based approaches and the ability to silence previously un-druggable targets make such therapies very attractive for many disease areas. The first siRNA-based therapies are currently in various stages of clinical development. Despite those advances, however, the major challenges towards realizing the full potential of RNAi-based therapeutics are the identification and characterization of highly active, specific and biocompatible siRNA molecules and - even more important - the development of efficient, safe and patient compliant delivery systems. In order to identify siRNA drug products suitable for clinical development, the best siRNA molecules targeting a gene of interest have to be comprehensively characterized and the accompanying carrier

system has to be selected based on a) its ability to deliver the siRNA to the selected target cell type and b) on a comprehensive safety assessment. Different approaches on how to address those key questions will be discussed.

INFECTION AND INFLAMMATION BEYOND THE IMMUNE SYSTEM / DRUG RESISTENCE

ANDREAS WIDMER

The discovery of antibiotics in the 1930s fundamentally transformed the way physicians care for patients, shifting their approach from a focus on diagnoses without means to intervene into a treatment-focused approach that saves lives. Now, nearly 70 years later, we've reached a critical point in treating infectious diseases: new drugs are not being developed at anywhere near the pace necessary to keep ahead of the natural ability of bacteria to evolve and defend themselves against antibiotics. The result is that some of our most powerful drugs are becoming useless.

- Antimicrobial resistance is recognized as one of the greatest threats to human health worldwide.
- Drug-resistant infections take a staggering toll in the United States and across the globe. Just one organism, methicillinresistant Staphylococcus aureus (MRSA), kills more Americans every year than emphysema, HIV/AIDS, Parkinson's disease, and homicide combined.
- Nearly 2 million Americans per year develop hospital-acquired infections (HAIs), resulting in 99,000 deaths – the vast majority of which are due to antibacterial-resistant pathogens.
- Two common HAIs alone (sepsis and pneumonia) killed nearly 50,000 Americans and cost the U.S. health care system more than $8 billion in 2006.
- Based on studies of the costs of infections caused by antibiotic-resistant pathogens versus antibiotic-susceptible pathogens, the cost to the U.S. health care system of antibiotic resistant infections is $21 billion to $34 billion each year and more than 8 million additional hospital days.
- Antibiotics are becoming less and less effective, in part due to overprescription and inappropriate use.
- New antibiotic development has slowed to a standstill due to market failure and regulatory disincentives. Antibiotics aren't as profitable as other drugs (e.g., drugs to treat diabetes or asthma, which patients take for years). Also, the US Food and Drug Administration has long delayed publishing workable guidances describing how companies should design antibiotic clinical trials. Moreover, once a new antibiotic makes it to market, physicians hold it in reserve for only the worst cases rather than rushing to use it on all their patients due to fear of drug resistance. These economic and regulatory disincentives have made it far too difficult for companies to continue developing new antibiotics.

Today, gram-negative bacteria expressing broad-spectrum betalactamases (ESBL) K.pneumoniae carbapenemases (KPC) and metallobetalactamases (e.g.NDM-1) emerged and have started the postantibiotic time. It will take more than 15 years to develop new classes of antibiotics: In the mean time, infection control and sophisticated use of current and very old antibiotics remain the only option.

Adapted from the Infectious Diseases Society of America, April 2011

NANOMEDICINE MEETS REGENERATIVE MEDICINE: DOES THE MARRIAGE HAVE A SILVER LINING?

KENNETH KAK YUEN WONG

Regenerative medicine is an emerging field aiming to the development of new strategies to treat degenerative diseases, injury, and trauma to rebuild the architecture and function of the original injured organ. Stem cells represent a great hope for regenerative medicine. In the skin for example, constant regeneration is achieved due to stem cell differentiation within the epidermis and the hair follicle. This is of paramount importance in the treatment of skin wounds. In this respect, nanotechnology may be able to provide the perfect tools to control and guide the regenerative process through actions on stem cells. Indeed, we have previously shown that the use of silver nanoparticles could enhance skin wound healing through their effects on epidermal-derived stem cells.

Taking our findings further, we have been focusing recently on the field of regenerative orthopedics. The use of stem cell applications has been explored and aimed at regenerating new bone. Our recent studies indicated that silver nanoparticles could promote the proliferation of mesenchymal stem cells (MSCs), as well as sensitizing the MSCs to differentiate into bone forming cells (osteoblasts) in response to osteoinductive factors.

This talk will discuss the positive impact of nanosilver particles on mesenchymal stem cells, from the promotion of cell survival and steering differentiation into eventual somatic pathways, as well as potential uses in future clinical applications. More importantly, this new knowledge can hopefully be translated into the design of future clinical trials, with significant benefits to patient care.

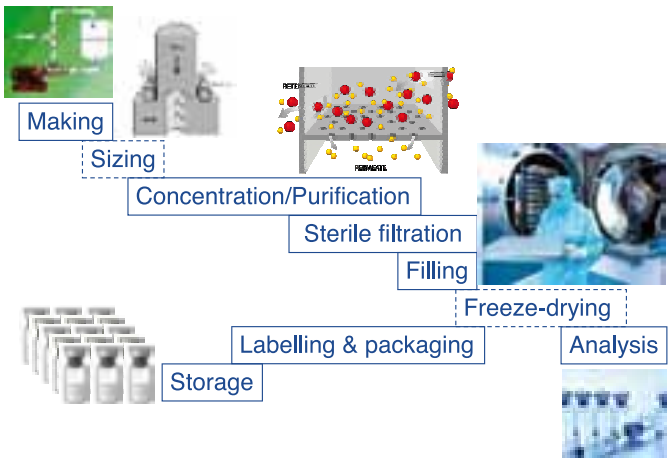
UPSCALING AND STERILE MANUFACTURE OF NANOCARRIER-BASED DRUG PRODUCTS

MARIE-ANDRÉE YESSINE

During the past decades, a substantial amount of research has been conducted on lipid-based nanoparticulate formulations (e.g. liposomes and lipid-drug complexes). Although considerable knowledge on nanocarrier-based drugs has been generated throughout the years, a small number of all the products investigated is or has been under clinical evaluation, and only a few have reached the market.

The limited number of marketed products can be explained by the complexity of both the product and of the manufacturing process. Lipid nanoparticles such as liposomes must have a reproducible particle size, size distribution, and drug loading from batch to batch, as a variation in these critical parameters can jeopardise the product efficacy and toxicity in vivo. Furthermore, they must have an acceptable chemical and physical shelf life. As shown in Figure 1, the processes used for the large scale production of liposomes/lipid-drug complexes most of the time consist in several steps. From particle formation to freeze-drying, each step that is performed can affect the end product properties and thus must be carefully controlled. This leads to major scale up challenges.

Figure 1. An overview of the manufacturing process for lipid-based nanoparticulates. The dashed boxes represent auxiliary steps that can be skipped depending on the process and end product stability. If sterile filtration is not possible, an aseptic process must be conducted.



To ease large scale production, the method used to prepare the particles in the development stage should be carefully chosen taking into accounts different parameters such as the physicochemical properties of the drug and its stability. Ideally, a scalable process should be used to prepare the particles as early as possible in the development

phase. Critical process parameters should be identified, hold times should be determined, and the need for a freeze-dried formulation should be evaluated. Target product profile should be defined, including size and polydispersity as these critical product attributes will guide process development as well as define the need for aseptic manufacturing. Process conditions must allow the production of sterile and pyrogen-free products. If sterile filtration is not possible due to the size and/or polydispersity of particles, (part of) the process must be conducted aseptically. In the presentation, the way successful large scale manufacturing of lipid-based nanoparticulate formulations can be achieved will be illustrated with a state-of-the art example in which process design was initiated early in the development stage.
In conclusion, the manufacturing and scale up of lipid-based nanoparticulate formulations are major challenges and the importance of thorough formulation and process development cannot be overemphasised. The manufacturing process should ideally be scalable, simple, and robust to avoid conducting extensive development work each time scale up is required. Applying these principles will result in faster development, more straightforward scale up, better process knowledge and control, and increased probability of success.

CANCER NANOTECHNOLOGY: PRISON NOT POISON CANCER CELLS

YULIANG ZHAO[1,2,3]
[1]Professor, Deputy Director-General, National Center for Nanoscience and Technology of China, Beijing 100190.
[2]Professor, Director, Chinese Academy of Sciences Key Laboratory for Biomedical Effects of Nanomaterials, Beijing 100049
[3]Professor, Director, The Research Center for Cancer Nanotechnology, (A Joint Research Center of Tianjin Cancer Hospital & Chinese Academy of Sciences)

A novel approach, using Gd@C82(OH)22 nanoparticles (f-NPs) that involves manipulating the tumor microenvironment that results in local control and inhibition of tumor metastasis, has been developed. In tissue culture condition, we demonstrated that f-NPs were non-toxic to normal cells and also tumor cells, but capable of reducing matrix metalloproteinase (MMP) level. In an in vivo tissue invasion model, we demonstrated that primary tumors treated with f-NPs showed significantly less metastases (with an inhibitory rate of around 90%) to the lungs through induction of a thick fibrous cage capable of cancer cell entrapment that cuts the communication between tumors and tumor-associated macrophages (TAMs). The animal model experiments simulating bloodstream metastasis, f-NP treatment was also capable of preventing establishment of tumor foci in lung with a high inhibitory rate of around 88%. The findings may result in a strategy shift for cancer chemotherapeutics: Prisoning cancer cells instead of Poisoning them by engineered nanomedicines. The detail will be reported at the conference.

POSTER ABSTRACTS FOR CLINAM 5/12

NATURAL PRODUCTS AND NEWLY DEVELOPED NANOSTRUCTURED BASED MATERIALS WITH ANTICANCER ACTIVITY. RESEARCH TRENDS AT BABES-BOLYAI UNIVERSITY.

MIHAELA ALUAS, E. Vanea, C. Gruian, S. Pinzaru, V. Simon, S. Simon
Faculty of Physics, Kogalniceanu 1 Cluj-Napoca 400084, Babes-Bolyai University, Romania

This paper presents three directions concerning our recent developments on nano-bio-materials with anticancer activity.

1. NATURAL PRODUCTS EXTRACTED FROM BETULA PENDULA ROTH BIRCH TREE. PHYSICO-CHEMICAL ANALYSIS.

The natural products have been known and used with great results since ancient times, having an important contribution to the development of the modern drugs.

Pentacyclic triterpenes with lupan skeleton such as betulinic acid, betulin and lupeol are important antitumor agents with a very low solubility. The substances isolated from plants have to be investigated using modern analytical methods and subjected to in vitro and in vivo biological assays. Among the modern analytical techniques Solid-State Nuclear Magnetic Resonance Spectroscopy and Raman Spectroscopy are the most useful for characterizing especially the insoluble forms of the systems.

The Betula Pendula Roth birch tree, a wide spread plant especially in Northern temperate climates, has proved since ancient times to have a broad range of beneficial medicinal properties. The outer bark of this tree is rich in pentacyclic triterpenes such as betulin, betulinic acid, lupeol, oleanolic acid and others. Different extraction products from birch bark have been obtained using various protocols and solvents. The products with the highest content of betulin (97%) were determined by HPLC methods [1].

Using Raman Spectroscopy based on accurate vibrational characterization of betulin [2] we were able to discuss and quantitatively asses different extracts obtained with different protocols and solvents. Using small extract amount we were able to detect betulin species with high reproducibility.

Betulin (lup-20(29)-ene-3β, 28-diol) is found mainly as crystalline deposits in the outer layers of the bark, consisting of large cells with thin walls and can be easily obtained by sublimation or by extraction with organic solvents. Betulin was used in skin treatment as anti-inflammatory for a long time and it was recently found to be active on some types of cancer cells or even to induce apoptosis, therefore, a special attention was paid to the possibility of creating pharmaceutical formulations based on betulin and its metabolic successor betulinic acid, to be tested on skin malignancies, including melanoma and skin cancer. Betulin can be easily converted to BA, which possesses a varied spectrum of biological and pharmacological activities, too. The extraction products have been accurately characterized using sensitive 13C solid-state NMR, FT-Raman and Surface-Enhanced Raman Scattering (SERS).

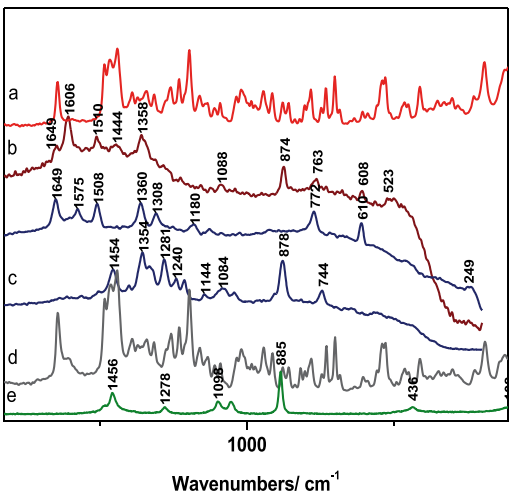


Fig. 1 SERS spectra of the extract sample (c) and of betulin dissolved in ethanol (b) in comparison to the FT-Raman spectra of betulin (a) and the extract sample (d) and the SERS spectrum of an ethanol solution (e)

2. THE STUDY OF THE ADSORPTION OF THE PROTEINS TO THE SURFACE OF THE BIOMATERIALS USING EPR SPECTROSCOPY COMBINED WITH SITE-DIRECT SPIN LABELING.

The first step in evaluating the blood and tissue compatibility of any medical device is to study their behavior when interacting with proteins. The total amount of adsorbed protein and the overall protein-implant surface area interactions are of primary importance for the biocompatibility of bioengineered materials. Adsorption of protein molecules onto solid surfaces frequently results in conformational and/or orientational changes within the adsorbed layer. EPR spectroscopy combined with site-directed spin labeling was used for investigating adsorption of proteins on solid surfaces. In addition to cw-EPR, the inter spin distances in proteins adsorbed on solid surfaces by means of double electron electron resonance (DEER) pulsed EPR was determined, from which a more detailed picture of the protein conformation and conformational changes could be deduced. Also, the effect of glutaraldehyde as protein coupling agent in protein adsorption on bioactive glasses was analysed. For this purpose, interaction of two model proteins: horse methemoglobin and MnmE (5-methyl-aminomethyl-uridine forming enzyme) with bioactive glass which is commonly used as biomaterial for bone defects repair was investigated in terms of conformational changes and quantitative adsorption properties [3].

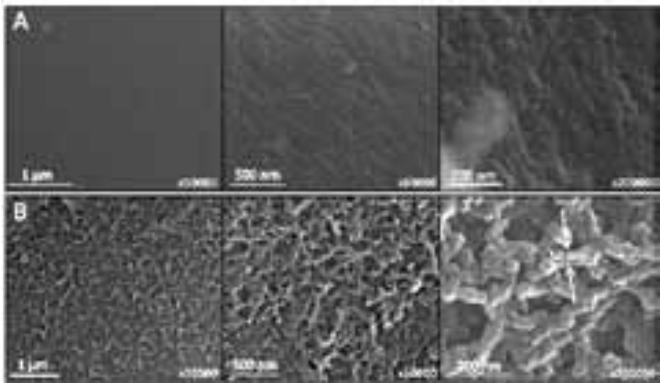


Fig. 2 SEM images of the BG (bioactive glass) with GA (glutaraldehyde), before (A) and after immersion in methemoglobin solution (B). The images in the center and right panels are recorded with higher magnification. Higher porosity can be observed on sample treated with GA, which is thought to be the result of microporosity induced by ion leaching during heat-treatment in water at 80°C

3. SPECTROSCOPIC STUDIES ON ALUMINOSILICATE BIOMATERIALS CONTAINING IRON AND RARE EARTHS

Glass systems are very often used as bone repairing and substituting materials in many dental and orthopedic applications because of their excellent biocompatibility and osteointegration characteristics. The aluminosilicate glass ceramics are highly stable in the body and, by addition of iron oxide, they could be optimized for hysteresis heating of interest in hyperthermia.

Hyperthermia has been gaining a lot of interest recently as a method for curing cancer especially as an adjunct to other more conventional methods such as radiotherapy and chemotherapy. The simultaneous application of radiotherapy and hyperthermia considerably enhances the therapeutic effects of the two cancer treatment methods.

It is well known that it is necessary to sterilize all medical implants after fabrication and prior to their use to reduce the risk of infections and associated complications.

Despite the availability of a wide range of sterilisation techniques, it is generally agreed that no single sterilisation process is capable of sterilising without adverse effects, all processes having their own advantages and disadvantages. An effective sterilization method must guarantee the required sterility assurance level with a minimum effect on the chemical, physical and biological properties of the biomaterial.

This study describes the gamma irradiation effects, for sterilization purpose, on aluminosilicate compounds of biomedical interest. The paramagnetic defect centers in silica–based sol-gel materials were produced at room temperature. A promising technique that can be used to describe and explain the radiation-induced paramagnetic centers is the Electron Paramagnetic Resonance (EPR) [4].
Another objective of this study was to functionalise the aluminosilicates compounds containing iron and yttrium/dysprosium in order to improve the systems biocompatibility. The proteins adsorption was investigated by means of XPS and FT-IR spectroscopy. X-ray Photoelectron Spectroscopy (XPS) is a very useful analysis tool dedicated to investigate the atomic composition and chemical environment of the outermost 2-10 nm layer of a surface and can accurately determine the surface coverage. Transform Infrared Spectroscopy (FTIR) is one of the most used techniques for studying protein secondary structures.

Keywords: SEM, natural products, NMR, FT-Raman and SERS, rare-earth aluminosilicate glasses, hyperthermia, EPR, X-ray Diffraction.

REFERENCES

[1] C. M. Şoica, C. A. Dehelean, M. Aluas et al, Natural Product Research, 1478-6427, (2011).
[2] A. Falamaş, S. Cînta Pînzaru, C. A. Dehelean et al., J. Raman Spectrosc. 42 (2011), 97-107.
[3] C. Gruian, PhD Thesis and the references therein
[4] E. Vanea, PhD Thesis and the references therein

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DETERMINATION OF FACTORS CONTROLLING THE SIZE OF PLA NANOPARTICLES CARRYING HYDROPHOBIC DRUG MOLECULES USING ARTIFICIAL NEURAL NETWORKS

MOHAMMAD ALI AMINI[1], Mohammad Ali Faramarzi[2,3,4], Dariush Mohammadyani[5], Elina Esmaeilzadeh-Gharedaghi[6], Amir Amani[4,6]
[1]Department of Microbiology, Faculty of Veterinary Medicine, Islamic Azad University, Karaj branch, Karaj, Iran
[2]Department of Medical Biotechnology, School of Advanced Medical Technologies, Tehran University of Medical Sciences, Tehran, Iran.
[3]Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 14174, Iran
[4] Biotechnology Research Center, Tehran University of Medical Sciences, Tehran 14174, Iran
[5]Materials and Energy Research Center (MERC), 14155-4777, Tehran, Iran
[6] Department of Medical Nanotechnology, School of Advanced Medical Technologies, Tehran University of Medical Sciences, Tehran, Iran

INTRODUCTION

During the last two decades, based on a number of distinct advantages of submicron sized particles over conventional ones, a great deal of research has been focused on the formulation of methods for preparation of PLA nanoparticles. Some of techniques prevailingly employed include nanoprecipitation, interfacial deposition, salting out and solvent evaporation.
Due to complexities usually observed in process of particles formation, non-linear relationships often exist between components and/or processing conditions. Thus, formulation and preparation of nanoparticles, as with other complex processes, commonly fail to be comprehensively modeled by classical statistical techniques. In recent years, Artificial Neural Networks (ANNs), the biologically inspired approaches to analyze information, have been widely used in dealing with non-linear processes usually observed in nano-science and technology.
The aim of this study was to model the parameters involved in preparation of PLA nanoparticles, carrying budesonide as a model hydrophobic drug, based on solvent evaporation technique using ANNs. The model would provide comprehension about the critical factors and their interactions affecting the size of PLA nanoparticles and help predicting and optimizing the processing conditions and outcomes.

METHODS

PLA nanoparticles were prepared according to the modified method of Krause et al., [1] based on emulsification/solvent evaporation. Briefly, 2.5 mg budesonide was dissolved in 2 ml chloroform containing different concentrations of PLA. The solution was then added drop wise to 10 ml gelatin 0.5% containing Tween 80 with predetermined concentration under sonication using an ultrasonic probe (diameter 0.5 Cm and 750 W intensity ultrasonic processor, operating at 20 kHz,. (Lab Sonic®, B.Broun, Germany). Subsequently, the temperature was raised to 40°C during the emulsification using bath sonicator for 30 min, allowing a slow evaporation of the chloroform. The mean particle size was determined by photon correlation spectroscopy (PCS), using a Zetasizer (Malvern Instruments, United Kingdom) INForm v4.0 (Intelligensys, UK), a commercial ANNs software, used to model the non-linear and complex relations between inputs and output, was employed in this study.
38 samples were prepared under random processing conditions. The preparation process of nanoparticles involved four processing variables (ie. input parameters) for each experiment: concentration of PLA (mg/ml) and Tween 80 (% w/v), amplitude and sonication time (Sec). The remaining three individual data sets were used as "test data" to prevent overtraining. Furthermore, 9 additional experiments were performed to validate the predictability of the trained network.

RESULTS AND DISCUSSION

It could be observed from Figure 1 that increasing the Tween concentration, the particle size decreases; a phenomenon which is expected due to the high adsorption potential of Tween 80 as a nonionic nonpolymeric surfactant. Interactions of nonionic surfactants such as Tweens at organic solvent/water interface reduce the interfacial tension and cause the optimal conformation of polymer chains extending into the external phase [2] consequently prevent aggregation of the newly formed droplets, thus, keeping the particles small.

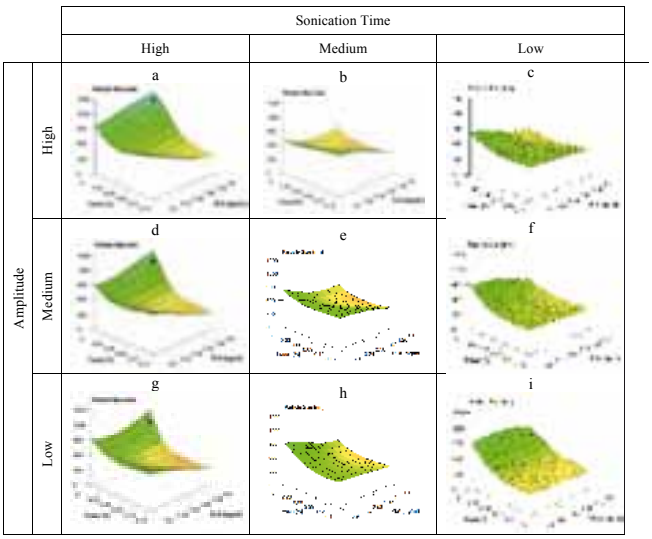


Fig. 1. 3D plot of particle size (nm) predicted by the ANNs model at low, medium and high values of sonication time and amplitude.

Besides, the increase in PLA concentration causes a rise in particles diameter. Chorny et al. [3] and Gornera et al. [4] also have explained this effect by the fact that at higher polymer concentrations, the organic phase would become more viscous rendering it more resistant to shear forces. The exception is at low value of Tween and high value of sonication time and amplitude, when particle size rises as PLA concentration diminishes. This phenomenon is more obvious in Figure 2 when the amplitude reaches its highest values. It is speculated that this phenomenon is a result of two simultaneous processes: (I) the disassociation of surface attached budesonide from PLA nanoparticles at low PLA/budesonide ratio and under high shear stress, followed by aggregating the free budesonide molecules together, (II)

the detachment of the weak bonds between hydrophobic parts of PLA and Tween caused by high applied energy, which may lead to aggregation and causing larger particles of PLA.
It is also perceived that using high sonication time and amplitude concurrently, has an adverse effect on the size of particles obtained by solvent evaporation method. It is believed that high energies applied to the solution may lead in aggregation of budesonide as well as PLA particles as described above.

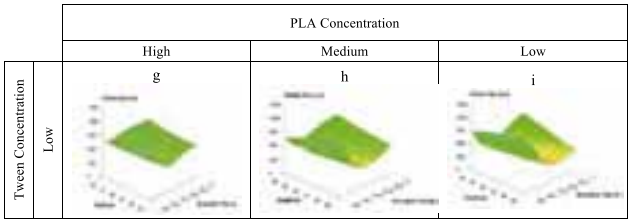


Figure 2. 3D plot of particle size (nm) predicted by the ANNs model at low, medium and high values of PLA and low values of Tween concentration.

By defining the effects of each parameter on the size of PLA nanoparticles loading a hydrophobic drug, this study estimated the optimum condition to obtain the minimum particle size.

REFERENCE

[1] Krause HJ, Schwarz A, Rohdewald P. Polylactic acid nanoparticles, a colloidal drug delivery system for lipophilic drugs. Int J Pharm. 1985;27:145–155.

[2] Garti N. A new approach to improved stability and controlled release in double emulsions, by the use of graft-comb polymeric amphiphiles. Acta Polym. 1998;49:606–616.

[3] Chorny M, Fishbein I, Danenberg HD, Golomb GJ. Lipophilic drug loaded nanospheres prepared by nanoprecipitation. Effect of formulation variables on size, drug recovery and release kinetics. J Control Release. 2002;83:389–400.

[4] Gorner T, Gref R, Michenot D, Sommer F, Tran MN, Dellacherie E. Lidocaine-loaded biodegradable nanospheres. I. Optimization of the drug incorporation into the polymer matrix. J Control Release. 1999;57:259–268.

NANOLIPOSOMAL CHEMOTHERAPY COMBINED WITH THERMAL ABLATION IN ANTICANCER THERAPY

ALEXANDER ANDRIANOV[1], Daniel Zucker[1], S. Nahum Goldberg[2], and Yechezkel Barenholz[1]

[1]Department of Biochemistry, Institute for Medical Research Israel-Canada,
Hebrew University – Hadassah Medical School,

[2]Radiology Department, Hadassah Medical Center, Jerusalem, Israel

We hypothesized that combining thermal ablation and nanoliposomal chemotherapy would act synergistically to improve therapeutic efficacy of these two anticancer therapy modalities. The former anticancer treatment is based on in-situ tumor heating using radiofrequency (RF). Two different nanoliposomal formulations were fabricated and evaluated: liposomes encapsulating a combination of vincristine and topotecan as two drugs in one liposome, named LipoViTo, and liposomes encapsulating doxorubicin (Doxil®).
Nude mice were injected with either lung cancer (A549) or medulloblastoma (Daoy) cells. After 4-5 weeks, the mice developed tumors with a diameter of ~14-15 mm. Two days after treatment with RF ablation and/or chemotherapy, the mice were sacrificed. The tumors were excised and sliced to thin disks. Histopathological studies included staining for mitochondrial enzyme activity and measuring the necrosis area.
Combination as modality treatment (nanoliposomes and RF) was compared to single modality treatments (nanoliposomes or RF).
For medulloblastoma, the most efficacious treatment was the combination of LipoViTo with RF, while for lung cancer it was the combination of Doxil with RF.
Concentrations of drugs in tumor tissue after chemotherapy with RF were higher compared to single therapy with liposomes alone.
Survival curves for 90 days in animals bearing human medulloblastoma were determined. Both combinations, LipoViTo plus RF and Doxil plus RF, were superior in improving survival to each single treatment, chemotherapy or RF.

In conclusion, the combination of RF and nanoliposomal chemotherapy is superior in both types of cancer to RF or nanoliposomes alone. However, for optimization of the chemotherapeutic drug has to be selected according to the cancer type.

THERAPEUTIC RESPONSE IN A XENOGRAFT MICE MODEL BY FOLATED PEG-PCL-PEI/TAM67 COMPLEXES

ROHIDAS AROTE[1], Soon-Kyoung Hwang[3], Hu Lin Jiang[3], You Kyoung Kim[2], Myung Haing Cho[3], Chong Su Cho[2]

[1]Department of Dentistry, School of Dentistry, Seoul National University, Seoul 151-921, Korea

[2]Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Korea

[3]College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea

INTRODUCTION

Gene therapy comprises a novel form of molecular medicine that will have a major impact on human health in next century. The needs for safe and efficient methods for gene delivery still remain a critical obstacle to the routine clinical implementation of human gene therapy. Polyethylenimine (PEI) is one of the successful polymers used for gene delivery because of density of primary, secondary and tertiary amines although several groups have reported that PEI is toxic in many cell lines. Various studies have shown that genes have been successfully delivered to the cells in in vitro and in vivo by exploiting receptor-medicated endocytosis. Our studies have been performed to improve gene delivery efficiency and to reduce their cytotoxicity by synthesizing a gene carrier based on low molecular weight PEI and biodegradable polycaprolactone (PCL). This also introduced biodegradable ester bond leading to low cytotoxicity compared with PEI 25K. In continuation with above approach, we coupled folic acid moiety to poly (ester amine)s (PEAs) with PEG as a spacer for receptor-mediated (FR) endocytosis for cancer therapy.

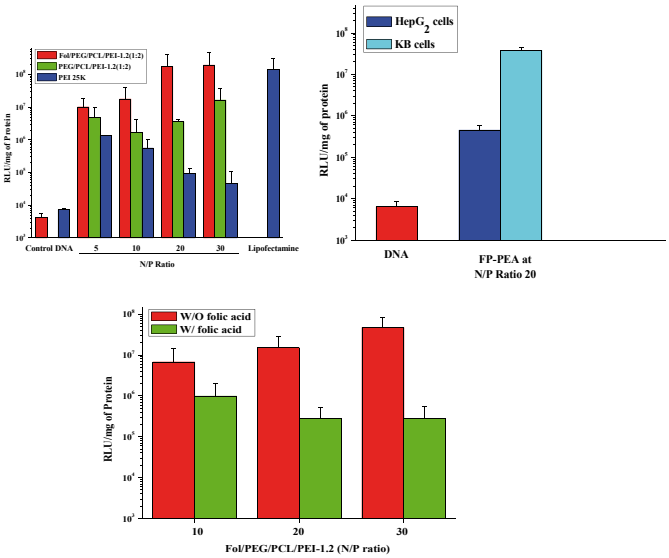
METHODS

In our previous study, we synthesized PEAs by Michael addition reaction, to improve gene delivery efficiency and to reduce their cytotoxicity, based on low molecular weight PEI and biodegradable PCL. Folate conjugated-PEA (Fol/PEG/PCL/PEI-1.2) (FP-PEA) was synthesized by coupling folic acid with PCL/PEI copolymers with dicyclohexyl carbodimide (DCC)/N-hydroxyl succinamide (NHS) chemistry using bifunctional PEG (MW: 2000 Da). The complexation of FP-PEA/pDNA was characterized by gel retardation assay, dynamic light scattering (DLS) and transmission electron microscopy (TEM) to determine the complex forming ability, particle sizes and morphology, respectively. FP-PEA and non-folate PEA (P-PEA) were analyzed for their cytotoxicity and transfection efficiency on cultured KB and A549 cell lines in vitro. Tumor volume was drastically decreased in xenograft mice when the polyplexes containing FP-PEA and a therapeutic gene, TAM-67 gene were injected by intratumoral injection.

RESULTS

After the successful synthesis of PEA by Michael addition reaction, folate moiety was coupled to it using DCC and NHS. Synthesis of FP-PEA was confirmed by 1H NMR spectroscopy. The polymer showed suitable biophysical characteristics and excellent transfection efficiency in KB and A549 cells compared with PEI 25K. FP-PEAs showed typical receptor mediated enhanced transfection than P-PEAs or PEI 25K in the cell lines containing folate receptors such as KB and A549 cells. Remarkable decrease in tumor volume after the intratumoral injection of polyplexes in xenograft mice indicated the in vivo success of FP-PEAs through folate receptor mediated-

endocytosis. Furthermore, antitumor activity with PEA without folic acid moiety (P-PEA) proved not to be effective against xenograft mice model with KB cells when administered at the same dose to that of FP-PEA. Taken together, these results indicate that FP-PEA is highly effective gene carrier capable of producing therapeutic benefit in xenograft mice model without any sign of toxicity.



CONCLUSIONS

The approach described in this work represents an easy and efficient method to get fairly stable gene delivery system with folate receptor mediated endocytosis. We have shown that FP-PEA/TAM67 complexes could inhibit tumor growth through diverse functions such as angiogenesis and apoptosis etc thus demonstrating the target specific gene delivery. We propose that biocompatible FP-PEA system is fit for repeated administration to maintain sustained gene expression, thereby opening the possibility for cancer gene therapy. Taken together, these results indicate that FP-PEA is highly effective gene carrier capable of producing therapeutic benefit in xenograft mice model without any sign of toxicity.

NOVEL STRUCTURED LIPID MATRIX IMPROVED LOADING EFFICIENCY AND PERMEATION OF TIMOLOL HYDROGEN MALEATE

ANTHONY A. ATTAMA

Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka 410001, Nigeria

INTRODUCTION

Delivery of drugs to the tear film is routinely done with eye drops, which are well accepted and, for most patients, easy to use. However, attainment of an optimal drug concentration at the site of action is a major problem. Poor bioavailability of drugs from ocular drug delivery systems is mainly due to the pre-corneal loss factors and relative impermeability of the corneal epithelial membrane. In this study, solid lipid nanoparticles containing timolol hydrogen maleate were formulated with a novel lipid matrix composed of 30%w/w of phospholipon 90G in a 1:1 mixture of natural fats obtained from Theobroma cacao and beeswax and evaluated for sustained ocular delivery using bioengineered human cornea.

METHODS

Solid lipid nanoparticles were prepared by melt emulsification with high pressure homogenisation using 1 %w/w polysorbate 80, 0.005 %w/w thiomersal, 1 %w/w THM, 7.5 %w/w the novel lipid matrix and enough bi-distilled water to make 100 %w/w. The nanoparticles were characterized and the permeation assessed using freshly bioengineered using immortalized human corneal endothelial cells (HENC), stromal fibroblasts, and epithelial cells (CEPI 17 CL4).

RESULTS

The prepared lipid nanoparticles had particle size range of 116.67 ± 0.58 nm to 217.67 ± 1.53 nm with low polydispersity indices and zeta potential range of -9.47 ± 0.24 mV to -24.40 ± 1.78 mV after 1 month. There was 25.45 % increase in encapsulation efficiency when the structured lipid matrix was used. The release of the THM was also sustained. The lipid nanoparticles prepared with novel lipid matrix containing THM recorded the highest flux together with high permeation coefficient compared with non-structured lipid matrix.

CONCLUSIONS

The presence of Phospholipon 90G in binary lipid matrix core nanoparticles improved the drug loading and dynamics of transport across bioengineered cornea. Phospholipid thus improves the biophysical characteristics of drugs encapsulated in mixed lipid core nanoparticles.

FLEXIBLE CARBON NANOTUBE BASED MICRO-ELECTRODE ARRAY FOR RETINAL IMPLANT APPLICATIONS

GIORA BEIT-YAAKOV[1,2], Moshe David-Pur[1,2], Lilach Bareket[1,2], Dorit Raz-Prag1, Arieh Solomon[3] and Yael Hanein[1,2]

[1]Tel-Aviv University Center for Nanosciecne and Nanotechnology, Tel-Aviv, Israel

[2]School of Electrical Engineering, Faculty of Engineering, Tel-Aviv University, Israel

[3]The Goldschleger Eye Research Institute, Faculty of Medicine, Tel-Aviv University, Israel

PURPOSE

To create an artificial device that can replace the physiological function of retinal photoreceptors as a way to save sight in conditions of photoreceptor degeneration. Such implants consist of a micro-electrode array (MEA) and are implanted in the sub-retinal or epi-retinal space. The electrodes are intended to inject low electrical currents that activate the remaining healthy retinal ganglion cells (RGC).

Methods: For this project we developed a flexible implant with medical tape as a substrate and a carbon nanotube (CNT) film that served as high-capacitance electrodes and low-resistance traces to the power source. Silicone membrane (polydimethylsiloxane, PDMS) was used for electrical passivation (figure 1).

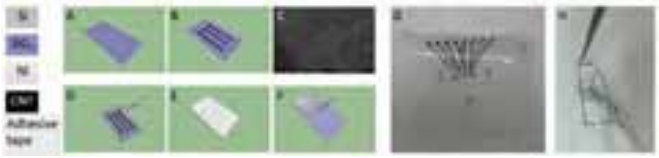


Fig. 1: Process flow: 2.5nm of Ni layer on SiO2 served as the catalyst layer for the CNT growth using chemical vapor deposition (A-C). Then the CNT film was faithfully transferred to a flexible substrate (D-H).

RESULTS

In-vitro tests were performed with embryonic chick retina under physiological conditions (figure 2). Activation thresholds similar to those obtained with standard commercial MEA were achieved.

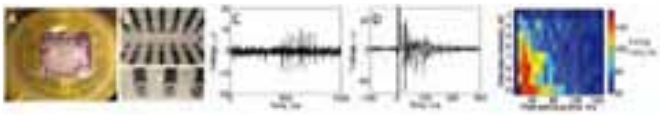


Fig. 2: The retina was extracted and flattened on the MEA (retinal ganglion cells down) (A, B), and showed both spontaneous (C) and electrically stimulated activity (D) with low activation threshold (E). Preliminary epi-retinal implants in rat and rabbit presented no inflammation and no damage to the retina and other structures of the eye during a two months period of follow up (figure 3).

Fig. 3: Rat eyecup (A) and retina cryosections (B, C) with the implant. Pieces of PDMS are evident near the ganglion cell layer (arrows), and pieces of CNT are integrated in the tissue (asterisks).

CONCLUSION

These data suggest that the flexible implants with CNT micro-electrodes may potentially be used as a new type of retinal prosthesis.

SETUP OF QUANTITATIVE MAMMOSPHERE ASSAYS FOR NANODRUG SCREENING

SIDSEL BERING OLSEN[1,2], Pernille Lund Hansen[1,2], Steffen Schmidt[1], Angela Riedel[1], Carolin Müller[1], Ines Block[1], Jakub Trojnar1, Aleksandra Jaskot[1], Markus List[1], Helle Chistiansen[1], Birgitte Olsen[3], Poul-Fleming Høilund-Carlsen[3], Helge Thisgaard[4], Jan Mollenhauer[1]

[1]Lundbeckfonden Center of Excellence in Nanomedicine NanoCAN and Molecular Oncology, University of Southern Denmark, Odense, DK
[2]The two authors contributed equally and should be considered as shared first authors
[3]Dept. of Nuclear Medicine, Institute of Clinical Research, Odense University Hospital, Odense, Denmark
[4]PET and Cyclotron Unit, Odense University Hospital & Institute of Clinical Research, University of Southern Denmark, Odense, DK

Cancer stem cells (CSC) have been documented in a variety of cancer types, including breast cancer, and are a specialized and rare subpopulation, comprising only few percent of the tumor cells. They are thought to give rise to recurrence of therapy-resistant and metastatic breast cancer after treatment, leading to patient death due to incurable cancer. Therapeutic targeting of breast cancer stem cells (BCSCs) therefore is thought to lead to more effective treatment strategies. In vitro mammosphere formation assays are commonly used as a measure for the number of BCSCs in a given sample, but are time-consuming because of sphere-counting, so that high-throughput screening, e.g. for novel BCSC-targeting nanodrugs is not feasible. Moreover, the present data about BCSC-phenotype-inducing genes are anecdotical and lack a standardized comparison of the gene's BCSC-inducing potential. We report on the setup of a quantitative mammosphere assay, omitting cell counting and thus being appropriate for high-throughput screening. In an initial approach, we use this assay for systematically comparing the BCSC-inducing capacity of a set of 30 selected candidate genes. Further, we demonstrate general suitability to identify nanodrug components with BCSC-killing activity.

DEVELOPMENT OF EXPANDED HIGH-THROUGHPUT SCREENING OPTIONS FOR NUCLEIC ACID-BASED NANODRUGS VIA CELL LYSATE BIOCHIPS

INES BLOCK[1,2], Marlene Lemvig Pedersen[1,2], Markus List[1,2], Steffen Schmidt[1,2], Pernille Lund Hansen[1,2], Angela Riedel[1,2], Carolin Müller[1,2], Aleksandra Jaskot[1,2], Jakub Trojnar[1,2], Helle Christiansen[1,2], Jan Mollenhauer[1,2]

[1]Lundbeckfonden Center of Excellence NanoCAN
[2]Molecular Oncology, University of Southern Denmark, Odense, DK

Short interfering RNAs (siRNAs) offer an attractive option for the next generation of anti-cancer nanodrugs because of their ability to selectively inactivate a particular gene. Furthermore, genome-wide siRNA libraries are available, such that in a differential screen using e.g. cancer cells versus normal cells, selectively each human gene can be inactivated and the effect of the respective siRNA on the cells can be monitored. Such systematic screens have the potential to recover novel starting points for personalized cancer medicines.

Due to the large number of compounds in these libraries such screens are performed in an automated fashion with a single differential screen commonly comprising about 120,000 individual cell experiments. The readout options, however, are generally limited to one or very few parameters.

We started with the implementation of chip-based readouts for changes in protein quantities into automated screening in order to increase the information gain and to accelerate drug discovery approaches in nanomedicine. We set up a process, allowing to place 15,000 samples on a microscopic glass slide. Using this technique, we so far succeeded to perform protein detection in 0.25 cells or less in a volume of 350 pl. Furthermore, automated analysis routines were developed, so that the knockdown of the target protein could easily be detected on the miniaturized chips. Present work concentrates on integration in a fully automated process and detection of a larger panel of cancer-relevant proteins.

INVESTIGATION OF THE PHASE TRANSITION IN SLM FABRICATED NITI SAMPLES

THERESE BORMANN[1,2], R. Schumacher[1], B. Müller[2], and M. de Wild[1]

[1]University of Applied Sciences Northwestern Switzerland, IMA, Muttenz, Switzerland.
[2]Biomaterials Science Center, University of Basel, Basel, CH.

INTRODUCTION

NiTi belongs to the shape memory alloys (SMA). The phase transition of 50%-50% NiTi can be induced either by thermal or by mechanical stimuli. The thermally induced transition is termed shape memory effect, whereas the mechanically induced transition is referred to superelasticity. The two effects are reversible phase transitions taking place at physiologically relevant temperatures. The crystallographic structure of the material changes between two phases, the low-temperature martensite and the high-temperature austenite. FDA-approved NiTi [1] shows its phase transition depending on the Ni/Ti ratio in the temperature range between -150 °C and 100 °C. As this temperature range includes body temperature, the SMA materials are perfectly suited for medical implants and instruments. We fabricate complex-shaped porous NiTi scaffolds, which should exhibit improved interactions with the surrounding biosystem. Here, we use the additive manufacturing technique of selective laser melting (SLM) to fabricate free-form NiTi parts (Fig. 1) with the shape memory properties [2]. In addition, the phase transition temperatures have been directly tailored applying appropriate parameters within the SLM process [3]. As the SMA-effects are intrinsically linked to the crystal structure, x-ray diffraction (XRD) and differential scanning calorimetry (DSC) are used to characterize the micro-structured NiTi-specimens.

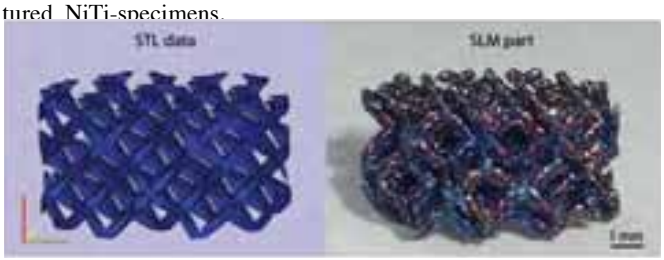


Fig.1: STL data and SLM part of a NiTi lattice structure.

MATERIALS AND METHODS

NiTi-specimens were manufactured from NiTi-powder (MEMRY GmbH, Weil am Rhein, Germany) using SLM. Different energy densities within the fabrication processes as well as subsequent heat treatments at annealing temperatures of 800 °C were used to generate specimens with different phase transition temperatures. All process steps were carried out under inert gas atmosphere of Ar 4.8 quality. Differential scanning calorimetry (DSC) and x-ray diffrac-

tion (XRD) measurements were accomplished on NiTi-powder and on samples whose phase transition temperatures lie beneath (Sample 1) and above (Sample 2) room temperature, respectively. XRD measurements were done at room temperature using CoKα radiation.

RESULTS AND DISCUSSION

The DSC measurements showed that the austenite peak temperature Ap of Sample 1 was -3 °C whereas Ap of Sample 2 corresponded to 50 °C. Sample 1 therefore should have superelastic properties, whereas Sample 2 should exhibit the shape memory effect. As expected, the preliminary XRD measurements reveal differences in the spectra depending on the measured phase transition temperatures. The peaks of Sample 1 mainly relates to austenite with a cubic crystal lattice (see Fig. 2). This is not surprising, since the phase transition takes place below room temperature, i.e. the material is in its high-temperature austenitic phase at room temperature. For Sample 2, whose austenite peak temperature lies above room temperature, mainly martensite phase with a monoclinic crystal lattice is expected. The spectrum, however, not only shows martensite but also austenite peaks, as illustrated in Fig. 2. That means in Sample 2 both phases are in coexistence. The DSC investigation on Sample 2 reveals that the starting point of the phase transition lies already at 26 °C. A reason for the austenite phase in Sample 2 could therefore be a start of phase transition due to a slight increase in the sample surrounding temperature, caused e.g. by the heat of the x-ray tube during the XRD investigations itself.

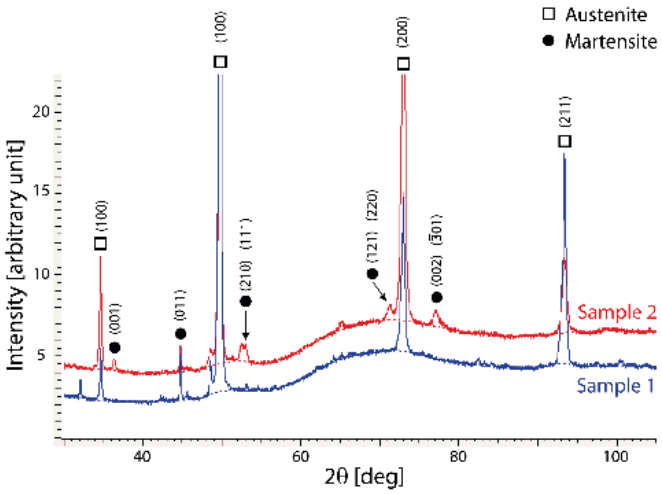


Fig. 2: XRD-spectra of two SLM samples with different process histories.

CONCLUSION

In this preliminary study, we find differences in the crystallographic structure of our SLM samples, which have been prepared by different processing routes. Quantifying the two phases, the nanostructure of SLM fabricated NiTi samples can be tailored towards sophisticated medical applications.

ACKNOWLEDGEMENTS

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REFERENCES

[1] ASTM International, F 2063 - 05.

[2] T. Bormann, R. Schumacher, B. Müller, M. Mertmann, U. Pieles, M. de Wild, Properties of NiTi-structures fabricated by selective laser melting, eCells and Materials Journal 20 Supplement 1, 13 (2010).

[3] T. Bormann, S. Friess, M. de Wild, R. Schumacher, G. Schulz, B. Müller: Determination of strain fields in porous shape memory alloys using micro computed tomography. Proceedings of SPIE 7804 (2010) 78041M.

NEW LIPID-BASED NANOMEDICINE FOR AMPHOTERICIN B DELIVERY: PREPARATION, CHARACTERIZATION AND EVALUATION OF ANTIFUNGAL ACTIVITY

ROBERTA CAVALLI[1], E. Vigna[1], A. Bisazza[1], A. Civra[2], M. Donalisio[2], D. Lembo[2]

[1]Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, Via P. Giuria 9, 10125, Torino, Italy

[2]Dipartimento di Scienze Cliniche e Biologiche, Università degli studi di Torino, ospedale S.Luigi Gonzaga, 10043 Orbassano, Torino, Italy

Amphotericin B (AmB) is an effective antifungal and antileishmanial agent associated with low oral bioavailability (0.3%) and severe nephrotoxicity [Italia 2009]. Parenteral lipid-based formulations of AmB, such as Abelcet® and Ambisome® significantly decreased the toxicity associated with the treatment. However, the need to administer the drug as an intravenous infusion and the high costs associated with these formulations may be serious drawbacks of the current treatments [Asghari 2010, Sivak 2011]. This work aims at developing a new nanomedicine formulation as Amphotericin B carrier. The drug was encapsulated in purposely prepared nanocapsules consisting of an oil core and a cyclodextrin derivative shell. The amphotericin-loaded nanocapsules showed a mean diameter of about 350 nm, a drug loading of 33 % and a prolonged release kinetics. The stability of the formulation was proved for one year stored at 4°C. The new nanomedicine showed no haemolytic activity on red blood cells.

The efficacy of this formulation was tested in vitro against clinical isolates of C. albicans, C. glabrata, C. tropicalis, C. krusei and C. parapsilosis and compared with the one of free AmB. Moreover, to test the in vitro efficacy of the formulation against C. albicans biofilm, the Minimal Sessile Concentration that Inhibits of the 50% the growth of the biofilm (SMIC50) was evaluated.

Results obtained with all the species tested demonstrate that the nanocapsule formulation is able to stably inhibit fungine growth for 72 hours, at concentrations ten times lower than AmB and AmBisome®. These data have been confirmed also for a C. glabrata strain resistant to AmB.

Interestingly, preliminary experiments in mice showed that in vivo efficacy of AmB encapsulated in the new formulation is similar to the one of AmBisome®.

Finally, SMIC50 of AmB delivered by nanocapsules was ten times lower than the one of plain AmB and comparable to the one of AmBisome®.

Considering the antifungal efficacy and biocompatibility, besides high drug loading and stability, the nanocapsule might be proposed as an alternative nanomedicine for the delivery of AmB.

- J. L. Italia, M. M. Yahya, D. Singh and M. N. V. Ravi Kumar, Pharm. Res. 2009, 26, 1324-1331.
- Asghrai et al. 2010, Modares Journal of Medical Sciences, 13(3):1-10.
- Sivak et al. Lipids in Health and Disease 2011, 10:135

NANOSTRUCTURE OF RED BLOOD CELL MEMBRANE UNDER CRITICAL STATE. ATOMIC FORCE MICROSCOPY AND CALIBRATED ELECTROPORATION

ALEXANDER M. CHERNYSH, Elena K. Kozlova, Victor V.Moroz

V.A. Negovsky Scientific Research Institute of General Reanimatology RAMS, Moscow, Russia

INTRODUCTION

The purpose of the present work is to study the alterations of nanostructures of red blood cell (RBC) membranes under intoxication of the blood caused by acute massive hemorrhage, by action of pharmaceutical chemicals in high doses, oxidation processes due to ionizing radiation, long-term storage of the blood in vitro.

METHODS

Blood and Solutions. Blood donations were performed of healthy donors. In accordance with ethics commission of the Scientific Research Institute of General Reanimatology RAMS all donors signed the informed consent form. The experiments of blood loss and hypotension were performed using white rats. The study was approval by Institutional Animal Care and Use Committee. From the selected probes there were prepared monolayers of red blood cells. The intoxication was modeled by adding into blood hemin, furosemide, chlorpromazine and zinc ions in high concentrations, in vitro. All of them influence on protein structures of RBC. The long-term storage of donated blood was studied. There were used UV and gamma-radiation as the sources of ionizing radiation. Atomic force microscope (AFM) imaging. Space Fourier transform for analysis of membrane surface. The images of the cells, membrane surface and of their fragments were obtained using AFM (NTEGRA Prima, NT-MDT, Russia) in semi-contact and contact regimes. We used Space Fourier transfrom for the obtaining of a detailed image and estimation of quantitative parameter of the surface nanostructure.

Calibrated electroporation. The high voltage electrical impulse (E=1100 V/cm) was used for diagnostics of nanostructure of cells membrane. There were measured the kinetic curves of hemolysis due to electroporation after different influences.

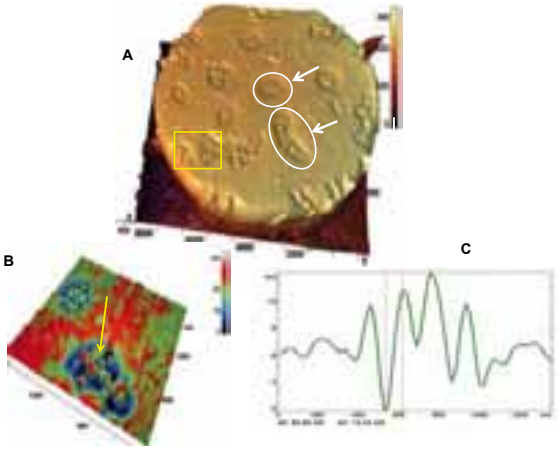

Fig 1. The influence of hemine on red blood cell membrane. A – The cell with domains of damages at the membrane surface. B – The fragment of membrane with domains. C – The profile of the domain surface

RESULTS

Images of membranes nanostructure under various influences were obtained. All these influences acted on membrane proteins and change the structure of membranes.

The character of alterations was specific to each of them. All defects increased with growth of concentration of the agent. The quantitative estimation of defects is given in the work. For example, at influence of hemin C=1.7 mM specific structures in the form of domains and "buttons" on the membrane of RBC appeared (Figure 1). The diameter of elementary "button" was 150-200 nm, height 3-15 nm.

In Figure 2 there are represented control cell and the cell after hypotension and the fragments of their membrane. The heights of the surfaces of first and second orders are essentially increased after massive hemorrhage.

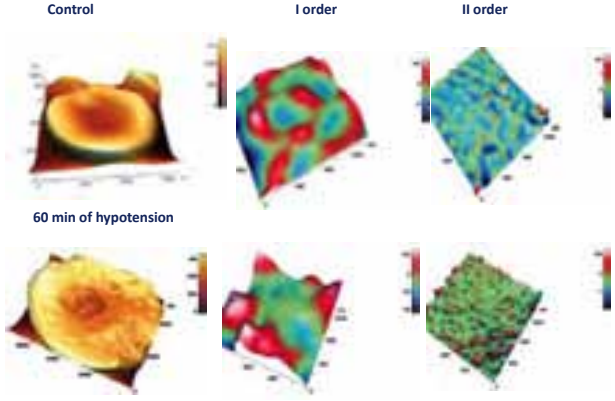

Fig.2. The change of red blood cells membrane after hypotension

Experimentally it was shown the damages of membrane nanostructure during storage of donated blood (30 days), under action of heavy metals ions, under influences of ionizing radiation.

CONCLUSIONS

It was experimentally established by the AFM and calibrated electroporation that intoxication essentially influenced on the membrane nanostructures of RBC. Further studying of features of the membrane nanostructure changes is useful as the basis for the establishment of mechanisms of intoxication of organism. This, in turn, will make it possible to choose correct tactics for treatment of critical states.

NANO-IMAGING IN DENTISTRY

HANS DEYHLE[1,2], Oliver Bunk[2], and Bert Müller[1]
[1]Biomaterials Science Center, University of Basel, c/o University Hospital, 4031 Basel, Switzerland
[2]Swiss Light Source, Paul Scherrer Institut, 5232 Villigen, CH

INTRODUCTION

Nanodentistry deals with the prevention, diagnosis and treatment of oral diseases by applying materials tailored on the nanometer level. For any of these techniques to be effective, a deep understanding of the tissue of concern is needed.

A variety of imaging techniques is used in clinical dentistry and dental research for diagnosis, treatment planning, and characterization of dental tissues. Cone beam computed tomography allows for the three-dimensional (3D) visualization of teeth and jaw-bone with sub-millimeter resolution [1]. Higher resolution can be achieved post mortem with micro-computed tomography (μCT). For example, micrometer-wide cracks in tooth hard tissues or the 3D morphology of the dentinal tubules can be visualized [2]. For even higher resolutions, scanning probe techniques as scanning electron microscopy (SEM) or atomic force microscopy (AFM) are routinely used [3].

Despite their unrivaled resolution capabilities, these techniques suffer from a series of drawbacks. For instance, they only grant information on a restricted area of the specimens, rendering data acquisition over larger portions highly time consuming. Often, extended specimen preparation is needed, leading to far from physiological conditions.

Scattering measurements, which are an example of reciprocal-space techniques, are characterized by a inverse relationship between the size of the inspected nanostructures and scattering angle. Thus, by tuning the inspected angular range of the scattered X-rays, one can obtain insight in the morphology of the inspected specimen in the range below one micrometer down to the atomic structures. According to the angles of interest, scattering can be divided into small-angle X-ray scattering (SAXS), dealing with the ranges approximately between 2 and 200 nm, and wide-angle X-ray scattering (WAXS), which allows for the inspection of atomic species.

Exploiting the high brilliance of synchrotron facilities and high sensitivity/fast readout detection systems, scattering experiments in a scanning setup recently became available. In this setup, the specimen is scanned through a focused X-ray beam in steps of several micrometers. A scattering pattern is recorded at each raster point. The PILATUS detector [4] available at the cSAXS beamline (SLS, PSI, Villigen) allows acquiring of scattering patterns with a frame-rate of several ten Hz, thus providing the means to scan macroscopic areas with micrometer resolution in reasonable time [5]. This report aims to give an overview of the imaging and data analysis possibilities and results obtained on various scans of human teeth at the cSAXS beamline.

EXPERIMENTAL DATA

Four human molars, extracted for clinical reasons, were cut into 200 to 500 μm thin slices orthogonal and parallel to the tooth axis. The tooth slices were scanned at the cSAXS beamline in SAXS and WAXS setup, respectively.

Scattering techniques provide a statistical average over the illuminated volume and do therefore not allow resolving individual nanometer-sized features. However, information about the abundance,

orientation, shape, size and degree of anisotropy or degree of crystallinity of the nanocomponents can be obtained. For example, the slope of the scattered intensity as a function of the scattering vector q or radius r is closely related to the shape of the scattering nanoparticles. Spherical scatterers exhibit an intensity decay proportional to q-4, while 2D disc-like structures present a decay proportional to q-2 and needle or rod-like structures proportional to q-1 [6]. Figure 1 shows this exponent for one tooth slice in the ranges corresponding to 20 to 30 nm, 50 to 60 nm and 90 to 100 nm.

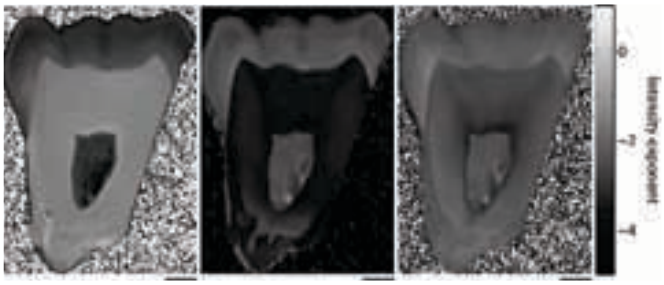

Figure 1. Exponent of I(q) in the ranges to 20 to 30 nm, 50 to 60 nm and 90 to 100 nm, from left to right. The length bar corresponds to 2 mm.

The area without specimen should yield an exponent 0, however, due to noise a residual signal is detected. In the smaller range, between 20 and 30 nm, the dentin appears to contain more volume-like structures with no dimension significantly larger than the other, while in the enamel disc to rod-like scatterers can be found. In the range between 50 and 60 nm the situation is inverted, here the volumetric scatterers are found in the enamel while disk or rod-like structures are predominant in the dentine. In particular, along the lines connecting tooth cusps to the pulp the shape of the main components in the dentin changes from disk to rod-like. When looking at even larger structures around 100 nm, a rather uniform signal of volumetric scatterers is obtained for the whole specimen.
In addition, components presenting distinctive periodicities generate scattering signals that can easily be identified. For example, collagen can easily be identified due to its characteristic periodicity of about 67 nm [7]. Another feature is the 002 of the hydroxyapatite (HA) crystallites in tooth enamel [8].

OUTLOOK

X-ray scattering in scanning mode is a powerful technique to uncover the nano and sub-nano-structure of human teeth over macroscopic areas. The identification of the specific features from the scattering patterns requires a certain a priori knowledge of the specimen. However, under specific conditions individual components can be easily identified. In human teeth, strong anisotropies are found along the whole nanometer range and below. The organization of tooth hard and soft tissues clearly relates to the mechanical properties.
The insight gained on the morphology of organic and inorganic components should provide the means to further develop nanodentistry treatment possibilities, such as biomimetic fillings or remineralization procedures with nanoparticulate bioglasses.

REFERENCES

[1] Luckow, M., Deyhle, H., Beckmann, F., Dagassan-Berndt, D., and Müller, B., "Tilting the jaw to improve the image quality or to reduce the dose in cone-beam computed tomography", European Journal of Radiology in press, (2011).

[2] Deyhle, H., Bunk, O., Buser, S., Krastl, G., Zitzmann, N.U., Ilgenstein, B., Beckmann, F., Pfeiffer, F., Weiger, R., and Müller, B., "Bio-inspired dental fillings", Proceedings of SPIE 7401, 74010E (2009).

[3] Lenz, H., "Elektronenmikroskopische Untersuchungen der Mineralisation des Zahnschmelzes und der beginnenden Schmelzkaries", Archives of Oral Biology, special supplement 4, 34-39 (1961).

[4] Heinrich, B., Bergamaschi, A., Brönnimann, C., Dinapoli, R., Eikenberry, E.F., Jhonson, I., Kobas, M., Kraft, P., and Mozzanica, A., "PILATUS: A single photon counting pixel detector for X-ray applications", Nuclear Instruments and Methods in Physics Research A 607, 247-249 (2009).

[5] Bunk, O., Bech, M., Jensen, T.H., Feidenhans'l, R., Binderup, T., Menzel, A., and Pfeiffer, F., "Multimodal x-ray scatter imaging", New J Phys 11, 123016 (2009).

[6] Guinier, A. and Fournet, G., "Small angle scattering of X-rays". 1955, New York: John Wiley & Sons, Inc.

[7] Kinney, J.H., Pople, J.A., Marshall, G.W., and Marshall, S.J., "Collagen orientation and crystallite size in human dentin: a small angle X-ray scattering study", Calcified Tissue Int 69, 31-27 (2001).

[8] Leventouri, T., Antonakos, A., Kyriacou, A., Venturelli, R., Liarokapis, E., and Perdikatsis, V., "Crystal structure studies of human dental apatite as a function of age", International Journal of Biomaterials 2009, 698547 (2009).

MULTI RESPONSIVE TARGETING MICROCONTAINERS AS DRUG DELIVERY SYSTEMS: RELEASE AND CYTOTOXICITY STUDIES

ELENA K. EFTHIMIADOU[1], L. – A. Tziveleka[1], C. Tapeinos[1], A. – F. Metaxa[1], A. Chatzipavlidis[2], N. Boukos[1] and G. C. Kordas[1]
[1]Institute of Materials Science, NCSR "Demokritos", GR-15310 Aghia Paraskevi, Athens, Greece
[2]National Technical University of Athens, School of Chemical Engineering, 9 Heroon Polytechniou St, Zografos, Athens GR-15780, Greece

In recent years, considerable efforts have been developed towards the design and controlled fabrication of nanostructured materials displaying specific functional properties [1]. Bio-functionalized organic nanocontainers have been synthesized through efficient synthetic approaches aiming at producing, monodispersed, shape-controlled, stable and biocompatible nanocontainers. In particular, organic nanocontainers have been firstly synthesized, and then coated with organic polymeric shell. At a second step, their surface has been functionalized with targeting ligands in order to reach specific cancer cells. Targeting ligands conjugated on the microcontainers surface can also strongly affect their affinity to specific cell- surface receptors. Introducing of tumor targeting groups onto polymers surface can further enhance their accumulation in cancer cells, through active targeting [2]. In this respect, we have introduced a combination of folate moiety as a target group and magnetic nanoparticles on the nanospheres surface improving the targeted cancer therapy through hyperthermia. Generally, the exposure of magnetic nanoparticles to a high-frequency magnetic field, results in heat generation due to various magnetic relaxation mechanisms [3]. It is reported in various studies that direct injection of magnetic nanoparticles into solid tumors and the sequential exposure to an alternating magnetic field is capable of inducing tumor regression [4]. Our approach for the employment of magnetic hyperthermia is focused on the combination of magnetic nanoparticles with thermo-responsive polymer nanosystems. Specifically, we developed hybrid nanocontainers with a variety of interesting properties and perspectives, including instant dispensability, thermoreversible behavior, and novel magnetoresponsive properties.
In this work we have synthesized and characterized Yolk-type core-shell microspheres based on HPMA. The Yolk-Type microspheres were synthesized via seed emulsion polymerization and structurally characterized by FT-IR spectroscopy. The morphology of the microspheres was studied through Scanning (SEM) and Transmission (TEM) electron microscopy. The hydrodynamic diameter of the isolated microspheres was studied by Dynamic light scattering (DLS). Vibrating sample magnetometer (VSM) and hyperthermia was used to evaluate the magnetic properties of the multi targeted microspheres. Moreover, the obtained nanoparticles were characterized by X-Ray powder diffraction (XRD). The afforded microspheres were further modified chemically both, with magnetic nanoparticles deposition and folate moiety through EDC coupling, aiming at introducing a combination of hyperthermia and targeting properties. The loading and controlled release behavior has been investigated with Doxorubicin hydrochloride (DOX) at different pH conditions while

the in vitro cytotoxicity of the DNR-loaded or empty modified hollow microspheres was also examined on MCF-7 breast cancer cells.

ACKNOWLEDGEMENTS

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REFERENCES

1. X. Yang et al., Biomaterials, 32, (2011) 4151-4160.
2. C. P. Leamon et al., Pharmaceutical Research, 26, (2009) 1316-1323.
3. Laurent et al., Advances in Colloid and Interface Science 166 (2011) 8-23.
4. M. Whelan et al., Drug discovery Today 8, (2003) 253-258.

CELLULAR UPTAKE OF DNA NANOSTRUCTURES IS SHAPE DEPENDENT

JOHANS FAKHOURY[1], C. McLaughlin[1], and H. Sleiman[1]
[1]Department of Chemistry, McGill University, Montreal, Quebec, H3A 3K6, Canada

ABSTRACT SUMMARY

Here we report that DNA nanostructure plasma membrane penetration and cytoplasmic accumulation is highly shape dependent. We assembled a progression of one-, two- and three-dimensional DNA structures and studied their potential for cellular internalization and accumulation. We demonstrate that only rigid DNA cubes decorated with Cy3 and/or Cy5-labelled complementary strands can efficiently accumulate in the cytoplasm of human cervical cancer cells (HeLa) without the aid of transfection agent. These molecules could represent a new class of selective cellular probes and drug delivery tools, and would assist the development of nucleic acid therapeutic routes.

INTRODUCTION

Nucleic acid therapeutics represent a promising avenue for the treatment of a spectrum of diseases. Several approaches are currently examined, including the incorporation of gene units to compensate for the deficiency in a protein, the delivery of small interfering RNA or antisense oligonucleotides to achieve gene silencing, or the introduction of genetic material from various pathogens as vaccines.[1-4] However, the success of nucleic acid therapeutic modalities has been severely hampered by physiological barriers that prevent delivery of these anionic macromolecules into cells.[5, 6] Several strategies have been employed to overcome these challenges, including modifications to the phosphate backbone or the sugar moieties, and the use of transfection agents, such as viral vectors, cationic polymers, dendrimers or liposomes.[7, 8]

Recently, emerging studies have demonstrated that, if DNA strands are arranged into a dense core-shell structure, such as oligonucleotide-gold nanoparticle conjugates,[9, 10] they can be efficiently taken up by a variety of cells without the need for viral or non-viral vectors. Purely DNA-based constructs, such as DNA nanotubes[11] or origami[12] have also been shown to accumulate intracellularly, and cellular uptake for smaller DNA-based structures, such as tetrahedra[13, 14] or icosahedra[15, 16] was also observed. Although uptake efficiencies vary, this raises the possibility that packaging DNA strands into compact three-dimensional structures can enhance their cellular uptake and intracellular accumulation.

We were interested in investigating whether cellular internalization and accumulation of DNA nanostructures is shape dependent. We previously reported the synthesis of a range of two- and three-dimensional DNA structures, with fine control of shape, size, porosity, and with the ability to encapsulate and release cargo in response to specific biological molecules.[17-19] If these structures are able to enter cells, they could represent a new class of selective cellular probes and drug delivery tools, and would assist the development of nucleic acid therapeutic routes.

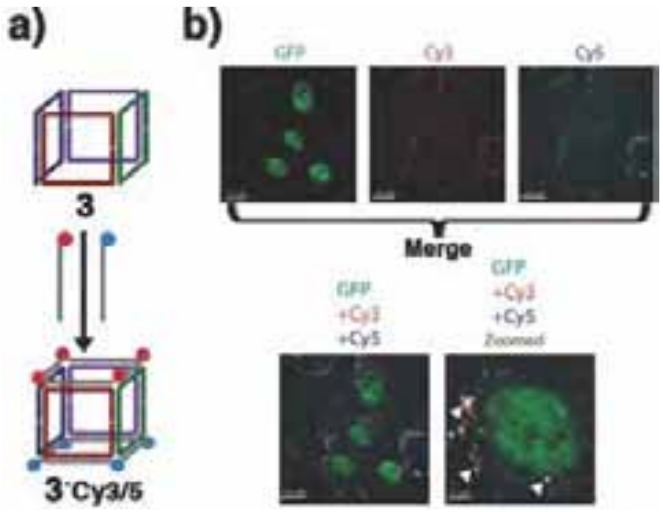
EXPERIMENTAL METHODS

In the present study, we assembled a progression of one-, two- and three-dimensional DNA structures and studied their potential for cellular internalization and accumulation (Figure1a). We decorated DNA cubes with Cy3 and/or Cy5-labelled complementary strands and studied their intracellular accumulation in human cervical cancer cells (HeLa) with confocal fluorescence microscopy (CFM) and fluorescence assisted cell sorting (FACS).

RESULTS AND DISCUSSION

We demonstrated that compact naked DNA cubes travel across the plasma membrane of human cancer cells without the aid of any transfection reagent (Figure1b). Additionally, their integrity remains intact upon crossing the plasma membrane and subsequent cytoplasmic accumulation. In contrast, single and double-stranded DNA shows little or no cellular uptake. Interestingly, open two-dimensional intermediate structures are unable to penetrate cells and neither does a DNA cube structure that is mostly single-stranded. We hypothesize that in the absence of complementary DNA strands, mostly single-stranded DNA cube particles are dynamic and may collapse to form two-dimensional structures. We observed some intracellular accumulation (10% of counted cells) of triangular double-stranded DNA particles. We propose that these particles are slightly more efficient in forming compact structures that may be more amenable to plasma membrane transport and cytoplasmic accumulation.

We observed DNA cube accumulation exclusively in the cytoplasm into distinct foci, suggesting that cellular uptake is achieved through receptor mediated endocytosis.[20] We hypothesize that pattern recognition receptors, such as scavenger receptors that are able to selectively distinguish size, shape, and density could be involved in the cellular uptake of rigid DNA cube particles, but the exact mechanism involved here is under exploration.



CONCLUSION

We have thus demonstrated that DNA nanostructure plasma membrane penetration and cytoplasmic accumulation is highly shape dependent, and is significantly enhanced when DNA is packaged in compact, well defined cubes. We suggest that these DNA cages can be used as vehicles to achieve efficient and selective delivery of therapeutic oligonucleotides and drugs, as well as switchable, responsive probes of intracellular activity.

REFERENCES

1. Watanabe, T., T. Umehara, and M. Kohara, Therapeutic application of RNA interference for hepatitis C virus. Adv Drug Deliv Rev, 2007. 59(12): p. 1263-76.
2. Schiffelers, R.M., et al., Cancer siRNA therapy by tumor selective delivery with ligand-targeted sterically stabilized nanoparticle. Nucleic Acids Res, 2004. 32(19): p. e149.
3. Rice, J., C.H. Ottensmeier, and F.K. Stevenson, DNA vaccines: precision tools for activating effective immunity against cancer. Nat Rev Cancer, 2008. 8(2): p. 108-20.
4. Meyer, M. and E. Wagner, Recent developments in the application of plasmid DNA-based vectors and small interfering RNA

therapeutics for cancer. Hum Gene Ther, 2006. 17(11): p. 1062-76.
5. Harada-Shiba, M., et al., Polyion complex micelles as vectors in gene therapy--pharmacokinetics and in vivo gene transfer. Gene Ther, 2002. 9(6): p. 407-14.
6. Kariko, K., et al., Small interfering RNAs mediate sequence-independent gene suppression and induce immune activation by signaling through toll-like receptor 3. J Immunol, 2004. 172(11): p. 6545-9.
7. Sliva, K. and B.S. Schnierle, Selective gene silencing by viral delivery of short hairpin RNA. Virol J, 2010. 7: p. 248.
8. Singha, K., R. Namgung, and W.J. Kim, Polymers in Small-Interfering RNA Delivery. Oligonucleotides, 2011.
9. Cutler, J.I., et al., Polyvalent nucleic acid nanostructures. J Am Chem Soc, 2011. 133(24): p. 9254-7.
10. Giljohann, D.A., et al., Oligonucleotide loading determines cellular uptake of DNA-modified gold nanoparticles. Nano Lett, 2007. 7(12): p. 3818-21.
11. Ko, S., et al., DNA Nanotubes as Combinatorial Vehicles for Cellular Delivery. Biomacromolecules, 2008. 9(11): p. 3039-3043.
12. Schueller, V.J., et al., Cellular Immunostimulation by CpG-Sequence-Coated DNA Origami Structures. ACS Nano, 2011.
13. Walsh, A.S., et al., DNA Cage Delivery to Mammalian Cells. ACS Nano, 2011. 5(7): p. 5427-5432.
14. Li, J., et al., Self-Assembled Multivalent DNA Nanostructures for Noninvasive Intracellular Delivery of Immunostimulatory CpG Oligonucleotides. ACS Nano, 2011.
15. Bhatia, D., et al., A synthetic icosahedral DNA-based host-cargo complex for functional in vivo imaging. Nat Commun, 2011. 2: p. 339.
16. Chang, M., C.-S. Yang, and D.-M. Huang, Aptamer-Conjugated DNA Icosahedral Nanoparticles As a Carrier of Doxorubicin for Cancer Therapy. ACS Nano, 2011. 5(8): p. 6156-6163.
17. Aldaye, F.A., et al., Modular construction of DNA nanotubes of tunable geometry and single- or double-stranded character. Nat Nanotechnol, 2009. 4(6): p. 349-52.
18. Aldaye, F.A. and H.F. Sleiman, Dynamic DNA templates for discrete gold nanoparticle assemblies: control of geometry, modularity, write/erase and structural switching. J Am Chem Soc, 2007. 129(14): p. 4130-1.
19. Lo, P.K., et al., Loading and selective release of cargo in DNA nanotubes with longitudinal variation. Nat Chem, 2010. 2(4): p. 319-28.
20. Gu, Z., et al., Tailoring nanocarriers for intracellular protein delivery. Chem Soc Rev, 2011. 40(7): p. 3638-55.

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UPCONVERTING NANOCRYSTALS AS CONTRAST AGENT FOR IMPROVED IN VIVO FLUORESCENCE IMAGING

SARAH FREDRIKSSON[1], Haichun Liu[2], Anna Gisselsson[1], Pontus Kjellman[1], Linda Andersson [1], Rene in't Zandt[1], Pontus Svenmarker[2], Fredrik Olsson[1], Yu Can[2], and Stefan Andersson-Engels[2]
[1]Genovis AB, Lund, Sweden, [2]Biophotonics Group, Medical Laser Centre, Lund University, Sweden

BACKGROUND

Imaging has become an increasingly important tool in drug discovery and development. In order to broaden and enhance the understanding of a drug candidate's mechanism of action, it is important to study the impact on basic pathological physiology changes at the organ, tissue, cellular and molecular level in animal models monitored over time. Longitudinal studies over days, weeks and months on each individual are possible with repeated screening procedures. Molecular imaging using biomarkers have taken imaging even further down to cellular levels.

Taken together the latest development increases the reliability and accuracy of imaging and can further facilitate translational research. All imaging modalities are available for preclinical imaging and optical imaging is the most common modality used today in small animal in vivo imaging.

Optical imaging using fluorescent probes methods is cost effective and relatively easy to operate. The main limitations of the technique are tissue autofluorescence background, poor resolution and poor light penetration, making it difficult to image deep tissue. Furthe more, traditional fluorescent probes are sensitive to multiple screening over time due to bleaching of the probes. An upconverting phosphor crystal (UPC) is composed of a transparent host lattice doped with certain trivalent lanthanide ions or transition metals. Common materials are ytterbium (Yb) and yttrium (Y) in combination with small amounts of other ions like for instance erbium (Er) or thulium (Tm). The Tm-doped UCP is illuminated with a wavelength of 980 nm and emits light at 800 nm. This wavelength window is optimal for in vivo imaging in tissue, figure 1, when it comes to minimize tissue absorption and autofluorescence, which in turn increase sensitivity and resolution. This study was set up to investigate the performance of upconverting nanostructures as probes in fluorescent optical in vivo imaging. The goal is to find probes that can facilitate optical imaging in deeper tissue.

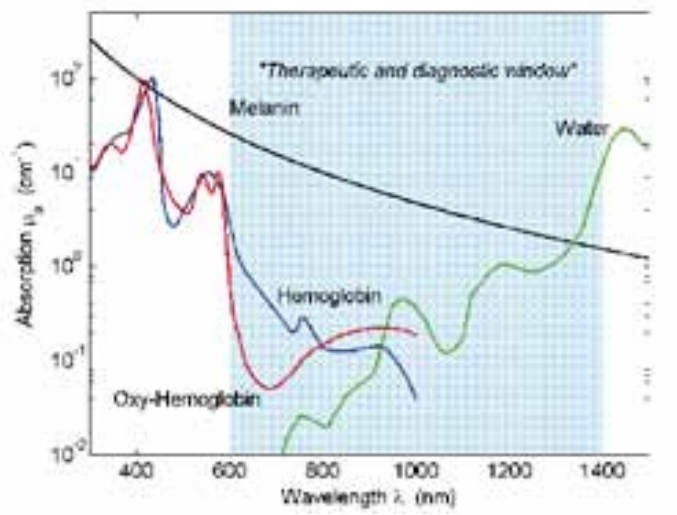


Figure 1: UPC emits light at 800 nm, which is favourable for in vivo imaging applications.

RESULTS AND DISCUSSION

UPCs were synthesised using seed growth in a mixture of YCl3, YbCl3, ErCl3 and TmCl3 in oleic acid and octadecene in 156°C. The crystals were washed and precipitated before coating with a combination of PMAO (poly(maleic anhydride-alt-1-octadecene) and polyoxyalkyleneamine (MW 2000). The final nanostructures had a diameter of 18 nm (hydrodynamic diameter) and were stored in 150 mM NaCl until use. Initial tox and proliferation tests were performed on hepatocellular carcinoma Hep G2 cells. The proliferation profile and toxicity profile did not indicate any adverse effects within the concentration range tested (up to 170 pg/cell).

The detection of UPC in vivo was evaluated by a subcutaneous injection in the right hind paws of rats (female Wistar, weight 200-250 gram, concentration UCP 0.26 mg Y3+/ml, injection volume 100 µl). The retention of the particles in the lymphatic system was then visualized in vivo, 6 hours post injection, for the popliteal (SLN) and the inguinal lymph nodes.

The lymph nodes were visualized using a diode laser at 975 nm, illuminated through an optical fiber with a fiber diameter of 400 µm. The illumination area was approximately 3 mm in diameter. The detection was done using a CCD camera (Andor iXon). The localization of the lymphnodes and the uptake of the UCPs within the lymphnodes were verified by subsequent resection.

After 6 hours the results from the imaging study show that the nanostructures have reached the inguinal node, figure 2.
The size of the particles suggests that these results are in accordance with studies performed on superparamagnetic nanostructures of similar sizes and coatings. The intensity is dependent on localization of the structures where tissue depth is an important parameter and hence it is not straightforward to determine the relative distribution of the nanostructures between the popliteal and the inguinal nodes using imaging only.
Further optimization of the imaging settings is needed in order to understand optimal dosing and to fully interpret the potential amount of information given in the imaging study. The low toxicity in combination with optimal wavelength window is promising for future in vivo imaging even in deeper tissue.

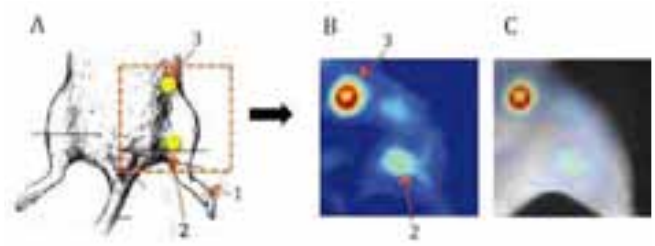


Figure 2: The first lymph nodes are the popliteal (SLN) (A-2) and the inguinal node (A-3). The popliteal and the inguinal nodes can clearly be seen in the optical image (B) and in the image where the fluorescence signal is fused with the bright view image (C).

AN IMPROVED PEGYLATED LIPOSOMAL DOXORUBICIN WITH SIGNIFICANTLY LOWER PPE THAN DOXIL®

DORON FRIEDMAN, Yaelle Felsen, Tal Berman, Yaacov Toledo and Yechezkel Barenholz
LipoCure, Ltd. and Laboratory of Membrane and Liposome Research, Hebrew University – Hadassah Medical School, Jerusalem.

INTRODUCTION

The high demand for Doxil® during the current shortage is a good demonstration of its high utility. Doxil® has many advantages over free doxorubicin (Solomon and Gabizon (2008) Clin. Lymphoma Myeloma 8:21-34, Barenholz (2012) J. Control. Release, in press). The excellent drug retention, combined with the very long circulation time of Doxil® allows the liposomes to accumulate in the skin in high doses, thereby inducing skin toxicity, a phenomenon known as "palmar-plantar erythrodysesthesia" (PPE) or "hand-foot syndrome" (Gabizon et al (1994) Cancer Research 54:987-992).
In clinical studies and current routine treatments at 50 mg/m2 dosing every 4 weeks, more than 50% of patients treated with Doxil® developed hand-foot syndrome. The prevalence of this side effect limits the maximum tolerated dose (MTD) of Doxil® to 50 mg/m2 (compared with 60 mg/m2) for free doxorubicin.

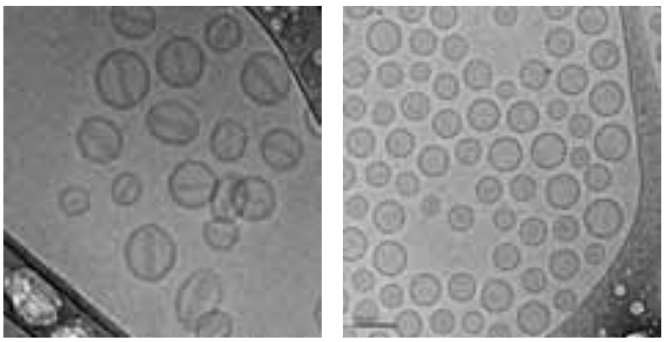
GOALS

To introduce a novel formulation of doxorubicin encapsulated in pegylated long circulating liposomes (DOX003) designed to reduce the PPE effect. To demonstrate in preliminary experiments with rats that DOX003 shows an improved safety profile with regard to PPE and gross toxicity using Doxil® as a comparator.

RESULTS

Our results demonstrate that under the same treatment conditions tested and at equal doses and total amounts of doxorubicin, the treatment with DOX003 resulted in a much lower severity of rat PPE and toxicity compared with the rats injected with Doxil® in terms of general health (body weight, appearance) and clinical PPE symptoms.
Rats injected with DOX003 had better "humane" criteria than rats injected with Doxil®, which can be interpreted as a better quality of life.
Based on the above results (and on unimpaired efficacy), DOX003 is on its way to clinical trials.

Figure: ACryoTEM picture of Dox003 versus Doxil®.



NEAR-INFRA RED FLUORESCENT SOLID LIPID NANOPARTICLES FOR TUMOR TARGETING IMAGING

SIMONA GHIANI[1], Lorena Beltrami[1], Lara Caminiti1, Roberta Bonafè[1], Alessandro Maiocchi[1]
[1]Bracco Imaging SpA, Via Ribes 5, I-10010 Colleretto Giacosa, Torino, Italy

The development of Near-Infra Red (NIR) fluorophores embedded in nanomaterials is expected to have a significant impact on the future of tumor-targeted diagnostic agents due to the opportunity offered by these systems in the non-invasive and high sensitive visualization of cancer lesions. However in vivo clinical applications of NIR agents are limited to surgical, endoscopic procedures or to surface detections (i.e. skin cancer, optical mammography1 or sentinel lymph nodes imaging)2, due to the limitation of low penetration of light. In order to overcome the above usage restraints, many efforts are currently spent in the design of multi-functionalized nanoparticles focusing on improving fluorescent efficiency and the target binding affinity.
In the present work, fluorescent Solid Lipid Nanoparticles (f)-SLNs are proposed as a promising delivery system for lipophilic cyanine. In order to decorate the surface of SLNs with an high payload of fluorescent molecules, a cyanine phospholipid derivative was synthesised. A hydrophylic cy5.5-NHS dye was conjugated to a 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE) moiety before the nanoparticle formulation. Afterwards the (f)-SLNs were prepared and characterized in term of optical and general physicochemical properties (i.e. particle size distribution, surface charge, fluorescent quantum yield, brightness and fluorescence lifetime). Moreover in vitro cytotoxicity of the Cy5.5-DPPE loaded SLNs was preliminarily assessed on HUVEC cells line.
The obtained fluorescent nanoparticles showed small particle size distribution, high fluorescence efficiency as well as good in vitro tolerability. Hence, in vivo experiments were performed to explore the mechanism of passive tumor targeting of the (f)-SLN through the EPR effect on ovarian cancer xenografts (IGROV-1) in Balb/C nu/nu mice. The optical imaging experiments showed a significant accumulation of the nanoparticles in the tumor tissue until 48 h.
In conclusion, the proposed nanoparticles can be seen as a promising platform that can be further improved by an appropriate molecular vector to increase the specificity of the imaging probe and possibly the sensitivity of the whole procedure.

(Endnotes)

1 van de Ven SM, Elias SG, van den Bosch MA, Luijten P, Mali WP. Optical imaging of the breast. Cancer Imaging 2008, 8, 206-215.
2 Troyan, S.L. et al. The FLARETM intraoperative near-infrared fluorescence imaging system: a first-in-human clinical trial in breast cancer sentinel lymph node mapping. Ann. Surg. Oncol. 16, 2943–2952 (2009). Proulx ST, Luciani P, Derzsi S, et al. Quantitative imaging of lymphatic function with liposomal Indocyanine green. Cancer Res. 2010; 70(18): 7053–62.

OPTIMIZING UPTAKE OF LIPID COATED SPION PARTICLES IN CELL CULTURES

ANNA GISSELSSON, Linda Andersson, Malin Mejàre, Pontus Kjellman, René in 't Zandt, Fredrik Olsson and Sarah Fredriksson
Genovis AB, POB 790, SE-22007 Lund, Sweden

INTRODUCTION

Cell therapy holds a promising future in regenerative medicine within fields such as cardiovascular diseases, diabetes, neurological disorders and hepatocyte transplanantation as well as in cancer therapy. Multipotent mesenchymal stromal cells (MSC), also called mesenchymal stem cells, are increasingly used as cell therapy agents due to the establishment of isolation protocols from a variety of tissues, the possibility to expand large amounts of cells in vitro and to their inherent therapeutic plasticity.

Apart from survival rate and function of the cells, the spatial distribution is of major importance in evaluating the success of transplantation. As the field of cell therapy grows, there is an increasing demand for non-invasive imaging methods to track the engrafted cells. In this work we present labelling of cells in vitro for dual detection by fluorescence and MR imaging. Endocytosis of a lipid coated superparamagnetic iron oxide nanoparticle (SPION), bearing a fluorescing marker, was studied in a variety of cell types and special interest was directed to labelling of mesenchymal stem cells.

METHODS

Cells presented in table 1 were cultured according to recommendations. An 11 nm solid core FeO particle was coated with DOTAP lipid and conjugated to Texas Red fluorophore, resulting in a SPION with a hydrodynamic diameter of 50 nm. Prior to labelling experiments, all cells were counted, and cells were allowed to start proliferating over night before addition of nanoparticles. SPIONs were added to cell growth medium to a final concentration of 50 pg Fe/seeded cell, followed by 1-2 h incubation at 37oC. STEMPRO human adipose-derived stem cells (Invitrogen) were labelled with up to 300 pg Fe/seeded cell for 1-16 hours. Labelling of cells was verified by fluorescence microscopy, excitation 615 nm. The amount of Fe taken up per cell was determined using a bathophenanthroline method (Lewis, 1971. Am J Clin Pathol 56(4):543-5). Cell proliferation was determined by CellTiter 96® AQueous proliferation assay (Promega) and was used as an indication of potential cell toxicity. To establish that MSCs retained their pluripotency after labelling, differentiation was performed using STEMPRO® osteogenesis differentiation kit and STEMPRO® adipogenesis differentiation kit (Invitrogen).

RESULTS

All cell types tested in this study were labelled with fluorescing SPIONs simply by adding nanoparticles to the cell culture medium (Table 1). The fluorescence of some of these cells is presented in Figure 1. Lipid coated particles are distributed within a large number of endosomes throughout the cytoplasm.

Human adipose-derived stem cells had an uptake of 10 pg Fe/cell when adding 100 pg Fe/seeded cell to the cell culture medium, and 20-25 pg Fe/cell when adding 150 pg Fe/seeded cell to the cell culture medium. Proliferation was intact at these amounts of nanoparticles, compared to non-labelled cells. However, higher particle load resulted in a decreased proliferation rate (Fig 2A). Incubation time up to 3 hours of labelling resulted in a higher uptake of SPIONs. Even though the amount of SPIONs/cell did not increase after that, cells continued to take up particles following cell division, resulting in a constant degree of labelling at later time points (Fig 2B). Not only the amount of Fe/cell but also the concentration of SPIONs in the cell culture medium proved to be of importance (results not shown here). Therefore, medium was 0.2 ml/cm2 in all experiments. One criterium for successful labelling of mesenchymal stem cells is that they retain their ability to differentiate into different cell types. After incubation with 100 pg Fe/seeded cell for 18 hours, MSCs were successfully differentiated into adipocytes and osteocytes under respective growth conditions (Fig 3).

Table 1. Cell types labelled with lipid coated SPIONs.

Species	Tissue	Cell line	Carcinoma	Morphology	Adherent
Human	Adipose tissue	MSC	-	Fibroblast	+
Human	Bone marrow	MSC	-	Fibroblast	+
Human	Bone marrow	K562	Myelogenous leukemia	Lymphoblast	-
Human	Bone marrow	RS4; 11	Lymphoblastic leukemia	Lymphoblast	-
Human	Bone	U-2 OS	Osteocarcinoma	Epithelial	+
Human	Blood	Jurkat E6.1	T-cell leukemia	Lymphoblast	-
Human	Blood	Granta-519	B-cell lymphoma	Speroid	-
Human	Pleural effusion	U937	Histiocytic lymphoma	Monocyte	-
Human	Embryonic kidney	HEK-293	-	Epithelial	+
Human	Kidney	786-O	Adenocarcinoma	Epithelial	+
Human	Mammary gland	MCF-7	Adenocarcinoma	Epithelial	+
Human	Pancreas	PANC-1	Epitheloid carcinoma	Epithelial	+
Human	Pancreas	HPAF-II	Adenocarcinoma	Epithelial	+
Human	Pancreas	MiaPaCa2	Adenocarcinoma	Epithelial	+
Mouse	Bone marrow	LADMAC	Expr. Oncogene	Lymphoblast	-
Mouse	Bone marrow	MSC	-	Fibroblast	+
Mouse	Brain	EOC 20	-	Macrophage	+
Mouse	Embryo	MEF	-	Fibroblast	+
Mouse	Blood	EL-4	Lymphoma	Lymphoblast	-
Mouse	Pancreas	Primary β	-		+
Monkey	Kidney	COS-7	-	Fibroblast	+

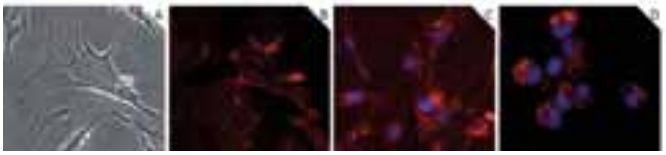


Figure 1. Cell cultures labelled with lipid coated SPIONs. A and B: Bright field view and Texas Red fluorescence of human MSCs. C: murine MSCs and D: murine primary β cells with Texas Red fluorescence and counter-stained cell neclei.

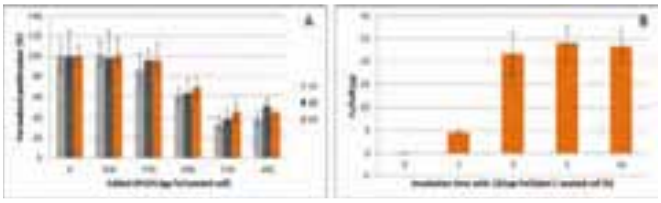


Figure 2. A. Labelling of human MSCs with lipid coated SPIONs did not significantly reduce cell proliferation, at incubation with up to 150 pg Fe per cell, and for up to 6 hours. B. Uptake of SPIONs at incubation with 150 pg Fe per cell and for up to 16 hours.

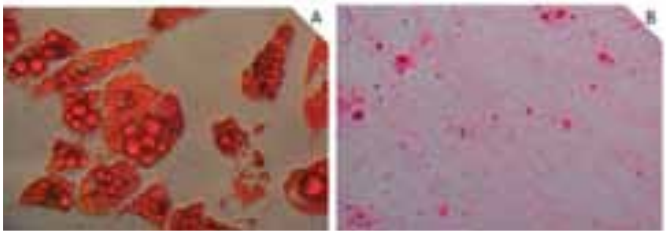


Figure 3. Mesenchymal stem cells, labelled with 10 pg Fe of lipid coated SPIONs per cell, retain their capability to differentiate into (A) adipocytes and (B) osteocytes in vitro.

DISCUSSION:

Cell labelling with FeOlabel resulted in significant uptake in cell cultures, originating from a number of different species and tissue types, both diseased and healthy cells, immunocells and stem cells, grown in suspension as well as adherently. These results indicate a general endocytosis of the lipid coated SPIONs rather than a specific, receptor-mediated uptake. Also the uptake does not require any transfection agent for a rapid uptake of large amounts of nanoparticles, a major benefit, since usage of transfection agents can lead to altered cell morphology.

The optimized cell labelling protocol retains the ability for stem cells to as well as their pluripotentcy. The dual modality for imaging allows for a rapid verification of cell labelling before delivery of therapeutic cells in vivo, and provides an additional marker for evaluating histology, apart from immunostaining and staining of iron.

MAGNETIC NANOPARTICLES COUPLED TO MICRO-BUBBLES ENABLE MULTIMODAL IMAGING

JOHAN HÄRMARK[1], Silvia Margheritelli[2], Letizia Oddo[2], Gaio Paradossi[2] and Hans Hebert[1]
[1]School of Technology and Health, KTH Royal Institute of Technology and Department of Biosciences and Nutrition, Karolinska Institutet, Huddinge, Sweden; [2]Department of Chemical Sciences and Technologies, Universita di Roma Tor Vergata, Rome, Italy.

INTRODUCTION

Microbubbles (MB) are used in the clinic as injectable ultrasound contrast agents (UCA). Polymer shelled UCAs can potentially be more versatile compared to lipid shelled UCAs. They are more robust, i.e. they have longer shelf life and circulation time, and the surface provides different modification possibilities that allow enhanced targeting and local drug delivery.1 The next generation of contrast agents has to meet the criteria of working as a multimodal imaging device, combining different imaging techniques, such as ultrasound, magnetic resonance imaging (MRI) and single-photon emission computer tomography (SPECT). A modified poly vinyl alcohol (PVA) MB, that includes super paramagnetic iron oxide nanoparticles (SPION) embedded in the shell, can work as both ultrasound and MRI contrast agent. Transmission electron microscopy (TEM) has been used to confirm the binding ability of SPION to the MB.

METHODS

Synthesis of PVA based MB have been previously described.1 The protocol was extended by adding unmodified SPION to the solution during the PVA MB production.2,3 Distribution of SPION, embedded in the MB, were observed by TEM. Samples were applied to glow-discharged 400 mesh copper grids coated with a thin carbon film without any additional staining or fixation solution. In order to find the positions of the SPION inside the MB shell, thin sections of MB were prepared according to the following protocol. An aliquot of MB was transferred to Eppendorf tubes containing a preheated 10% gelatin solution. The tubes were then placed in a stand for 15 min at room temperature, thereby the gelatin started to solidify and MBs were trapped in the gelatin. To further stabilize the gelatin, 3% paraformaldehyde in 0.1 M phosphate buffer was added to the tubes and placed in a refrigerator overnight. The gelatin pellet was then embedded in epoxy resin, LX 112 (Ladd, Burlington, VT, USA). Sections of, approximately 50 nm were cut using a Leica Ultracut UCT and placed on formvar coated 50 mesh copper grids coated with a thin carbon film. All imaging was performed using a JEOL JEM2100F electron microscope at an acceleration voltage of 200 kV. Micrographs were recorded on a 4k CCD camera.

RESULTS

TEM micrographs, as the one shown in figure 1, confirmed that SPION can be coupled to UCA MB, utilizing the previously described production protocol. According to the micrographs, the SPIONS were evenly distributed within the MB shell. A more in depth TEM investigation utilizing thin sections of MB, confirmed that the SPION were positioned inside the MB shell, figure 2. The size of the MB shell was determined to an average of 400 nm.

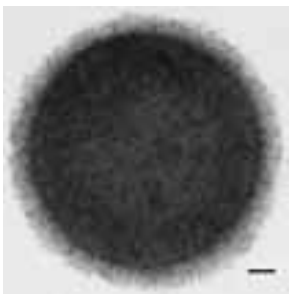


Figure 1. Transmission electron microscopy micrograph showing iron oxide nanoparticles (dark spots) coupled to a contrast agent microbubble. Scale bar represents 500 nm in length.

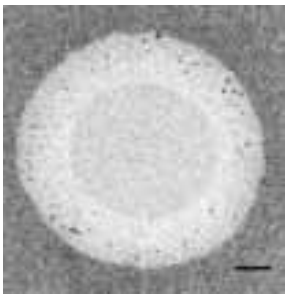


Figure 2. Cross section of a contrast agent microbubble that illustrated where the iron oxide nanoparticles (dark dots) were located in the shell. Scale bar represents 500 nm.

CONCLUSIONS

Utilizing TEM imaging, it was concluded that SPION were successfully coupled and located in the polymer shell of the MB.

REFERENCES

[1] Cavalieri, F.; El Hamassi, A.; Chiessi, E.; Paradossi, G. Langmuir 2005, 21, 8758–8764.
[2] Paradossi, G et al. Submitted paper. Biomacromolecules 2012.
[3] Dahne, L.; Egri, G., Preparation of magnetic microbubbles with PVA wall and use of them, DE
patent 10 2011 000 264.2-43, Jan 11, 2011.

BIODISTRIBUTION OF NEAR-INFRARED EMITTING CUINS2/ZNS QUANTUM DOTS BY MASS SPECTROSCOPY AND FLUORESCENCE IMAGING OF SENTINEL LYMPH NODE IN MICE

MARION HELLE[1], Thomas Pons[2], Lina Bezdetnaya[1], François Guillemin[1], Benoit Dubertret[2], Frédéric Marchal[1]
[1]CRAN, Nancy-University, CNRS, Centre Alexis Vautrin, Avenue de Bourgogne, 54511 Vandoeuvre-lès-Nancy Cedex, France
[2]Laboratoire Physique et Etude des Matériaux, CNRS UPR0005, ESPCI, 10 rue Vauquelin, 75005 Paris, France

BACKGROUND

The biopsy of the sentinel lymph nodes in breast cancer patients is now strongly recommended for the definition of the further therapeutic strategy. Although this approach has strong advantages, it has its own limitations (manipulation of radioactive products and possible anaphylactic reactions to the dye). As recently proposed, these limitations could in principle be by-passed if semiconductor nanoparticles (quantum dots or QDs) were used as fluorescent contrast agents for the in vivo imaging of sentinel lymph nodes [1]. QDs are fluorescent nanoparticles with unique optical properties like strong resistance to photobleaching, size dependent emission wavelength, large molar extinction coefficient and good quantum yield [2]. Most synthesized QDs are composed of toxic heavy metals (Cd, Te, Se,…) and as such could not be used in the clinical context. Recently, we have demonstrated excellent imaging properties of Cd-free QDs for in vivo lymph nodes detection along with the greatly diminished acute inflammation in healthy rodents [3, 4]. Far before the clinical settings could be envisaged, the study on biodistribution of CuInS2/ZnS QDs in pre-clinical models and the possibility of visualization of lymph nodes upon metastatic dissemination is mandatory.

MATERIAL AND METHODS

In vitro studies: The toxicity of CuInS2/ZnS core/shell QD on red blood cells has been tested using the haemolysis test. Red blood cells were incubated during 2h with different concentration of QDs and the release of haemoglobin was measured by spectrophotometry.
In vivo studies: Healthy or tumor-bearing mice received 20 µL of 1 µM CuInS2/ZnS core/shell QD solution subcutaneously in the right anterior paw. Right axillary lymph nodes (RALN) were visualized using a near-infrared imaging system (Fluobeam™) and healthy animals were sacrificed at different time points after QDs injection. Organs, blood and excretions were collected and their indium content

was measured by ICP-MS. The sentinel lymph node of tumor-bearing mice was imaged by fluorescence and the metastatic involvement of lymph nodes was assessed by the measurement of cytokeratin 19 by RT-qPCR or immunohistochemistry.

RESULTS AND DISCUSSION

In vitro assessed toxicity: No haemolysis of red blood cells was detected in the range of concentrations of CuInS2/ZnS QDs from 25 to 150 nM, while Cd-based QDs induced 50% of hemolysis with already 56.3 nM (data not shown).

In vivo studies: CuInS2/ZnS QDs were observed in RALN as soon as 5 min (Fig. 1) and up to 7 days after the subcutaneous injection of QD in the right anterior paw by in vivo NIR fluorescence imaging.

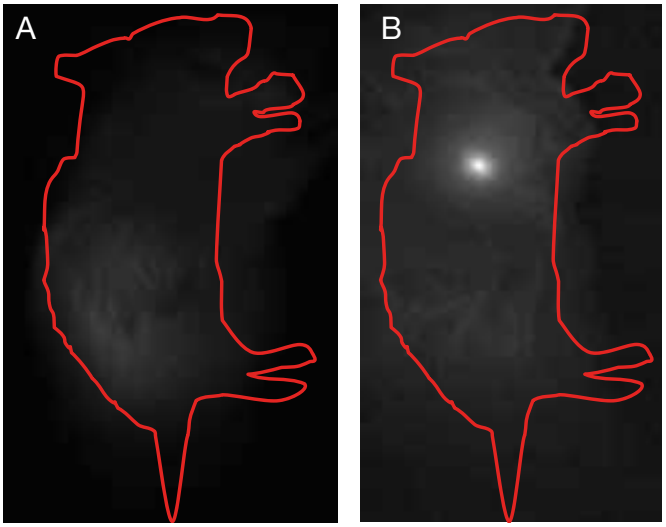


Fig 1: In vivo fluorescence imaging of mice before (A) or 5 minutes after (B) sc. injection of 20 pmol of CuInS2/ZnS QDs using Fluobeam™ system. Injection point was hidden for a better RALN visualisation. Exposure time was 100 ms for A and 10 ms for B.

The presence of QDs in the RALN was confirmed by ICP-MS analysis and a good correlation between two techniques (fluorescence and ICP-MS) was demonstrated during the first 72h after injection. At 7 days post-injection, the fluorescence decreases whereas the indium content in the RALN is constant (Fig. 2). This difference could be explained by the in situ degradation of the QDs after 3 days, causing decreased fluorescence intensity.

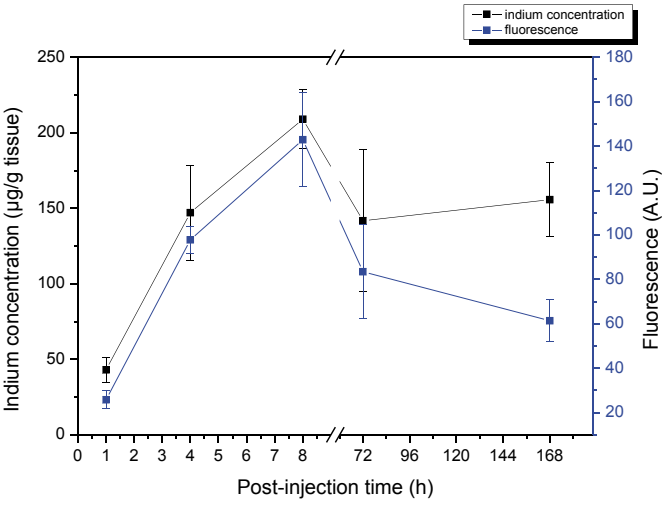


Fig 2: Correlation between indium concentration measured by ICP-MS and in vivo fluorescence signals assessed by Fluobeam™ system of RALN (n=3 per group)

We further conducted the mass-spectrometry analysis of the QDs in selected organs at different times after injection. Biodistribution study can provide the indications to the potential in vivo toxicity (Fig. 3).

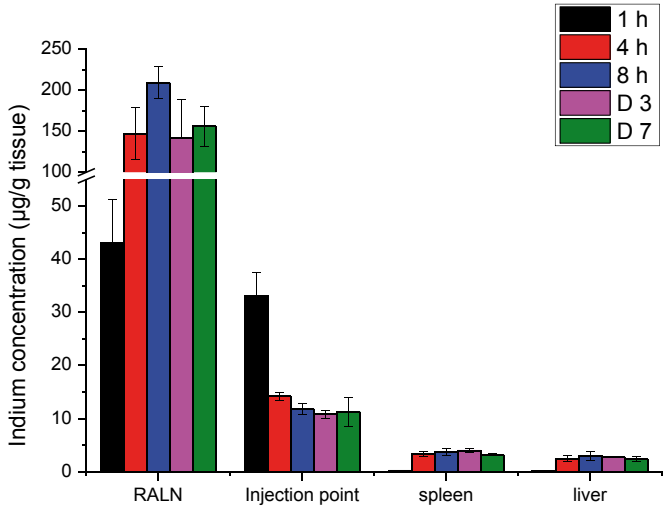


Fig 3: Tissue kinetics of indium concentrations in mice different time after s.c. administration of 20 pmol of CuInS2/ZnS QDs given by ICP–MS. Data are mean ± SD (n=3 per group).

Whereas RALN and injection point are the major organs of QD accumulation, they are also efficiently captured by the organs of the reticulo-endothelial system (liver and spleen). This way of accumulation points out to a possible hepatobiliary excretion rather than renal one. Indeed, we observed a cumulative excretion of 3% and 0.4% of injected dose in faeces and urine respectively at 96h after QDs administration (data not shown). Accumulation of QDs in both RALN and injection point is of minor concern since these organs can be removed during the first hour surgery in the operation theatre. In the next step, we attempted to visualise SLN with In-based QDs in a murine metastatic model of breast cancer. The reference marker of metastatic cells is the cytokeratin 19, which is found in epithelial cancerous cells and can be detected by immunohistochemistry (Table 1) and quantitative RT-PCR.

Table 1: Results of CK19 immunohistochemistry (IHC) and corresponding in vivo fluorescence intensity for 41 lymph nodes from tumor-bearing mice. Numbers in brackets indicate the number of node affected. NT: non tumoral, ITC: isolated tumor cells

	CK 19 IHC	*In vivo* RALN fluorescence (U.A.)
NT	68.29 % (28 / 41)	16.86 ± 7.51
ITC	21.95 % (9 / 41)	14.63 ± 4.28
micrometastasis	4.88 % (2 / 41)	25.60
macrometastasis	4.88 % (2 / 41)	32.39

Comparable to clinical studies, the metastatic murine model induces 22% of isolated tumor cells, 5% of micrometastasis and 5% of macrometastasis. The presence of metastasis in lymphatic vessels does not prevent the visualization of QDs in the sentinel lymph node in tumor-bearing mice (Table 1). Moreover, as follows from the Table 1, the fluorescence of QDs is not altered by the metastatic involvement of lymph node since the same signal was detected between positive and negative nodes.

CONCLUSION

Effective and rapid detection of SLN using fluorescence imaging of NIR emitting CuInS2/ZnS QDs in living animals was demonstrated in healthy mice and in a model of metastatic breast cancer. The surgery of the breast cancer, which has to take place shortly after QDs injection, will eliminate the main part (85 %) of injected nanoparticles. Nevertheless, further improvement of their surface chemistry is required to allow their excretion, and therefore, to eliminate any risk of toxicity.

REFERENCES

1. Khullar O, Frangioni JV, Grinstaff M, Colson YL: Image-guided sentinel lymph node mapping and nanotechnology-based nodal

treatment in lung cancer using invisible near-infrared fluorescent light. Semin Thorac Cardiovasc Surg 2009, 21(4):309-315.
2. Smith AM, Gao X, Nie S: Quantum dot nanocrystals for in vivo molecular and cellular imaging. Photochem Photobiol 2004, 80(3):377-385.
3. Pons T, Pic E, Lequeux N, Cassette E, Bezdetnaya L, Guillemin F, Marchal F, Dubertret B: Cadmium-free CuInS2/ZnS quantum dots for sentinel lymph node imaging with reduced toxicity. ACS Nano, 4(5):2531-2538.
4. Cassette E, Pons T, Bouet C, Helle M, Bezdetnaya L, Marchal F, Dubertret B: Synthesis and Characterization of Near-Infrared Cu–In–Se/ZnS Core/Shell Quantum Dots for In vivo Imaging. Chem Mater 2010, 22(22):6117–6124.

NANODENTISTRY

SIMONE E. HIEBER and B. Müller
Biomaterials Science Center, University of Basel, Switzerland

'Nanodentistry' is defined as the science and technology of diagnosing, treating and preventing oral and dental disease, relieving pain, and of preserving and improving dental health, using nanoscale-structured materials [1]. The nanotechnology generally considers entities between 1 and 100 nm leading to properties and functionalities of materials that fundamentally differ from what is known from larger scales. The surface of the nanoparticles dominates the materials properties, which are usually given by the bulk.
The common biomaterials are ceramics, metals, and polymers or any kind of combination. Nanoscale patterns on the surfaces and within the volume of the materials accomplish the dedicated functionalities. The fundamental knowledge of the human tissues on the nanometer scale is required to develop innovative and efficient technologies for patients. Imaging techniques to characterize nanomaterials include micro-tomography, electron microscopy, scanning probe microscopy, X-ray scattering and diffraction methods.
Figure 1 shows what phenomena can influence the biocompatibility. For a dental titanium implant the surface is made rough down to the molecular level by sandblasting and etching procedures to ensure osseointegration and to reduce the inflammatory reactions [2]. Surface morphology can offer certain angles for dedicated protein absorption and activity. In addition, the surface chemistry is commonly tailored concerning oxide thickness, stoichiometry and normally functionalized to obtain a hydrophilic surface. Further relevant factors are particle and ion release, surface charges, electrical conductivity and anisotropies as present on patterned surface micro- and nanostructures.

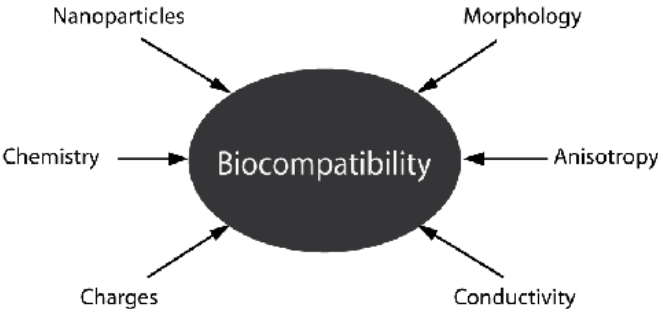


Fig. 1: Several parameters allow tailoring the biocompatibility of dental implants.
Filling materials for reconstructions, dental root implants, bone augmentation and dentin re-mineralization take advantage of nanotechnology already today, but still have increasing growth potential. Today's dental materials are expected to be replaced by nature-analogue, anisotropic tooth restorations. The nanostructures in dentin are orthogonally oriented to the ones of the same size in the enamel [3]. Bone augmentation performed with calcium phosphate phases gains more and more importance along with the increase in age of the population. The absorbable calcium phosphate phases or bioglasses support the growth of the natural bone being applied to larger defects. The materials have to be optimized on the micro- and nanometer scales to tweak the biocompability, the bioactivity and the osseoconductivity promoting tissue regeneration and resisting the mechanical loads. The micro- and nanostructured surfaces of tooth implants guarantee the osseointegration. Nanoparticles are already used in 'sensitive' toothpastes and will enable the re-mineralization of damaged teeth [4].
Nanotechnology has started to a new era of dental medicine that will change the current methods in diagnosis, treatment and prevention of the different patients. As medicine advances and people live longer, nanodentistry will play an increasing role in enabling people to keep their natural teeth and oral tissues healthy and functioning. The scientists will understand in detail how the teeth grow, develop and heal. The medical experts will understand the assembly of nanostructures in dentin and enamel to enable the development of biomimetic tooth repair and regeneration. Dentists will be able to reconstruct hard and soft periodontal tissues as well as to treat caries including biomimetic re-mineralization and repair of diseased teeth.

[1] H. Dosch, M. van de Voorde (Eds.) (2009) Gennesys White Paper: A new European partnership between nanomaterials science and nanotechnology and synchrotron radiation and neutron facilities. Max-Planck-Institut für Metallforschung, Stuttgart
[2] B. Müller Natural formation of nanostructures: from fundamentals in metal heteroepitaxy to applications in optics and biomaterials sciences Surf Rev Lett 2001;8:169-228.
[3] H. Deyhle et al. (2009). Bio-inspired dental fillings Proc. SPIE 7401:74010E
[4] F. Kernen et al. (2008). Synchrotron radiation-based micro computed tomography in the assessment of dentin de- and re-mineralization Proc. SPIE 7078:70780M

SHEAR STRESS SENSITIVE NANOCONTAINERS FOR TARGETED DRUG DELIVERY

MARGARET HOLME[1,2,3], B. Müller[1], A. Zumbuehl[2], T. Saxer[3]
[1]Biomaterials Science Center, c/o University Hospital Basel,
[2]Department of Chemistry, University of Geneva, Quai Ernest Ansermet 30, 1211 Geneva
[3]University Hospitals of Geneva, Rue Gabrielle-Perret-Gentil 4, 1211 Geneva

INTRODUCTION

Heart attack is the leading global cause of disease and mortality [1]. Ambulatory treatment includes the intravenous administration of vasodilators such as nitroglycerin to restore coronary blood flow and prevent further myocardial ischemia with ensuing arrhythmias and death. Unfortunately, systemic action of these drugs leads to complications such as vasodilation inducing severe hypotension and diminished blood perfusion of the suffering heart.

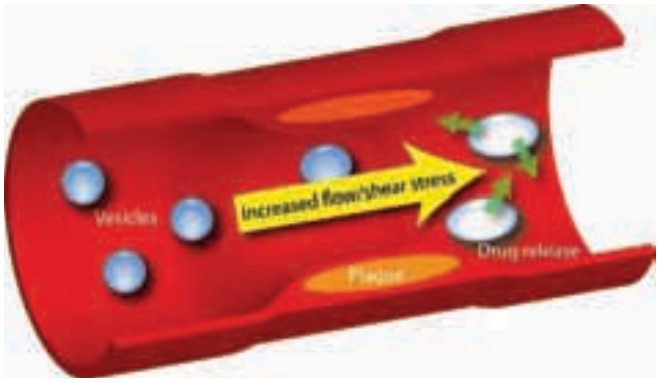


Figure 1: Principle of shear stress triggered drug release at the site of atherosclerotic plaques from vesicles circulating in the blood stream. Here, we show a fresh approach for targeted drug delivery; elevated shear stress, similar to that found in critically constricted coronary arteries, acts as a localized physical trigger for the release of vesicle-encapsulated drugs (Figure 1). In in vitro fluorescence release

studies, we investigated the properties of vesicles formulated from certain mixtures of Egg-PC and either the artificial phospholipid Pad-PC-Pad[2], or the surfactant Brij S10. The results show that the shear-induced release properties of vesicles can be tuned to allow for preferential release of drugs in regions of high shear stress.

METHODS

In order to assess the feasibility of drug release from a vesicle through shearing, vesicles of varying formulations were subjected to in vitro shear stresses found in physiological hemodynamic flow conditions. 50 mM 5(6)-carboxyfluorescein encapsulated LUVET100 vesicles were prepared by the thin film method [3]. Large uni-lamellar vesicles extruded through 100 nm polycarbonate filters (LUVET100) were prepared from 30 µmol Egg-PC with Brij S10 added to the lipid mixture in varying concentrations of 0 to 1 mol% in increments of 0.1 mol%. Vesicles with varying compositions (10, 25, 50, 75 and 100 mol%) of Pad-PC-Pad and Egg-PC were prepared by the same thin film method.

To simulate the physical conditions in the heart a model cardiovascular system was used, with vesicles pumped through either a common or constricted model artery constructed from PMMA (Elastrat Sàrl, Switzerland). Both artery models were formed from tubes with an inlet diameter of 2.5 mm, one with constrictions of up to 95% cross sectional area along a 2.5 cm segment. An extracorporeal circulation (ECC) pump (Medtronic Bio-Pump, Bio Console 540, Medtronic, Switzerland) with low intrinsic shear stress simulated the heart [4, 5].

RESULTS

1. Brij S10

Optimal Brij S10 concentration occurs where there the greatest difference between the fluorescence release of the common and constricted arteries is detected. This was observed at a concentration of between 0.5 and 0.6 mol%. After 40 arterial passes, the common model induced an additional release of 5% of the encapsulated dye, whereas the constricted model showed a 15% additional release. A Brij S10 concentration of 1% intrinsically released more encapsulated dye than pure Egg-PC vesicles, but at this concentration there was no significant difference in release between the constricted and common arteries.

2. Pad-PC-Pad

Compared with Egg-PC/Brij S10 formulations, vesicles containing only Pad-PC-Pad showed a much greater sensitivity between common and constricted artery models. They exhibited low intrinsic leakage but, after only the one pass through the artery model, half of the internal contents were released in the common model compared with 70% in the constricted model.

Mixtures of Pad-PC-Pad and Egg-PC were found to become intrinsically less stable with increasing concentration of Pad-PC-Pad. However, they did not exhibit a selective release of encapsulated dye in the constricted model.

CONCLUSIONS AND OUTLOOK

Egg-PC vesicles incorporating 0.5 to 0.6 mol% of Brij S10 have an increased sensitivity to shear stresses in long circulating systems, and could be tuned towards preferential release in constricted arteries. These findings build on studies performed by Bernard et al., who found that at shear rates of 10,000 s-1 there was a release of contents from Egg-PC vesicles containing 0.1% and 1% Brij S10 [6].

Vesicles formulated exclusively from Pad-PC-Pad are susceptible to shear-induced release. After only one pass, they showed an appreciable preference for release in the constricted artery. Such a result was not observed in the Egg-PC containing formulations, which became unstable, or 'leaky', with an increase in Pad-PC-Pad, but did not show preferential shear-induced release in the constricted artery. This Pad-PC-Pad formulation shows great potential for preferential shear-induced release of heart attack drugs near arterial stenoses in the first pass through the blood stream.

Further studies are ongoing to quantify the release characteristics of different formulations. We predict that these observations will act as a springboard for the development of liposomal formulations that exhibit specificity in drug delivery at elevated shear stresses, for example for cardiac, neurologic or angiologic applications. This targeted drug delivery using nano-containers is a powerful example in the field of nano-medicine.

ACKNOWLEDGEMENTS

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REFERENCES

1. Lopez, A.D., et al., Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. Lancet, 2006. 367(9524): p. 1747-1757.
2. Fedotenko, I.A., et al., The synthesis of 1,3-diamidophospholipids. Tetrahedron Letters, 2010. 51(41): p. 5382-5384.
3. Olson, F., et al., Preparation of liposomes of defined size distribution by extrusion through polycarbonate membranes. Biochimica et Biophysica Acta (BBA) - Biomembranes, 1979. 557(1): p. 9-23.
4. Curtis, J.J., et al., Centrifugal pumps: description of devices and surgical techniques. The Annals of Thoracic Surgery, 1999. 68(2): p. 666-671.
5. Paparella, D., et al., Blood damage related to cardiopulmonary bypass: in vivo and in vitro comparison of two different centrifugal pumps. ASAIO Journal (American Society for Artificial Internal Organs: 1992), 2004. 50(5): p. 473-478.
6. Bernard, A.L., et al., Shear-induced permeation and fusion of lipid vesicles. Journal of Colloid and Interface Science, 2005. 287(1): p. 298-306.

MRI IMAGING OF SENTINAL LYMPH NODES USING DIFFERENT SIZED SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES

RENE IN 'T ZANDT[1,3], Pontus Kjellman[1,2], Fredrik Olsson[1], Sarah Fredriksson[1], Sven-Erik Strand[2]

[1]Genovis AB, Lund, Sweden

[2]Medical Radiation Physics IKVL, Lund University, Lund, Sweden

[3]Lund University BioImaging Center, Lund University, Lund, Sweden

BACKGROUND

The metastatic spread, in breast cancer and malignant melanoma, mostly occurs through the lymphatic system. Histological examination of the first lymph node draining the tumor into (the sentinel lymph node, SLN) reveals if the tumor has metastasized [1]. The vision of this project is to be able to target and image the SLN of patients with breast cancer or malignant melanoma in order to facilitate the surgical procedure. Multimodal nanoparticles will be used that by design have a retention time in the SLN over 24 hours allowing for diagnostic imaging prior to surgery. When constructing nanoparticles for this purpose it is important to consider choice of material, surface charge, size, colloidal stability and biological compatibility. In this study, superparamagnetic iron oxide nanoparticles (SPIONs) of various sizes and similar charge labeled with fluorescent dye was used to target and image the SLN in rats using MR imaging. In this preclinical model, the SLN is the popliteal node while the iliac node can be considered to be the 2nd lymph node in the lymphatic system downstream from the hind paw with injected SPIONs.

MATERIALS AND METHODS

Two different sized SPIONs (27 and 67 nm) were constructed by coating 11 nm iron oxide cores with polyethylene glycol of varying molecular weight. The SPIONs were labeled with a fluorescent dye, DY-647, to enable detection in histology sections. The dynamics of the SPIONs were evaluated in vivo by a subcutaneous injection in the right hind paws of rats (female Wistar, weight 200-250 gram, concentration SPION 2.75 mg Fe/ml, injection volume 100 µl). The retention of the particles in the lymphatic system was then visualized in vivo, 24 hours post injection, for the popliteal (SLN) and the iliac lymph nodes. These lymph nodes were visualized by the acquisition of a 3D volume using MR imaging operating at 2.4T (Bruker Avance II system, 3D gradient echo, TE 6 ms, TR 27 ms). It is anticipated that higher levels of SPIONs will lead to larger susceptibility effects (higher T2-relaxivity) and as such to more signal loss in the MR im-

ages. For optimal detection of the lymph nodes, a 3D volume with and one 3D volume without lipid suppression were acquired. Animals were sacrificed after the MRI measurement and the popliteal and iliac lymph nodes were removed for histological analysis.

RESULTS

The T2-relaxivity of both particles showed to be similar dissolved in water (data not shown). The MRI results showed apparent differences in the build-up of the various SPIONs in the SLN 24 hours post injection (figure 1). Also a clear difference in the amount of particles was observed when comparing the lymph nodes downstream of the SLN. When examining tissue sections, of the lymph nodes, using fluorescence microscopy the particles were located in endosomes inside the cells whereas the distribution of the particles reflected a similar distribution as has been observed in the MR images (figure 2).

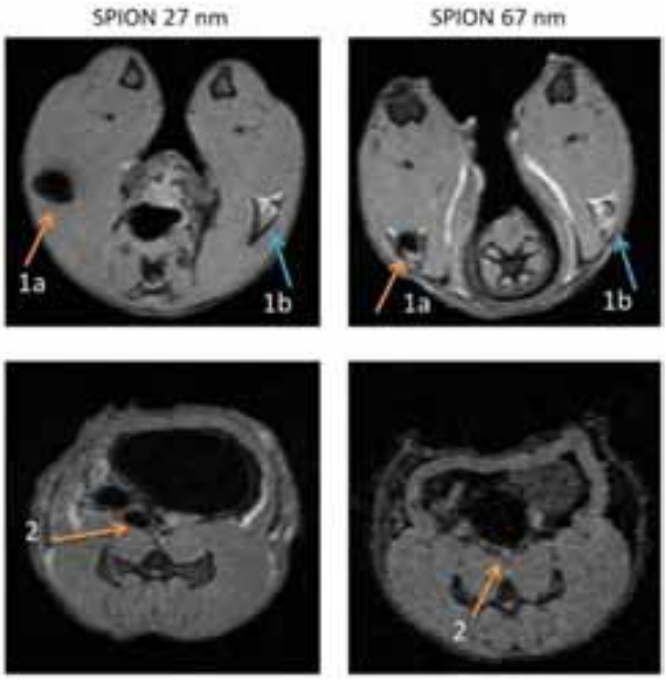


Figure 1: In vivo MR images of the popliteal nodes (1) which are the sentinel nodes after injection in the paw. At the control side (1b) no SPIONs are detected while at the injected side (1a) the presence of SPIONs leads to clear signal void in the MR images. Interestingly, the 27 nm particles seem to be present at a higher level than the 67 nm particles despite a higher relaxivity value for the large particles (data not shown). The 27 nm particles are clearly detected in the iliac node (2) while no particles can be seen in the iliac node after MR imaging when 67 nm particles were injected.

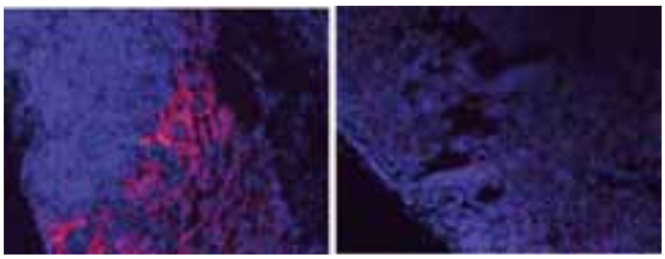


Figure 2: Histology section of two popliteal (SLN) nodes. The representative sections show the nuclei (blue) and the functional group dye DY-647 coupled to the nanoparticles (red) after the 27 nm injection (left) and the 67 nm injection (right).

CONCLUSION

Particle size plays an important role in the retention in the SLN. This facilitates the design of SPIONs that are optimized to target the sentinel lymph node (SLN) in patients with breast cancer or malignant melanoma. More detailed studies will focus on the dynamics of SPION transport from the hind paw into the SLN.

REFERENCE

1 The concept of sentinel node localization: how it started, Morton DL, Chan AD, Semin Nucle Med. 2000, 30(1), 4-10

OPTIMIZATION OF NUCLEIC ACID-BASED DRUGS FOR PERSONALIZED CANCER TREATMENT

ALEKSANDRA JASKOT [1,2], Steffen Schmidt [1,2], Helle Christiansen [1,2], Markus List [1,2], Ines Block [1,2], Marlene Lemvig Petersen [1,2], Jakub Trojnar [1,2], Pernille Lund Hansen [1,2], Angela Riedel [1,2], Carolin Müller [1,2], Antje Sucker [3], Dirk Schadendorf [3], Jan Mollenhauer [1,2]
[1]Lundbeckfonden Center of Excellence in Nanomedicine NanoCAN, University of Southern Denmark, Odense, Denmark
[2]Molecular Oncology, University of Southern Denmark, Odense, Denmark
[3]Department for Dermatology, Venerology und Allergology, University Hospital Essen, Essen, Germany

Synthetic lethality refers to situation, in which a drug causes cell death only in the presence of a cancer-specific mutation, whereas either the mutation or the drug alone does not provide a killing effect. The advantage of personalized cancer treatments based on synthetic lethality is that they would be predicted to have minimal toxicity, because only cells with the specific molecular alterations, i.e. the cancer cells would be affected. From a previous approach, we recovered a potential synthetic lethal drug target in cancer cells. We use this as a test case to derive initial routines for the optimization of siRNA design and for testing of siRNA delivery to cancer cells and cancer stem cells.
Here, we present initial data on the activity and selectivity of siRNAs with different designs. As a baseline, various non-targeting siRNAs exerted substantial unspecific toxicity, which was improved by siRNA modification. Only about 50% of the siRNAs designed to target the synthetic lethal gene exerted appreciable effects, pointing to still existing drawbacks in search algorithms. Further, when comparing different cell lines, the baseline susceptibility to unspecific siRNA toxicity is variable, which requires consideration.

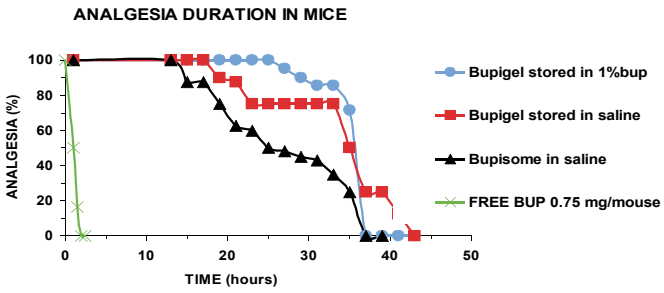
EXTENDING THE DURATION OF ANALGESIA FROM THE LOCAL ANESTHETIC BUPIVACAINE BY DELIVERY VIA THE LIPOSOME SYSTEM BUPISOME OR BY THE BUPISOME ENCAPSULATED IN HYDRO GELS (BUPIGEL)

HIBA KANAAN, Yechezkel Barenholz and Rivka Cohen
Laboratory of Membrane and Liposome Research, Hebrew University – Hadassah Medical School, Jerusalem, Israel

Long-acting local anesthetic formulations hold great promise for the management of acute pain after surgery or trauma. Slow drug release from liposomes would reduce the potential toxicity, allowing greater dosage and longer analgesia duration. Studies by Grant and colleagues showed promise for liposomal bupivacaine formulations (1), but they were not stable enough for clinical use. We aim to overcome this deficiency without compromising the efficacy achieved before.
In vitro, we studied drug leakage during storage at 4C° and release kinetics at 37C° to stimulate in vivo performance from large multivesicular vesicles (LMVV) remote loaded by an ammonium sulfate gradient to achieve a high (>1.0) drug -to -lipid mole ratio. The use of LMVV is crucial for obtaining the controlled slow drug release at 37C°. The first lipid composition used was the high Tm (53°C) hydrogenated soy phosphatidylcholine, HSPC, and cholesterol at 6:4 mole ratio (1), resulting in liquid ordered lipid bilayer. However,

while the 37C° release rate and biological efficacy were good, stability was poor. Studies to optimize lipid composition showed that HSPC: N- palmitoyl sphingomyelin: cholesterol (30: 30: 40 mole ratio) was the best. LMVV have to be prepared under iso-osmotic conditions. A further improvement was obtained by encapsulating LMVV into a Ca-alginate cross-linked hydro gel (Bupigel). In addition, Bupigel was stored in 0.5% - 2%- aqueous "free" bupivacaine to prevent leakage of the drug and increase storage life at 4C°. Analgesia duration was assessed using the pain model of Swiss Webster male mice (2).

All Bupisome and Bupigel formulations tested showed much superior and prolonged drug activity than free bupivacaine, with Bupigel being superior to Bupisome (the figure below). An additional advantage of Bupigel is that before injection the free bupivacaine can be easily removed, minimizing the amount of free drug injected. Further experiments are underway to determine the toxological outcomes of these formulations.



Taken from : Cohen et al. 2012, journal of control release " Prolonged anaglesia from Bupisome and Bupigel Formulations : From design and fabrication to improved stability", submitted

1. Grant et al. 2004, Anesthesiology; 101: 133-7. ; 2.Grant et al.2001, Pharmaceutical Research18: 336-343.

DESIGN AND CHARACTERIZATION OF CETUXIMAB IMMUNONANOPARTICLES PREPARED USING A NOVEL LINKER MOLECULE

NOUR KARRA[1], Taher Nassar[1], Juergen Borlak[2] and Simon Benita[1]

[1]The Institute for Drug Research of the School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem Israel

[2]Center of Pharmacology and Toxicology, Medical School of Hannover, Hannover Germany

INTRODUCTION

Cancer is the second leading cause of death worldwide. Yet, despite the significant progress in cancer research and the wide versatility of potent available drugs, treatment efficacy is still hurdled and often failed by the lack of pharmaco-selectivity to diseased cells [1]. Ligand targeted nanocarriers have become a widely investigated approach for tumor selective therapies and are perceived as a potential tool to overcome treatment limitations, primarily indiscriminate drug distribution, low drug concentration at the tumor site and systemic toxicities. The conjugation of nanoparticles (NPs) to targeting ligands promotes specific binding and internalization of the carrier cargo to cancer cells [2]. In this regard, polymeric nanoparticles such as PLGA NPs are of special interest due to their tunable biodegradability and favorable biocompatiblity. Unfortunately, an important drawback with PLGA NPs is the limited types of functional groups on their surface that are amenable for covalent conjugation to targeting ligands. An efficient ligand conjugation technique might therefore be of crucial importance in the formation and development of successful targeted drug delivery systems. Thus, we have synthesized an original amphiphilic molecule, Oleyl cysteineamide, which serves as a linker for the conjugation of targeting ligands at the surface of NPs. In the present study, we aimed to design and evaluate a delivery system to target the epidermal growth factor receptor (EGFR), over-expressed in many solid tumors.

METHODS

Paclitaxel palmitate loaded PLGA NPs were prepared by the interfacial deposition method [3]. Oleyl cysteineamide linker was synthesized and anchored at the interface of NPs to produce thiol surface activated NPs. Cetuximab (Erbitux®), an anti-EGFR monoclonal antibody was then covalently conjugated to NPs via a maleimide moiety, yielding stable thioether bonds and cetuximab immunonanoparticles (INPs). TEM, Cryo-TEM and AFM technologies were used for morphology and surface evaluation. Size and surface charges were measured by Malvern's zetasizer. The drug content was determined using an HPLC-UV method (at 227 nm). Cell culture studies were performed in the A549 human lung adenocarcinoma cell line overexpressing EGFR. The binding of INPs to cells was evaluated by flow cytometry. Rituximab (Mabthera®) INPs were prepared in the same technique and served as an isotype negative control. For cellular uptake studies, coumarin-6 loaded NPs and INPs were prepared and incubated with cells at different concentrations. The internalization of NPs and INPs to the cells was then evaluated by confocal microscopy.

RESULTS

1. Physicochemical characterization of NPs and cetuximab INPs

Blank and drug loaded cetuximab immunonanoparticles were prepared with the oleyl cysteineamide linker. This approach enabled antibody conjugation efficiency rates above 80%. Cetuximab immunonanoparticles with a mean size of 80 nm, spherical shape and homogenous size distribution were achieved, as represented by Figure 1. NPs and INPs presented a zeta potential of -40 mV and a drug content of 8%w/w.

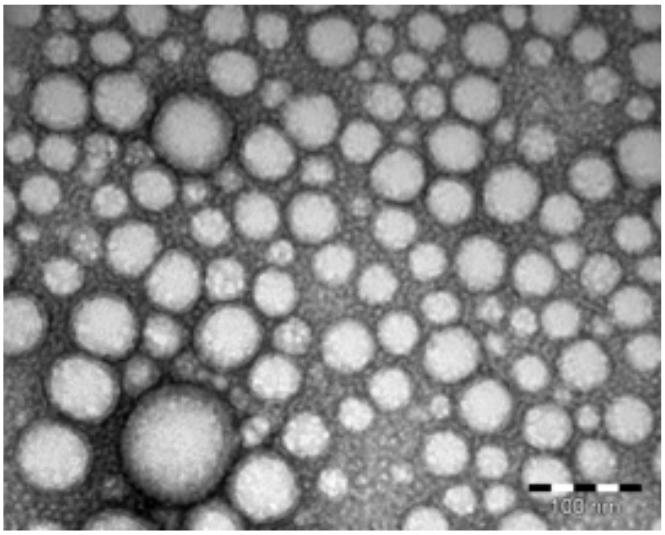


Figure 1: Transmission electron microscopy (TEM) of cetuximab INPs with uranyl actetae negative staining.

2. Cell culture studies in A549 cell line

Next we aimed to evaluate whether the conjugation of cetuximab to NPs via oleyl cysteineamide affects the ability of the monoclonal antibody to recognize and bind to the EGF receptor. Indeed, flow cytometry results showed that cetuximab covalently conjugated to NPs preserved its binding ability to EGFR positive A549 lung cancer cells, as compared to the native free antibody. Isotype matched negative control of rituximab INPs showed no binding to cells, thus confirming the binding specificity of cetuximab INPs to EGFR.

Confocal microscopy results also demonstrated the preferable intracellular uptake of cetuximab immunonanoparticles as compared to blank NPs following 4h incubation with cells. Indeed, in all tested concentrations, cetuximab INPs were internalized significantly more than the non-targeted control NPs, as represented by the strong cytoplasmic fluorescent staining in Figure 2.

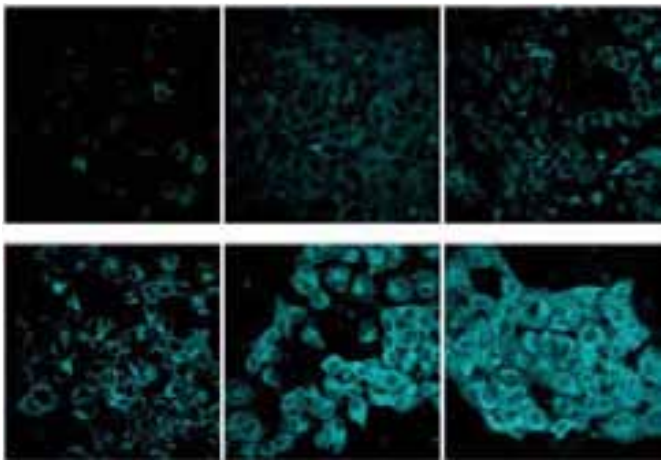


Figure 2: Cetuximab immunonanoparticles uptake in A549 cell line. Different concentrations (1:500, 1:200 and 1:100) of coumarin-6 loaded NPs (A) and cetuximab INPs (B) were incubated with A549 cells for four hours. Cetuximab INPs exhibited significantly higher intracellular uptake than the non targeted NPs.

CONCLUSIONS

Our results demonstrate that the amphiphilic linker molecule oleyl cysteineamide may provide a potential tool for the covalent attachment of targeting ligands, specifically monoclonal antibodies, to PLGA NPs in a reproducible and high efficiency conjugation method. Importantly, the resulting targeted NPs preserved their specific binding ability to EGFR and enhanced selective internalization to cancer cells. Overall results indicate the potential of this promising platform for the preparation of drug loaded targeted NPs for improved cancer treatment.

REFERENCES

[1] Karra N, Benita S.(2011) The Ligand Nanoparticle Conjugation Approach for Targeted Cancer Therapy. Curr Drug Metab. (Epub ahead of print, PMID: 21892918).

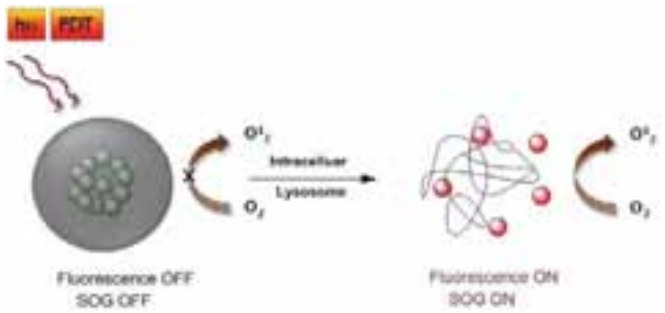
[2] Kirpotin DB, Drummond DC, Shao Y, Shalaby MR, Hong K et al.(2006) Antibody targeting of long-circulating lipidic nanoparticles does not increase tumor localization but does increase internalization in animal models. Cancer Res 66(13), 6732-6740.

[3] Fessi H, Puisieux F, Devissaguet JP, Ammoury N, Benita S.(1989) Nanocapsule formation by interfacial polymer deposition following solvent displacement. Int J Pharm 55, R1-R4.

PHOTOSENSITIZER-CONJUGATED POLYMERIC NANOPARTICLES FOR ACTIVATABLE FLUORESCENCE IMAGING AND PHOTODYNAMIC THERAPY OF CANCERS

HYUNJIN KIM, Yongdoo Choi

In this study, we developed activatable photodynamic therapy (PDT) agent by conjugating second generation photosensitizer chlorin e6 (Ce6) with hyaluronic acid (HA) through GSH-cleavable disulfide linker. The GSH-cleavable Ce6-HA conjugate forms nanoparticles with 254±51.5 nm diameters. Fluorescence and singlet oxygen generation of Ce6 were quenched after forming nanoparticles. We expected that this smart PDT agent can be accumulated into tumor tissues by EPR effect, and then its fluorescence and phototoxicity will be activated after entering cancer cells in the tumor tissues. When DTT at different concentration was added to Ce6-HA nanoparticle solution, fluorescence and singlet oxygen generation were increased depending on the concentration of DTT added. In particular, singlet oxygen generation was increased about 5 times after treating the Ce6-HA nanoparticles with 5 mM DTT while no increase in fluorescence and singlet oxygen generation were observed in the 5 μM DTT-treated sample. In vitro cell studies showed potential utility of this activatable PDT agent as a biocompatible optical theranostics for near-infrared fluorescence imaging and subsequent PDT of cancers.



Key words: polymeric nanoparticles, near-infrared fluorescence imaging, thiol reducing agent, photodynamic therapy, cancer

PROJECT NAPTIS: ANTIBACTERIAL ACTIVE COPPER DEPOSITED ON THE SURFACE OF TITANIUM IMPLANTS

JOACHIM KOESER[a], N. Ryter[a], W. Hoffmann[a,b], L. Straumann[a], N. Balimann[a], F. Meier[a], M. de Wild[a], F. Schlottig[c], I. Martin[b], U. Pieles[a] and C. Jung[d]

[a]School of Life Sciences, University of Applied Sciences and Arts Northwestern Switzerland, Muttenz, Switzerland

[b]Department Biomedicine, University Hospital Basel, Basel, CH

[c]Thommen Medical AG, Waldenburg, Switzerland

[d]KKS Ultraschall AG, Medical Life Center, Steinen, Switzerland

INTRODUCTION

Implant associated bacterial infections are a major cause for necessary revisions [1] with estimates in the range of 10% for dental implants [2]. Furnishing the implant surface with depots of antimicrobial active compounds is an attractive approach to tackle this problem. Here we present a method to deposit antimicrobial active copper on the surface of rough titanium implants and report on the release kinetic of active copper ions from such surfaces.

METHODS

Titanium samples were anodized according to the spark-assisted anodizing (SAA) method [3] to produce a rough and fine-porous surface which assists osseointegration. Copper was electrochemically deposited using proprietary electrolytes and process parameters. Fig. 1 A gives an overview of the surface of such modified titanium samples with particulate copper deposits highlighted in green.

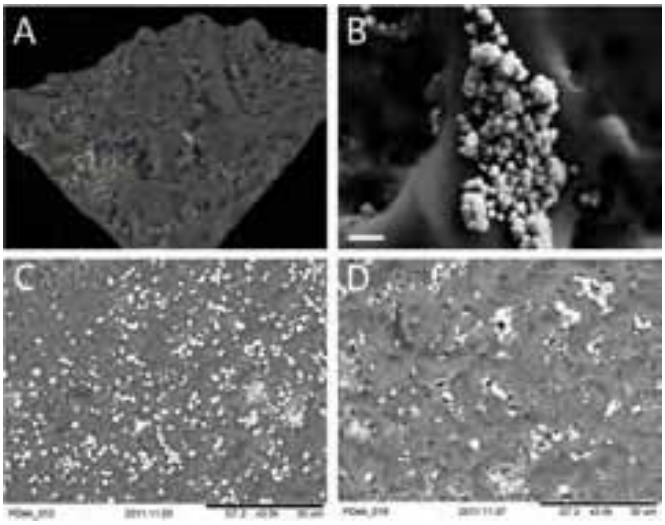


Fig. 1: Electron microscopic characterization of titanium surfaces with copper deposits. A) Overview picture at 2500x magnification; B) high resolution image of an copper particle agglomerate (scale bar: 1 μm); C) and D) show a variation of the copper deposition pattern depending on the process parameters.

Copper is present on the roughened titanium surface in the form of nano- to micrometer sized particles. The amount and deposition pattern of these copper deposits can be controlled by the process

parameters thus allowing the implant specific adaptation of the incorporated antimicrobial agent (see also Fig. 2).
To demonstrate both efficacy and safety of our approach we subsequently analyzed the release kinetics of copper ions in defined amounts of simulated body fluid and compared it to the concentrations needed to inhibit the growth of S. aureus bacteria or MG-63 osteosarcoma cells. Fig. 2 gives the release profile of copper from 5 different processed titanium samples. It can be estimated that copper is still released in considerable amounts after a simulated time of approximately half a year.

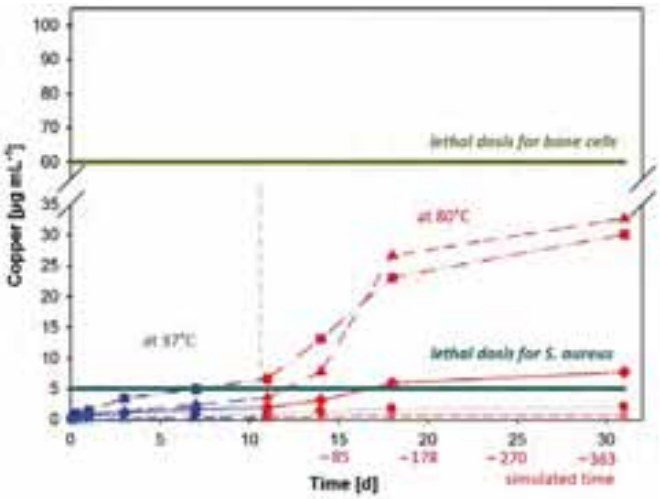


Fig. 2: Time course of copper release from different processed titanium samples in simulated body fluid at 37°C. After 10 days the temperature was increased to 80°C to simulate long-term effects [4]. The horizontal lines indicate lethal doses which have been determined in independent experiments.

CONCLUSION AND OUTLOOK

Here we report the successful deposition of antimicrobial active copper on spark-assisted anodized titanium samples. Furthermore this process allows us to tailor the amount and distribution of the copper deposits. In addition, we have demonstrated the potential long-term release of antimicrobial active copper ions in vitro. Future developments will focus on animal models for the determination of in vivo copper release rates and osseointegration as well as the adaptation of the copper deposition process to different types of titanium implants.

REFERENCES

[1] Hojo, K., Nagaoka, S., Ohshima, T., Maeda, N. (2009) Bacterial Interactions in Dental Biofilm Development, J. Dent. Res. 88: 982-990. [2] Straumann, F., Burkhardt, P. (2010) Zahnimplantate: eine tickende Zeitbombe. Sonntagszeitung 11: 44. [3] C. Jung (2010) European Cells and Materials, 19 (Suppl 2):4. [4] ASTM F-1980-02.

ACKNOWLEDGEMENTS

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PROJECT ONADA: OPTIMIZED NANOPARTICLES FOR DENTAL APPLICATIONS

JOACHIM KOESER[a], S. Goedecker[b], E. Meyer[b], S. Schweizer[c], M. Sicher[b] and M. Wasem[b]
[a]School of Life Sciences, University of Applied Sciences and Arts Northwestern Switzerland, 4132 Muttenz, Switzerland
[b]Department of Physics, Basel University, Klingelbergstrasse 82, 4056 Basel, Switzerland
[c]GABA International AG, Grabetsmattweg, 4106 Therwil, CH

INTRODUCTION

The use of fluoride containing dental care products has a beneficial effect on the reduction of caries progression1. On the enamel surface of teeth treated with an excess of soluble fluoride ions, calcium fluoride particles are formed which serve as a reservoir for fluoride in the time interval between the applications2. The project ONADA investigates the formation of calcium fluoride nanoparticles by a combination of theoretical and experimental methods. The aim is to better understand the formation of these particles and to use the gained knowledge to generate calcium fluoride particles with optimized dental adhesion and fluoride release rates for dental care applications.

METHODS

CaF2 particles are synthesized by mixing of precursor solutions with soluble calcium and fluoride salts in different ratios. The particle morphologies are analysed by scanning electron microscopy. DFT and forcefield-based global optimization runs are applied to determine the surface energies of different CaF2 assemblies. For the determination of particle adhesion forces on surfaces AFM based studies are performed.

RESULTS

When assembled on the enamel surface of teeth calcium fluoride particles exhibit globular shapes which contrasts to the reported cubic appearance of CaF2 particles formed in situ. Within the presented project we were able to generate in situ defined CaF2 particles resembling a variety of different shapes by tuning the mixing ratio of soluble calcium and fluoride ions. Particles ranging from approximately 50 nm to several μm diameter can be produced. Their morphologies range from perfectly cubic to more octahedral and spherical (examples see Fig. 1).

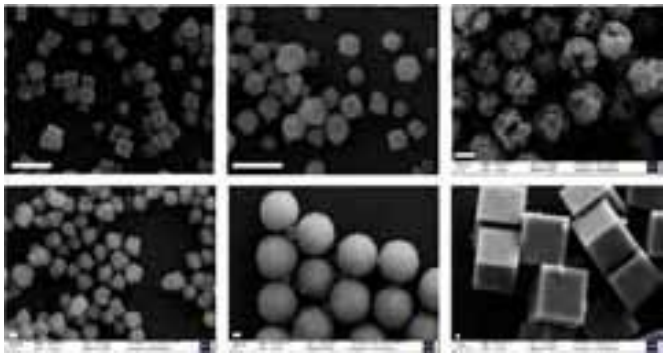


Fig. 1: SEM gallery of CaF2 particles obtained by variation of the parent salt concentrations.
NaF and CaCl2 salt solutions were mixed and the resulting particles purified and analysed by scanning electron microscopy. The white scale bars represent 200 nm.

Future work will analyze whether these synthesized particles are crystalline or amorphous. Interestingly, the simulation part of the project revealed the possible existence of both amorphous and crystalline stable and metastable assemblies (Fig. 2) with a prevalence of amorphous structures for smaller clusters.

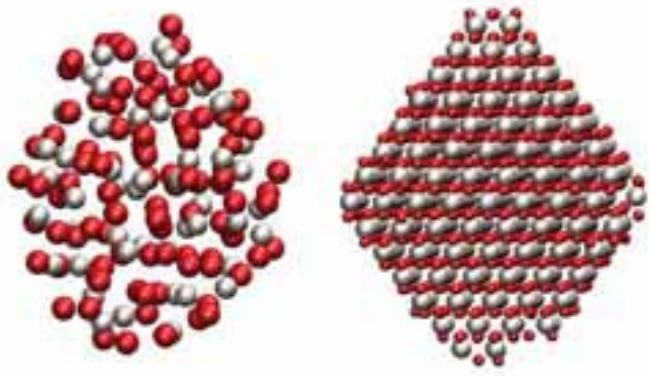


Fig. 2: Forcefield based optimization of Ca50F100 (left). The structure is amorphous with an ellipsoid shape. The ordered structure of Ca430F861 on the right is an (truncated) octahedron with defects.
AFM based adhesion studies allow the determination of CaF2 particle-surface interaction forces at the individual particle level and first results from these studies will be presented.

DISCUSSION & CONCLUSIONS

The above shown results demonstrate the possibility to tailor specific CaF2 particle morphologies. Future work will focus on the analysis of the adhesion of the different shaped particles to tooth enamel, their stability in physiological environments and the bioavailability of fluoride ions released from such particles over time. Furthermore simulations will further explore the possibility to obtain CaF2 clusters with tailor made properties by providing specific environments or excipients, which is of great interest for formulation studies.

REFERENCES

1 Mani (2009); Archives of Orofacial sciences 4:1-6; 2 Petzold (2001), Caries Res 35(suppl 1): 45–51.

ACKNOWLEDGEMENT

The authors acknowledge the financial support by the Swiss Nanoscience Institute and the help from the members of the Nanotechnology and Materials Sciences group of the FHNW.

NANOSTRUCTURE OF RED BLOOD CELL MEMBRANE UNDER CRITICAL STATE. ATOMIC FORCE MICROSCOPY AND CALIBRATED ELECTROPORATION

ELENA K. KOZLOVA, Alexander M. Chernysh, Victor V.Moroz
V.A. Negovsky Scientific Research Institute of General Reanimatology RAMS, Moscow, Russia

INTRODUCTION

The purpose of the present work is to study the alterations of nanostructures of red blood cell (RBC) membranes under intoxication of the blood caused by acute massive hemorrhage, by action of pharmaceutical chemicals in high doses, oxidation processes due to ionizing radiation, long-term storage of the blood in vitro.

METHODS

Blood and Solutions. Blood donations were performed of healthy donors. In accordance with ethics commission of the Scientific Research Institute of General Reanimatology RAMS all donors signed the informed consent form. The experiments of blood loss and hypotension were performed using white rats. The study was approval by Institutional Animal Care and Use Committee. From the selected probes there were prepared monolayers of red blood cells. The intoxication was modeled by adding into blood hemin, furosemide, chlorpromazine and zinc ions in high concentrations, in vitro. All of them influence on protein structures of RBC. The long-term storage of donated blood was studied. There were used UV and gamma-radiation as the sources of ionizing radiation. Atomic force microscope (AFM) imaging. Space Fourier transform for analysis of membrane surface. The images of the cells, membrane surface and of their fragments were obtained using AFM (NTEGRA Prima, NT-MDT, Russia) in semi-contact and contact regimes. We used Space Fourier transfrom for the obtaining of a detailed image and estimation of quantitative parameter of the surface nanostructure.

Calibrated electroporation. The high voltage electrical impulse (E=1100 V/cm) was used for diagnostics of nanostructure of cells membrane. There were measured the kinetic curves of hemolysis due to electroporation after different influences.

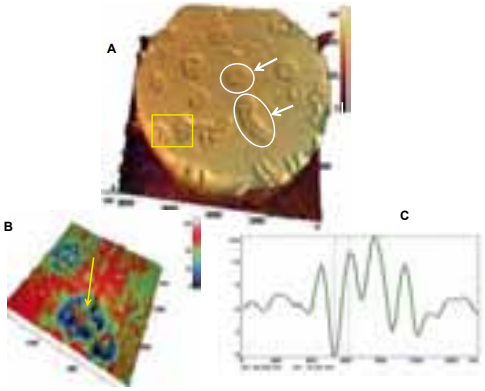


Fig 1. The influence of hemine on red blood cell membrane. A – The cell with domains of damages at the membrane surface. B – The fragment of membrane with domains. C – The profile of the domain surface

RESULTS

Images of membranes nanostructure under various influences were obtained. All these influences acted on membrane proteins and change the structure of membranes.

The character of alterations was specific to each of them. All defects increased with growth of concentration of the agent. The quantitative estimation of defects is given in the work. For example, at influence of hemin C=1.7 mM specific structures in the form of domains and "buttons" on the membrane of RBC appeared (Figure 1). The diameter of elementary "button" was 150-200 nm, height 3-15 nm.

In Figure 2 there are represented control cell and the cell after hypotension and the fragments of their membrane. The heights of the surfaces of first and second orders are essentially increased after massive hemorrhage.

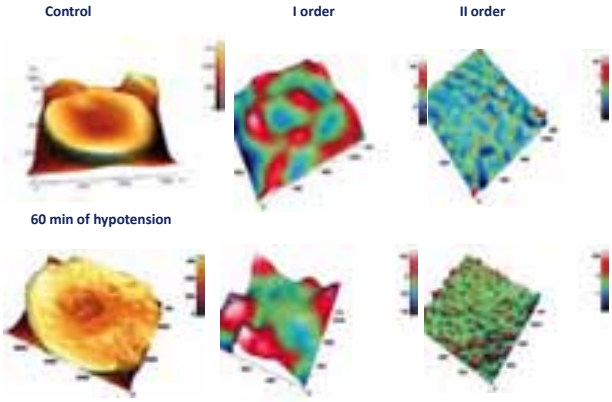


Fig.2. The change of red blood cells membrane after hypotension

Experimentally it was shown the damages of membrane nanostructure during storage of donated blood (30 days), under action of heavy metals ions, under influences of ionizing radiation.

CONCLUSIONS

It was experimentally established by the AFM and calibrated electroporation that intoxication essentially influenced on the membrane nanostructures of RBC. Further studying of features of the membrane nanostructure changes is useful as the basis for the establishment of mechanisms of intoxication of organism. This, in turn, will make it possible to choose correct tactics for treatment of critical states.

THE INTERACTION OF LIPID-LIKE DETERGENTS AND LIPIDS WITH THE EFFLUX TRANSPORTER P-GLYCOPROTEIN

XIAOCHUN LI-BLATTER and Anna Seelig
Biophysical Chemistry, Biozentrum, University of Basel, CH

Lipids and lipid-like detergents are often used as excipients to deliver or encapsulate drug molecules for cancer therapy to circumvent their extrusion by efflux transporters such as P-glycoprotein. It is known that certain lipids interact with the efflux transporter P-glycoprotein. If their affinity to P-glycoprotein is higher than that of the co-administered drug they act as modulators or inhibitors of P-glycoprotein and enhance drug absorption which could cause severe, unforeseen side effects. We therefore systematically investigated different lipid-like detergents and lipids (including Fos-Choline-m, lyso-phosphatidylcholines, phosphatidylcholines of different chain lengths and their charged analogs) with respect to their membrane binding propensity and their ability to bind to P-glycoprotein. We conclude that the lipid-like detergents and lipids are allocrits for P-glycoprotein and bind to the transporter via hydrogen bond formation as shown previously for drugs (1-3). The positive charge of the allocrits enhanced the transport rate of the compound by P-glycoprotein, and

the negative charge reduced it. Short chain analogs are more prone to an interaction with P-glycoprotein than very long analogs.

REFERENCES

1. Gatlik-Landwojtowicz E., Aanismaa P., Seelig A. 2006. Quantification and characterization of P-glycoprotein-substrate interactions. Biochemistry 45:3020-32.
2. Li-Blatter X., Seelig A. 2010. Exploring the P-glycoprotein binding cavity with polyoxyethylene alkyl ethers. Biophys J 99:3589-98.
3. Li-Blatter X., Seelig A. 2012. P-Glycoprotein-ATPase Modulation: The Molecular Mechanisms. Biophys. J. 102:1383-1393.

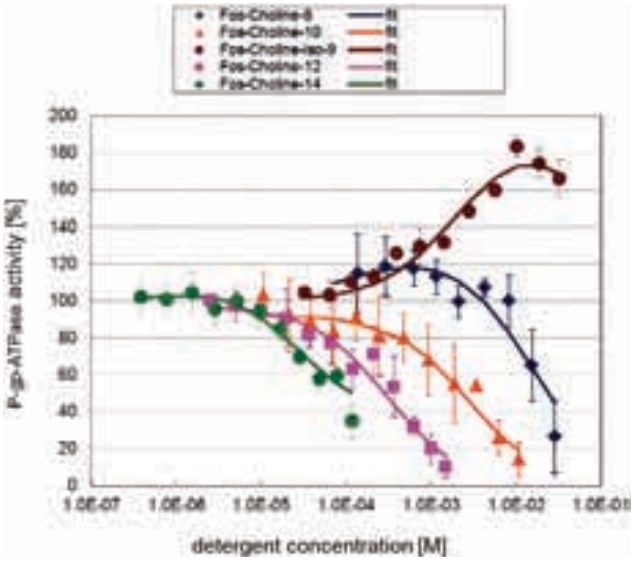
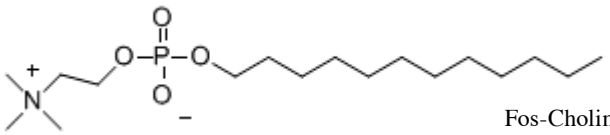
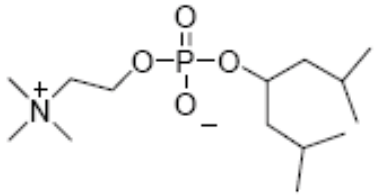


FIGURE 1. P-gp-ATPase activity measured in plasma membrane vesicles of NIH-MDR1-G185 cells as a function of the detergent Fos-Choline-m concentration. Measurements were performed at pH 7.0 and 37°C. Detergent concentrations remained always below 0.5 * CMC. Data are expressed as the average of two measurements. Solid lines represent fits to data using modified michaelis menten equation with two binding sites model (ref.1).



Fos-Choline-12



Fos-Choline-iso-9

A BIOINFORMATICS WEB-PLATFORM FOR INTEGRATION AND ANALYSIS OF HIGH-THROUGHPUT SCREENING AND PROFILING DATA IN CANCER RESEARCH

MARKUS LIST[1,2], Steffen Schmidt[1,2], Helle Christiansen[1,2], Ines Block[1,2], Marlene Lemvig Pedersen[1,2], Qihua Tan[3], Adrian Poffenberger[4], Mads Thomassen[3], Torben Kruse[3], Jan Mollenhauer[1,2]

[1]Lundbeckfonden Center of Excellence in Nanomedicine NanoCAN, University of Southern Denmark, Odense, Denmark

[2]Molecular Oncology, University of Southern Denmark, Odense, DK

[3]Clinical Institute and Institute of Public Health, University of Southern Denmark, Odense, Denmark

[4]Yale Center for High-Throughput Cell Biology, Yale University, New Haven, U.S.A.

Systematic, non-hypothesis driven high-throughput approaches are an appropriate strategy for discovering new starting points for personalized anti-cancer therapies. Within the NanoCAN center, a joint project between the Odense University Hospital (OUH) and the Health and Natural Sciences Faculties of the University of Southern Denmark, we have been building up one of Northern Europe's largest screening platforms for the discovery of new personalized treatment options in cancer. In these approaches, we obtain genomics and proteomics data in amounts that require a systematic approach for sample tracking and data analysis. To this end, we intend to create a collection of integrated web tools that allow for sample tracking between our genome screening and proteomics experiments. Furthermore, these tools shall enable researchers to directly perform sophisticated data analyses through a web interface, instead of having to deal with R packages that are difficult to access and lack integration. The proposed platform will then serve as a foundation for a combined analysis of gene-specific cell viability and proteomics data and thus lead to a better understanding of the complex gene relations that lead to cancer development. We also intend to further broaden our approach by integrating transcriptomics data collected at OUH and sequencing data of cancer cases.

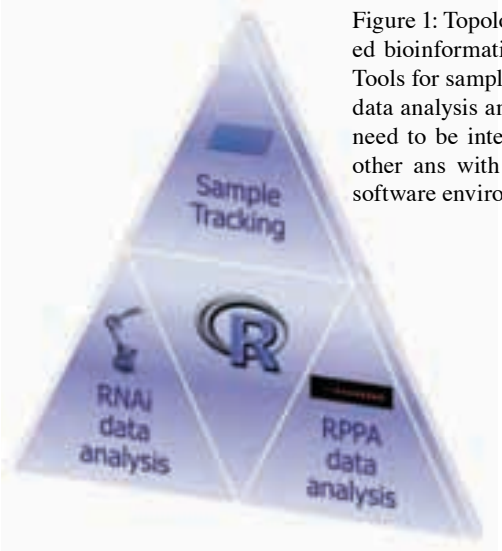


Figure 1: Topology of our intended bioinformatics web platform. Tools for sample tracking, RNAi data analysis and RPPA analysis need to be integrated with each other ans with the R statistical software environment.

CATIONISED RADIOLABELLED NANOPARTICLES FOR PERFUSION IMAGING OF THE LUNGS

SERGEY LOBOV, David King, Tim Senden and Ross Stephens

Department of Applied Mathematics, Research School of Physical Sciences and Engineering, Australian National University, Canberra, ACT 0200, Australia

Imaging of lung perfusion is currently performed using macro-aggregated albumin, which is mechanically arrested at limiting diameters of the capillary bed. While the proportion of blood flow obstructed is typically very low and temporary, it would seem more desirable to image lung perfusion by a method that produces no obstruction of vessels. We provide here cationised radiolabelled nanoparticles that have a strong charge-based affinity for the vessel walls of the lung, and which can be injected intravenously at very low concentration to provide a gamma camera image of lung perfusion without attendant obstruction of blood flow. The nanoparticles consist of a Technetium-99m core encapsulated in graphitic carbon, and passively coated with low molecular weight poly-lysine. The nanoparticle size and imaging concentration does not produce vessel occlusion, but the nanoparticles adhere to vessel walls without producing adverse events, even on repeated use in the same animal over several weeks.

N-PALMITOYL CERAMIDE DI (CARBAMOYL-SPERMINE) (PCDCS): A NOVEL CATIONIC SPHINGOLIPID FOR NUCLEIC ACIDS TRANSFECTION

KIRILL MAKEDONSKI[1], Yulia Boktov[1], Tirtsa Kleinman[2], Liza Silverman[1], Eylon Yevin[3] and Yechezkel Barenholz[1]
[1]Lab of Membrane and Liposome Research, Department of Biochemistry, Hebrew University - Hadassah Medical School, P.O.B.12272, Jerusalem 91120, Israel
[2]Bio-Lab Ltd, Jerusalem, Israel
[3]Institute for Drug Research, The School of Pharmacy, Hebrew University - Hadassah Medical School, P.O.B.12272, Jerusalem 91120, Israel.

This study was performed in the work frame of Consortium Rimonim, supported by Ministry of Industry, Trade and Labor of Israel

The use of cationic lipid/DNA complexes (lipoplexes) for productive nucleic acid delivery is based on the efficient association of the negatively charged nucleic acid to the cationic lipid or polymer and on the efficient adsorptive endocytosis of the positively charged complexes to the anionic plasma membrane of mammalian cells via electrostatic interactions. It will be advantageous if these lipids will also support "endosomal escape". The differences in the magnitude of their activity as a "proton sponge" may explain part of the difference between the transfection efficacy of various lipoplexes. Here we describe the use of N-Palmitoyl Ceramide di (Carbamoyl-spermine) (PCDCS) as a novel cationic component of in vitro and in vivo transfection reagent, a novel cationic lipid that was designed as a result of Prof. Barenholz collaboration with Bio-Lab Ltd and produced by Bio-Lab Ltd. This molecule has two primary amines that are positively charged in neutral pH and four secondary amines that could be only partially positively charged at neutral pH while being fully charged at acidic pH. These chemical properties of lipid enable it on the one hand to bind efficiently to negatively charged nucleic acid molecules and, on the other hand to act as an effective "proton sponge" at the endosomes where the complexes end up after internalization into cells, thereby facilitating effective endosomal escape. We used the PCDCS for preparing the cationic liposome with the helper lipid 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) that was shown to improve the lipoplex transfection efficiency and therefore it appears to be a favorable helper lipid for DNA transfection in vitro.
We used this PCDCS/DOPE formulation both for plasmid DNA and siRNA in vitro transfection in different cell types. Our results show that PCDCS/DOPE (1:3 molar ratio) liposome has a high potential as a transfection reagent both for siRNA (Fig.1) and a plasmid DNA in vitro.

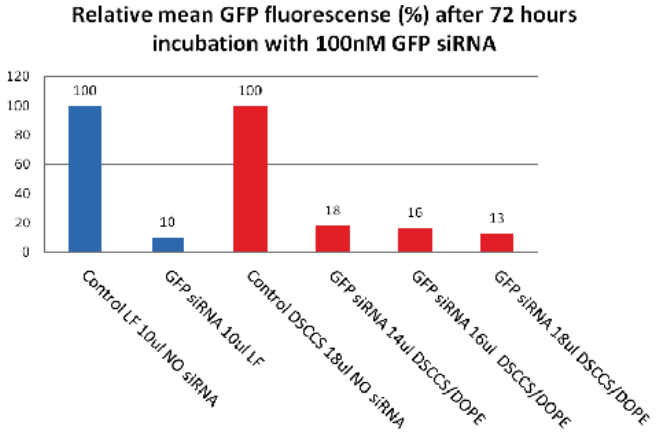


Figure 1: FACScan analysis of GFP expressing C26 cells 72 hours after transfection with anti GFP siRNA. 200nmole of anti GFP siRNA were mixed with 14, 16 and 18 µl of PCDCS/DOPE mix.

Moreover, our preliminary results show that PCDCS/DOPE formulation effectively knocked down Luciferase protein expressed in C26 murine carcinoma cell line in vivo.

NANOPARTICLES FOR MEDICAL PURPOSES – TECHNICAL, MEDICAL AND ETHICAL ASPECTS RESULTS FROM THE ELSI WORK PACKAGE OF THE NANODIARA PROJECT

JAN MEHLICH
Dr.; Europäische Akademie Bad Neuenahr-Ahrweiler

The Europäische Akademie, a well-established technology assessment institution, is leader of a large EU-funded consortium developing early diagnostics for rheumatic diseases on the basis of nanoparticles (NanoDiaRA). In scope of this research project we carry out accompanying research on ethical, legal and social implications of nanoparticles in medical applications.
The vast potential of nanotechnology is at least partly due to the specific physical and chemical properties of nanostructures. This "newness" has stimulated ethical concerns in the general public. But amongst experts too there is an ongoing debate on moral issues of nanotechnology and nanomedicine in particular. The most salient issue in this debate is risk: i.e. the question of how the fact-driven, technical risk assessment of nanomedical applications should be performed. Furthermore there is also the topic of adequate criteria of risk evaluation. The latter includes consideration of normative criteria and has, therefore, to include moral arguments. Further moral issues include fears that nanomedicine infringes the nature or naturalness of the human body, e.g. by augmenting or replacing certain bodily functions. In addition, there is considerable disagreement about the economic impact that a future nanomedicine will have on the health care system. Some authors fear that it may be very expensive and therefore not available for all patients thereby creating injustice in the health care system – or as it is sometimes called: a nano divide.
Another issue where normative expertise is required is the debate on how to deal with public concerns. In view of the responsibility researchers have opposed to society, it needs careful consideration how the field of nanomedicine can be developed in a both scientifically sound and morally responsible way.
The report that is written as the final outcome of the ELSI sub-group is based on the assumption that the sciences, in addition to providing specialised scientific information, also are responsible for furnishing orientational knowledge (normative Standards). To achieve this, an interdisciplinary approach is required, bringing together the results from life-sciences with thematically relevant studies in philosophy and the social sciences. Furthermore, the foreseeable results of research and development will be related transdisciplinarily to society's expected needs and positions. This working method takes up and develops approaches towards technology assessment, ethics of technology and medical ethics.

SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES FOR IN VIVO IMAGING WITH MRI

MALIN MEJÀRE[1], Fredrik Olsson[1], Pontus Kjellman[1,2], Linda Andersson[1], Hanna Toftevall[1] Rene in 't Zandt[1,3] and Sarah Fredriksson[1]
[1]Genovis AB, Lund, Sweden
[2]Medical Radiation Physics IKVL, Lund University, Lund, Sweden
[3]Lund University BioImaging Center, Lund University, Lund, Sweden

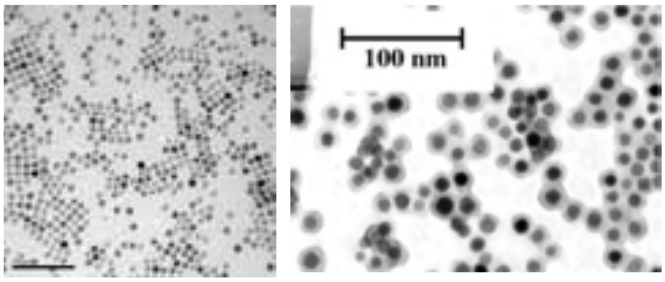
INTRODUCTION

MR imaging is widely used in biomedical applications. Labeling of cells, direct or ex vivo, with a contrast agent allows for non-invasive tracking in vivo. The fate of endogenous cells can be monitored through direct labeling of cells in the brain. Multimodal particles engineered with a targeting moiety can be used to follow tumor growth and spreading. In cellular therapy the study of cell migration, engraftment and distribution non-invasively is a prerequisite.
Superparamagnetic nanoparticles with an iron oxide core are used as contrast agents in MRI due to their high relaxivity. To ensure equal contribution from all particles in the MR image they need to be uni-

form with a narrow size distribution. Furthermore, for in vivo use stability in biological fluids, low toxicity and high biocompatibility are necessities.
The design and synthesis of superparamagnetic iron oxide nanoparticles (SPIONs) with high relaxivity and colloidal stability is described here. Coating of the SPIONs with biocompatible polyoxyalkyleneamine further allows for multimodality with fluorescence tagging and antibody conjugation. Lipid coating provides for high cellular uptake in ex vivo labeling of cells.

RESULTS & CONCLUSION

Superparamagnetic iron oxide nanoparticles (SPIONs) were synthesized and coated with PEG or lipid for ultimate use in in vivo imaging. Iron oxide cores, consisting mainly of magnetite, Fe3O4, were synthesized through a seed growth method according to Yu et al (1). By careful control of temperature and time during synthesis the size of the nanoparticles could be well defined (Figure 1). The size of the iron oxide cores was approximately 11 nm with a very narrow size distribution. SPIONs were coated with polyoxyalkyleneamine (POA) to make them colloidal stable and biocompatible. The coating procedure stems from the method used by Yu et al (2). In this reference paper the coating is built up with PMAO (poly(maleic anhydride-alt-1-octadecene) and PEG (polyethyleneglycol). The PEG-like molecule polyoxyalkyleneamine (MW 2000) was proven to be a good alternative to amino-PEG both in perspective of in vivo stability and production costs. SPIONs with a core size of 11 nm were coated with POA and the coating layer was 2-3 nm as determined with TEM (Figure 1). However, it is the hydrodynamic diameter that determines the properties and interactions of nanoparticles in biological systems. The hydrodynamic diameter of the SPIONs was determined to 30±10nm with TEM. The POA coated nanoparticles were colloidal stable in pH ranging from 2-12. No aggregation or flocculation was observed even after several months of incubation in human serum (Figure 2).



A. B.

Figure 1. TEM images of SPIONs. Iron oxide cores of the superparamagnetic nanoparticles with an approximate size of 11 nm (A). The coating forms a 2-3 nm thick layer (B).

Figure 2. The SPIONs were colloidal stable in human serum for several months.

POA coating promotes stability of the SPIONs in biological environments rendering them suitable for direct in vivo injection. To track cells labeled ex vivo and to be able to trace the fate of a few cells or individual cells, the iron load per cell needs to be maximized without being toxic or altering the intrinsic properties of cells. SPIONs were coated with DOTAP (N-[1-(2,3-Dioleoyloxy)propyl]-N,N,N-trimethylammonium methyl-sulfate) to get a lipid surface coating providing for high cellular uptake. The R2 relaxivity was 200 (mM-1*S-1) and the R1 relaxivity was 2 - 4 (mM-1*S-1) of the SPIONs at 2.4 Tesla.

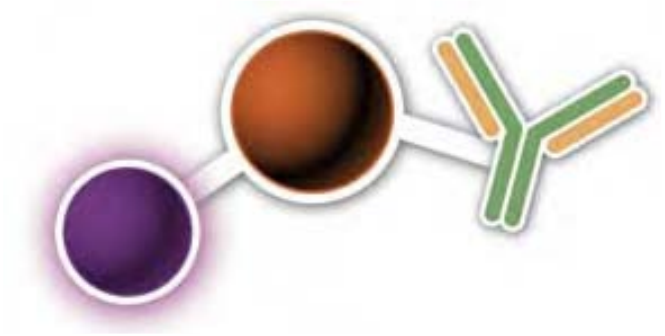
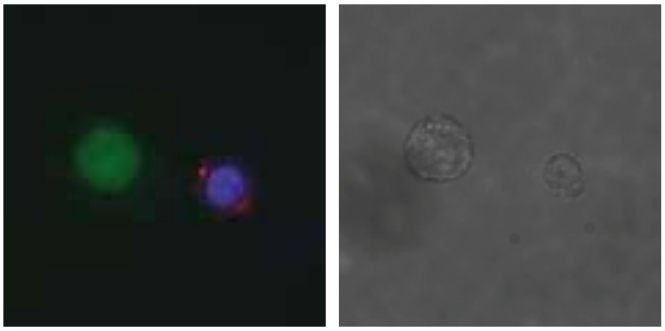


Figure 3. Schematic drawing showing a multimodal SPION with a fluorescent marker and a conjugated antibody.



A. B.

Figure 5. POA coated SPIONs, labeled with a fluorescent dye and conjugated with anti-rat CD45, bound selectively to primary rat CD45 expressing cells and not to human CD45 expressing cells, THP-1 (acridine stained, green in picture) (A). Bright field image of the same cells (B).
The POA coating also serves as an anchor for further conjugation of fluorescent markers and biomolecules such as antibodies or antibody fragments (Figure 3). Rat anti-CD45 antibody was covalently coupled to POA coated SPIONs containing the fluorescent dye (Dy-647). Selective binding of nanoparticles conjugated with antibodies was evaluated in vitro. Specific binding to rat cells expressing CD45 and no binding to the human cell line (THP-1) with high expression of CD45 was shown in vitro (Figure 4).

REFERENCES

1. Yu W.W et al "Synthesis of monodiperse iron oxide nanocrystals by thermal decomposition of iron carboxylate salts" Chem. Commun. 2004, 2306-2307.
2. Yu W.W et al "Aqueous dispersion of monodisperse magnetic iron oxide nanocrystals through phase transfer" INSTITUTE OF PHYSICS PUBLISHING Nanotechnology 2006 (17) 4483-4487.

BIOMATERIALS TO SUPPORT MSCS EX-VIVO CULTURING

BEATRIZ J.C. MONTEIRO

Instituto Superior Técnico, Universidade Técnica de Lisboa

The differentiation and proliferation of mesenchymal stem cell (MSCs) depends not only of the effect of soluble factors and in cell-cell interactions, but also on cell-extracellular matrix (ECM) interactions. ECM tridimensionality can be mimicked through the fabrication of nanofibers matrices that can be used, for instance, to engineer skeletal muscle tissues that could be used in human tissue transplantation. The strategy for our study consists on mimicking the ECM structure through a comparative study where electrospun polycaprolactone (PCL) and PCL/gelatin blend nanofibers matrices are used for MSCs culture. The main aim of this study is to investigate how MSCs proliferation and morphology can benefit from the presence of an inexpensive natural polymer, gelatin, in combinations with PCL, a robust biodegradable synthetic polymer. In order to understand how the fiber diameter and alignment affects the MSCs proliferation, random and aligned PCL and PCL/gelatin scaffolds were

electrospun with different nanofiber diameters. The cells morphology was observed after Rhodamin-Phalloidin fluorescence staining and their proliferation was measured by the Alamar Blue Assay. In this study, the use of aligned scaffolds showed to profoundly influence MSCs cells morphology while gelatin presence in the scaffold resulted in a higher proliferation rate than in PCL (alone) scaffolds.

Keywords: Mesenchymal stem cell, extracellular matrix, polycaprolactone, gelatin, electrospinning.

1.1 MSCs culturing on PCL and PCL/gelatin nanofibers matrices

Using hexafluoro-2-propanol solvent, PCL solutions with concentrations 6%, 8% and 10% (w/w) and PCL/gelatin (70:30, w/w) blend solution with concentration 4%, 6% and 8% were electrospun into random and aligned nanofiber matrices. Gelatin coated lamellas were prepared by coating each with a gelatin solution in phosphate-buffered saline (PBS) (0.1%(w/v)) for 2 hours. Then triplicates of each nanofibers meshes and gelatin coated lamellas were glued (Medical adhesive silicon type A, Dow Corning®) in 6-wells plaques. BM MSCs (male 69 years-old donor) were seeded at a density of 3000 cells/cm2 on top of the nanofibers scaffolds (5,76 cm2 surface area) and incubated at 37ºC (5% CO2, fully humidified) with Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% MSC-qualified FBS. Every 3 days the culture medium was changed with fresh medium, during 7 days of culture. The positive control consisted on cells cultured on gelatin flat sheets. At the 3rd day of cell culture, BM MSCs nuclei was stained with DAPI (Sigma) and cells actin cytoskeleton was stained with fluorescent dye tetramethylrhodamine (TRITC) conjugated with Rhodamine Phalloidin probe. Cells were permeabilized with a Saponin/PBS solution (50 μg/mL) during 45 min followed by staining with Phalloidin-TRITC solution (1:1000, v/v) during 60 min and a DAPI solution (1.5 μg/mL) during 5 min. After this procedure, the cells distribution and morphology was observed under an inverted fluorescence microscope (LEICA DMI 3000B, Germany) and images from each well were taken with a 100x magnification. The BM MSCs proliferation was measured at the 7th day of cell culture using AlamarBlue® Assay.

RESULTS

Then images obtained from MSCs DAPI and Rhodamine/Phalloidin staining were analyzed. Cells are normally growth on flat surfaces, therefore cell morphology of the positive control (where cells have grown in a gelatin flat surface) are compared with the ones observed for cells cultured in nanofiber matrices. In gelatin flat supports, the MSCs do not spread on the scaffold as they spread on fibers matrices, in fact, the spreading is so reduced that the nuclei is more pronounced than the actin cytoskeleton while in nanofibers the opposite occurs.

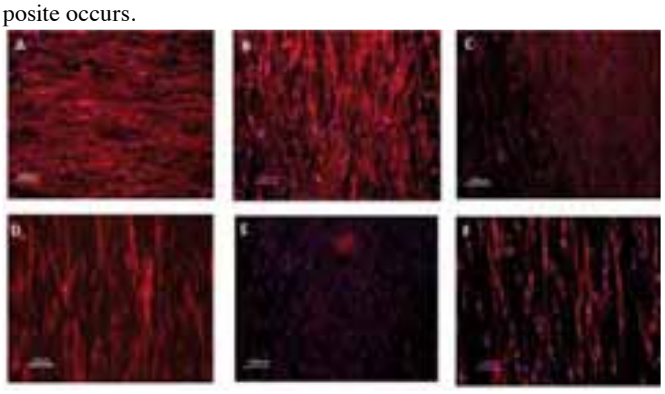


Figure 6: Images obtained by fluorescence microscopy of MSCs cultured in aligned PCL and PCL/gelatin matrices (100xmagnification). (A-B; E - F) DAPI and Rhodamine-Phalloidin staining. A – PCL 6 wt.% scaffold; Rhodamine-Phalloidin staining. B – PCL 8 wt.% scaffold. C – PCL 10 wt.% scaffold. D – PCL/gelatin 4 wt.% scaffold. E – PCL/gelatin 6 wt% scaffold. F – PCL/gelatin 8 wt.% scaffold. Images A-C and E-F were obtained by ImageJ overlapping of DAPI and Rhodamine-Phalloidin staining images.

The MSCs cultivated on random fibrous matrices have a spread shape in several directions. This might happen because they adhere to fibers with different alignments which do not give the same orientation. As expected, higher cell density is observed for PCL/gelatin than for the ones made of PCL alone. In the later matrices, it is observed large gaps between the cells populations while in PCL/gelatin 4 wt.% matrices and PCL/gelatin 6 wt.% matrices confluent cell culture is observed. This result may confirm that gelatin can have a beneficial effect on cells growth and adherence, since the inclusion of a protein in the scaffold can provide additional motifs for cell anchorage. Also, notice that this particular protein, results from denaturated collagen. Therefore, it can be speculated that when gelatin based electrospun nanofibers are produce, resembling the fibrous collagen geometry, and thus potentially providing beneficial properties for cell organization. In the aligned nanofibers the cells adopt the fibers orientation and stretched along it (Figure 1). This gives to the cells a unidirectional and better organization than in random scaffolds. Cell proliferation is quantified through indirect measure of cell metabolic activity using the AlamarBlue test. The relative percentage of cell metabolic activity was measured for each condition (Figure 2; A and B) at day 7 of culturing. From the data is noticed that cells proliferation is higher for both random and aligned PCL/gelatin fibrous matrices in contrast with PCL matrices. PCL fibrous matrices have a very low value of relative cell proliferation, particularly on random matrices. These high discrepancies between PCL random and aligned matrices may result from the unexpected fibers release from the wells.

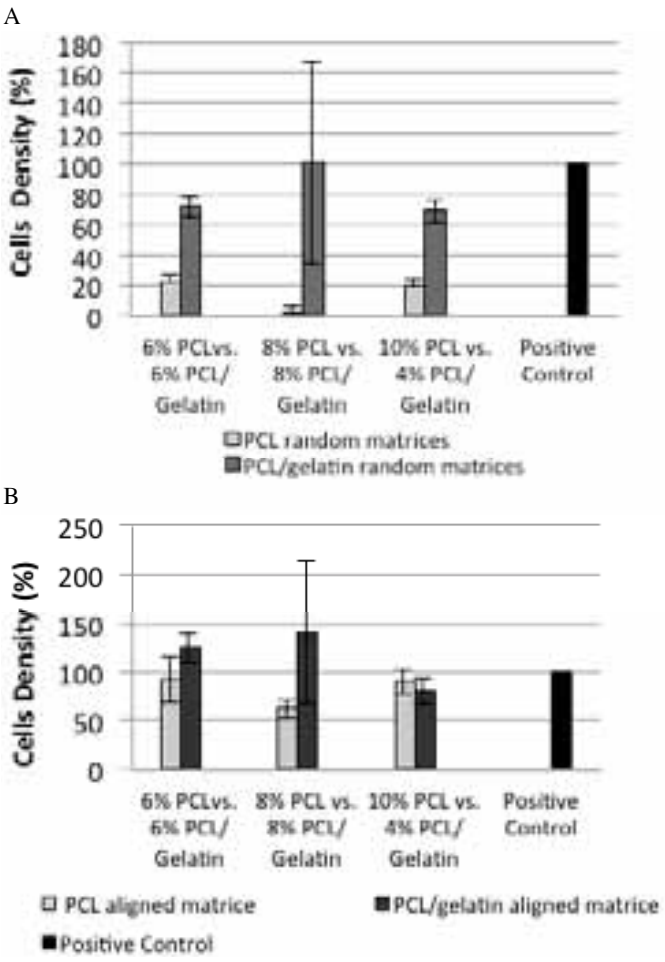


Figure 2: Graphs obtained for MSCs density on random and aligned PCL and PCL/gelatin scaffolds. Results obtained after MSCs culture on each establish condition and posterior cells density measurement by fluorescence measurement. A) PCL scaffolds vs. random PCL/gelatin scaffolds. B) Aligned PCL scaffolds vs. aligned PCL/gelatin scaffolds.

IDENTIFICATION OF NOVEL DRUG TARGETS FOR PERSONALIZED BREAST CANCER NANOMEDICINE

CAROLIN MÜLLER[1,2,4], Ines Block[1,2], Daniel Sdogati[1,2], Markus Worf[1,3], Mads Thomassen[1,4], Angela Riedel[1,2], Steffen Schmidt[1,2], Pernille Lund Hansen[1,2], Aleksandra Jaskot[1,2], Jakub Trojnar[1,2], Helle Christiansen[1,2], Marlene Lemvig Pedersen[1,2], Pernille Marie Madsen[1,2], Sidsel Buch Odgaard[1,2], Torben Kruse[1,4], Petra Kioschis-Schneider[1,3], Jan Mollenhauer[1,2]

[1]LundbeckfondenCenter of Excellence in NanomedicineNanoCAN, University of Southern Denmark, Odense, Denmark

[2]Molecular Oncology, University of Southern Denmark, Odense, DK

[3]Institute of Molecular Biology and Cell Culture Technology; University of Applied Sciences, Mannheim, Germany

[4]Clinical Genetics, Odense University Hospital, Odense, Denmark

Personalized cancer nanomedicine aims at the recovery of new nanodrugs that would match the molecular fingerprint of an individual patient`s tumor. Expression profiling and next generation-sequencing data represent rich resources for discovering new starting points for such approaches. Here, we selected a set of 140 genes, which recently have been proposed to show potentially relevant alterations in breast cancer. We serially analyze the normal and the aberrant gene variants for their effects on breast cancer growth via a novel strategy for the generation of standardized cell line panels.

To this end, we cloned the major part of these genes and have analyzed a total of 50 out of the 280 variants to be tested. This resulted so far in the identification of 8 new breast cancer growth modulators. Such new growth modulators offer for systematic screens to recover novel nucleic acid-based nanodrugs.

NANOTOXIC EFFECTS OF CRYSTALLINE SILICA NANOPARTICLES IN HUMAN PLACENTA

LIUDMILA NIKITINA[1], S.Krassnig[1], M.Gauster[1], G.Dohr[1] and H.Juch[1]

[1]Institute for Cell biology, Histology and Embryology, Medical University Graz, Harrachgasse 21/7, A- 8010 Graz

INTRODUCTION

Silica nanoparticles (nSP) have found extensive application in industry, as well as in biomedical and biotechnological fields as addictives of drugs, cosmetics, diagnostic assays ect. Thus the environmental and health impact of silica nanomaterials is of great interest. Toxicity of different size nSP has been demonstrated on a variety of different cell types. In addition, their association with autoimmune diseases and lung cancerogenesis in vivo and in vitro models has been described. Although prenatal stages of human development are suggested to be more sensitive to the toxic exposure, little is known about nanotoxic effects of nSP on reproduction and pregnancy. It was recently demonstrated in the mouse model that silica nanoparticles can pass the placental barrier depending on their size and cause toxic effects in the placenta and the embryo (Yamashita K, et al.2011). In our study we aimed to investigate toxic effects of crystalline SiO2 nanoparticles (Min-U-Sil5) in tissue explants of human placenta.

MATERIALS AND METHODS

Placental explants from first trimester and term placentas were cultivated in the presence or absence of crystalline silica particles (Min-U-Sil 5) for 6-42h under physiological pO2 (3% and 8% of oxygen for the first and third trimester explants respectively). Morphological changes and evaluation of nSP in placental tissue were analysed by light- and transmission electron- microscopy. Lactate dehydrogenase (LDH) activity was measured in the culture supernatants to evaluate cytotoxicity. Markers of cell proliferation (Ki67) and apoptosis (caspase 8) were analysed by immunohistochemistry and western blot. Secretory activity of placental tissue was analysed by measuring human chorionic gonadotropin (hCG) concentration in the culture medium.

RESULTS

Accumulation of silica crystals (approx. 300nm) was observed outside the microvillous membrane of the syncytiotrophoblast layer (Fig.1A), as well as damage of microvilli and apical membrane, and detachment of the syncytiotrophoblast. Single silica crystals approx. 20nm were found inside the placental tissue by electron microscopy (Fig. 1B). Ki67 expression (Fig.2B) and hCG secretion (Fig.4A) were decreased, while activated caspase 8 was increased (Fig. 3) in Min-U-Sil5 treated explants. No differences in LDH-release were observed between exposed placental villi and controls (Fig. 4B).

Figure 1

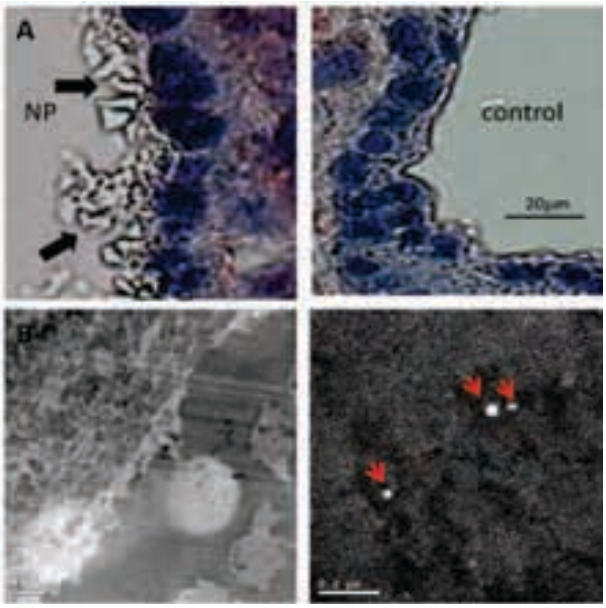


Figure 2

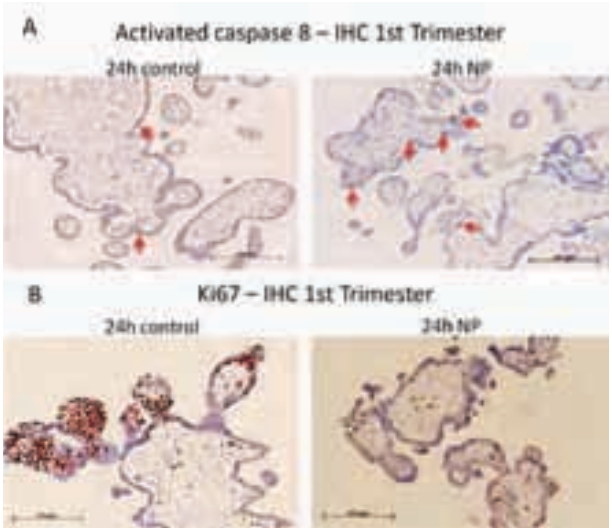


Figure 3

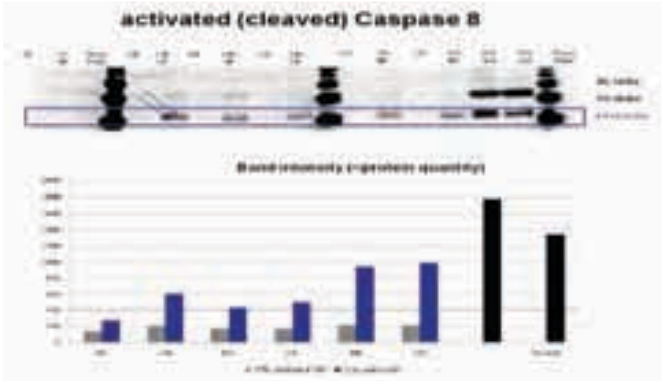
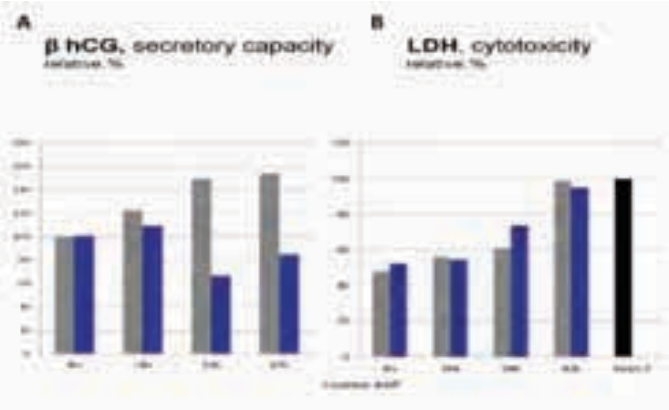


Figure 4



CONCLUSIONS

Placental explants exposed to SNP revealed morphological changes in the outer villous membrane (syncytiotrophoblast layer). Although no signs of acute cytotoxicity (LDH release) have been observed, there is evidence for toxic effects on placental cell proliferation (Ki67), hormonal secretory capacity (β hCG) and the early apoptotic cascade (caspase 8). There is evidence for potential toxic effects of crystalline silica nanoparticles on human reproduction.

MORPHOLOGICAL MARKERS OF CANCER CELLS FROM UTERINE NECK SMEARS

ADKHAMJON PAIZIEV, Khojiev, Sh., Hamidjonov D.
Arifov Institute of Electronics Uzbek Academy of Science, Tashkent , Uzbekistan

BACKGROUND

The cytological testing of the smears from uterine neck is the high-efficient screening test for carcinoma this localization. However, in oncocytology the main testing tool is optical microscopy of transmitted light, which usually can not reveal a fine morphology of the cell membrane surface of the native smears. Besides the surface membrane structures are destroying after smears preparation (fixing, cleaning, staining and others) what leads to artifacts too. Therefore the malignant cell do not display strongly specific morphologic features. For their revealing need to elaborate the new noninvasive express methods to test the native smears of difference localization by using reflected optical microscopy.

OBJECTS

The objective of the present work is revealing strongly specific morphologic features of the epithelial cells malignization from uterine neck of patients with diagnosis Cr. colli uteri.
Methods. Biospecimens - fresh, native smears endometrium of the healthy patients (10 samples) and sick women with diagnosis Cr. colli uteri (150 samples) has been investigated under reflected optical microscope Neophot-2 (Carl Zeiss , Jena). The samples was not treated with chemicals.

RESULTS

On the figure 1a typical healthy epithelial cells (control samples) from uterine neck are presented. As is seen from figure 1a, cell nucleus, their contours and cell content are clearly distinguished without any atypism. On the figure1b (centre) a pattern of the malignized epithelial cell from uterine neck of patients with diagnosis Cr. colli uteri is shown. On the cell surface clearly expressed multiple dispersed pathologic discharge (DPD) are observed. Same pattern is observed for difference stage of carcinogenesis but with difference density of distribution of DPD along cell surface and visible due to their good reflectance under reflected optical microscope. Diameter of the DPD is ranged from 0,3-0,5 to 1,2-1,5 mkm. Sometimes this DPD are clustered around nucleus or boundary of cell but always assembly of DPD are localized inside of cancer cells (figure 1b).

CONCLUSION

Application of the reflected optical microscopy let us clearly to visualize quite important morphological features of the malignized cells epithelial from uterine neck of patients with diagnosis Cr. colli uteri as aggregations of spherical multiple dispersed pathologic discharges on the cell surface with high reflectance.

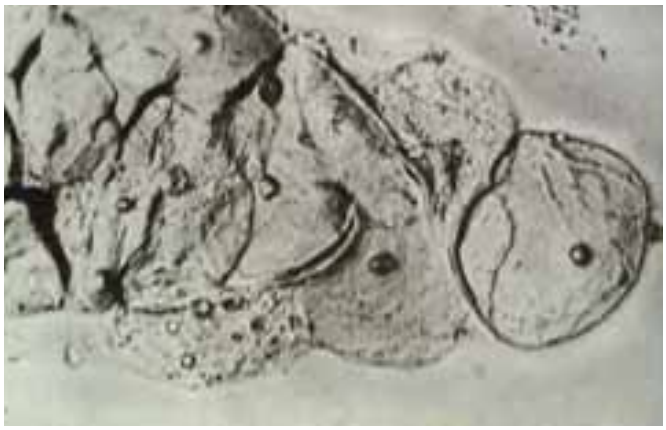


Figure1a

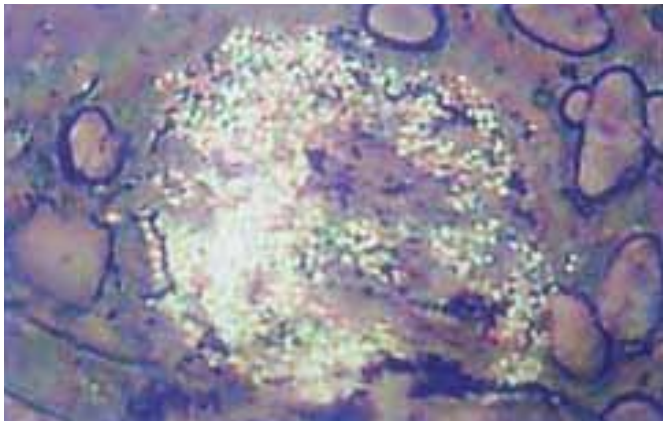


Figure1b

EXPLOITING THE SURFACE PLASMON RESONANCE OF THE FUNCTIONALIZED NANOPARTICLES IN SKIN PATHOLOGY AND THERAPY MONITORING

SIMONA CÎNTĂ PÎNZARU[a], A. Fălămas[a], C. A. Dehelean[b], C. Soica[b], S. Ciurlea[b], M. Aluaş[c], L. Barbu-Tudoran[d]
[a]Babes-Bolyai University, Bopmedical Physics, Theoretics and Molecular Spectroscopy Dept., Kogalniceanu 1, RO 400084 Cluj-Napoca, Romania
[b]Victor Bbaes-University of Medicine and Pharmacy Faculty of Pharmacy, Eftimie Murgu Square no.2, 300041, Timisoara, Romania
[c]Babes-Bolyai University, Faculty of Physics, Kogalniceanu 1, 400084, Cluj-Napoca, Romania
[d]Babes-Bolyai University, Electron Microscopy Centre, Clinicilor 5-7, 400006, Cluj-Napoca, Romania

As a response to the increasing demand to develop new, sensitive, noninvasive and accurate methods in early diagnostic and to track the molecular changes in early stage of malignancy or in the therapy monitoring, an interdisciplinary approach exploiting the surface plasmon resonance [1] of the pure or functionalized nanoparticles and ultrasensitive Raman spectroscopic techniques is reported. Due to its unique ability to provide ultrasensitive detection limits, surface enhanced Raman scattering (SERS) has been used to detect molecular modifications involved in disease, such as cancer [2, 3], diabetes [4], and others. A schematic representation of the spectral approach is shown in the Fig. 1, where the molecular species located in the close vicinity of the nanoparticles exhibit strong Raman scattering

which represents the SERS signal. We evaluated this process inside the tissue using animal models.

Melanoma tissue tracking at molecular level using both labeled and unlabeled silver and gold nanoparticles has been achieved using surface enhanced Raman scattering (SERS) technique. We used skin tissue from ex-vivo mice with induced melanoma as well as in-vivo mice in early stage of malignancy, where a new treatment based on betulin nanoemulsion formulation has been applied.

We evaluated the nanoparticles uptake ability of the skin tissues using transmission electron microscopy (TEM, Fig. 2) in conjunction with the SERS measurements (Fig. 3). Any optical evidence of nanoparticles accumulation was not observed. As suggested by the TEM images, the colloidal nanoparticles were able to penetrate the interstitial space between the cells and some of them to penetrate the cell membrane.

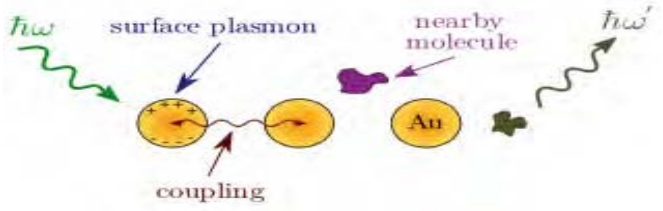


Fig. 1. Schematic representation of the Raman scattering enhancement of any molecular species from the close vicinity of the noble metal nanoparticles under the surface plasmon resonance condition.

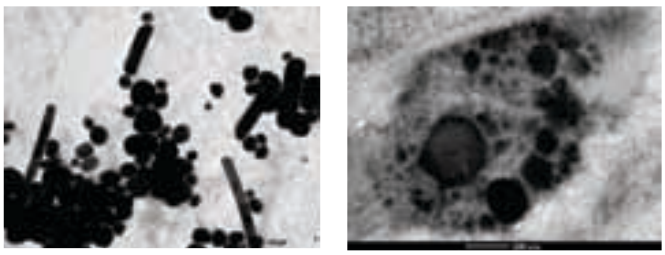


Fig. 2. TEM image of the Ag nanoparticles resulted upon centrifugation of the classical colloidal solution (left) and their tissue penetration (right).

Previous results reported by Larese et al. [5] found that in vitro nanoparticle skin permeation is possible and that silver nanoparticles as small as 25 nm stable for 24 h were located in the stratum corneum and the upper layers of the epidermis. The same passive fluid-phase uptake was accounted for the absorption of gold nanoparticles into cultured eukaryotic cells [6].

The silver colloidal solution prepared through the classical reduction and than centrifuged, generates a uniform size distribution of the nanoparticles as shown by the sharp extinction spectrum with a maximum at 410 nm. Adding a dilute solution of Raman reporter (cresyl violet perchlorate) the nanoparticles instantly exhibited aggregation and strong SERS signal fingerprint that is additionally enhanced by resonance Raman contribution when the red laser line is used for excitation. As such, in the red or even IR spectral range (that is optimal for laser tissue investigation), these functionalized nanoparticles act as optical sensors that provide spectral information from their close vicinity inside the tissue.Both labeled and unlabeled noble metal nanoparticles were employed to record and compare tissue SERS signal. Fig.3 presents a series of SERS spectra recorded from mouse tissue in early stage of disease. In the presence of the label we noted the intense sharp band at 590 cm-1 charateristic to the Raman reporter, which allowed tracking the optical nanosensors inside the tissue structure. The SERS signal from tissue with unlabeled nanoparticles is the sum of the auto-fluorescence background of the tissue resulted from the excitation with the visible laser line (632.8 nm) and several characteristic reproducible SERS bands located at 1618, 1455, 1359, 1283, 1119, 976, 899, 798 and 752 cm-1. According to Kneipp et al. [6] such bands are characteristic for the native chemical constituents in the cell nucleus and cytoplasm, like DNA, RNA, phenylalanine, tyrosine. Although the spectra collected from random points exhibit small band shape differences due to the tissue inhomogeneity, the overall spectral signature is similar, thus providing a good reproducibility of the signal.

As such, SERS molecular characterization of melanoma tissue was proposed for the first time [7]. Optical nanosensors based on Ag and Au nanoparticles with chemisorbed cresyl violet molecular species as labels revealed sensitive capability to tissues tagging and local molecular characterisation. Sensitive information originating from surrounding native biological molecules is provided by the tissue SERS spectra obtained either with visible or NIR laser line. Labeled nanoparticles introduced systematic diffrences in tissue response compared to unlabeled ones, suggesting that the label functional groups tag specific tissue components revealed by proteins or nucleic acids bands. The results obtained here open perspectives in applied plasmonic nanoparticles and SERS for the early cancer diagnostic based on the appropriate spectral databank. SERS signal was sensitive to the label penetration depth. The CV label was tracked inside the tissue and recognized due to its fingerprint bands.

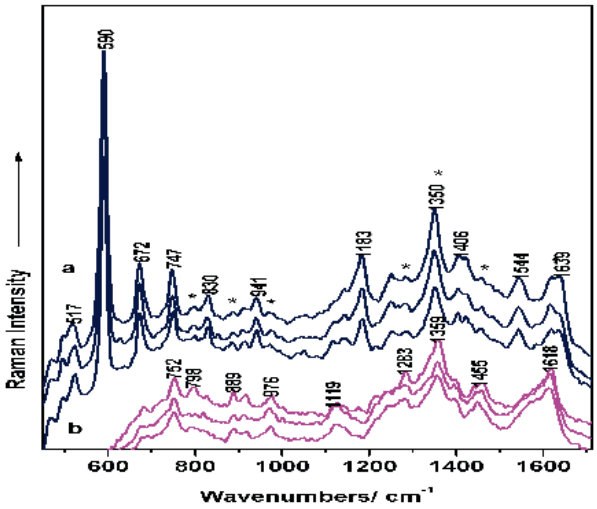


Fig. 3. SERS signal collected from tissue when the functionalized nanoparticles (CV-Ag nanotags) were inoculated into the mouse skin with B16 cells-induced melanoma (a) and SERS spectra from the unlabeled Ag nanoparticles (b). Skin tissue bands marked with " * "

Besides, SERS signal from the tissue molecular components was also observed. Moreover, additional bands appear which are absent from both the SERS signature of the label or that of the tissue, suggesting a clear interaction of the amino functional groups with specific receptors from the tissue molecular architecture. When applying a nanoemulsion formulation on the skin tissue, the spectral response revealed clear differences between the treated and untreated mice specimens, providing thus the proof of concept for in vivo SERS therapy monitoring. The nanoemulsion formulation based on betulin has been prior characterized using vibrational spectroscopy technique in order to probe the possibility to track its presence in tissue. Although the nanoemulsion bands were hampered by the tissue signal, the changes in the tissue spectral shape strongly suggested the structural perturbation induced by the betulin treatment [8].

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REFERENCES

1. Vo-Dinh T, Wang H.-N, and Scaffidi J, Plasmonic nanoprobes for SERS biosensing and bioimaging, J. Biophoton 2010; 3, 1–2: 89–102.
2. S. Cîntă Pînzaru, L. M. Andronie, I. Domsa I, et al. Bridging biomolecules with nanoparticles: SERS of colon carcinoma nd normal tissue J. Ram. Spectr 2008; 39 (3): 331-34.
3. Vo-Dinh T, Allain L, David R, et al. Cancer gene detection using SERS J. Raman Spectrosc 2002; 33 (7): 511-16.
4. Lyandres O, Yuen JM, Shah NC, et al. Diabetes Technology & Therapeutics 2008, 10(4): 257-265. doi:10.1089/dia.2007.0288.

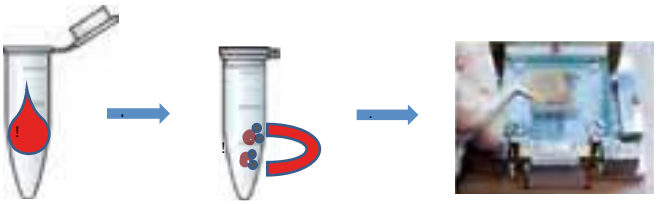
5. Larese F, Agostin FD, Crosera M, et al., Toxicol 2009; 255:33–37.
6. Kneipp J, Kneipp H, Wittig B et al. Novel optical nanosensors for probing and imaging live cells, Nanomedicine: Nanotechnology, Biology, and Medicine 2010; 6: 214–26.
[7]. S. Cîntă Pînzaru, A. Falamas, C. A. Dehelean, Molecular conformation changes along the malignancy revealed by optical nanosensors, J. Cell. Mol. Med. 2012, submitted.
[8]. A. Fălămaş, C. Dehelean, S. Ganta, M. Amiji, A. M. Cimpean, C. Cainap, S. Cîntă Pînzaru, J. Raman Spectr., submitted 2012.

"CHIP-BASED MOLECULAR PROFILING OF CIRCULATING TUMOUR CELLS FOR INDIVIDUALIZED CANCER TREATMENT"

SIBYLL POLLOK[1], Christine Steinbach[1], Karina Weber[1,2], Jürgen Popp[1,2]
[1]Institute of Photonic Technology Jena, Albert-Einstein-Str. 9, 07745 Jena, Germany
[2]Friedrich Schiller University Jena, Institute of Physical Chemistry and Abbe Center of Photonics, Helmholtzweg 4, 07745 Jena, Germany

A major challenge towards personalized cancer therapy is the availability of predictive biomarkers of sensitivity. Although, EGFR inhibitors yield anti-cancer activity e.g. in metastatic colorectal cancer, patients with mutated KRAS or BRAF do not benefit. Thus, the identification of cancer-specific mutations represents the basis for future targeted therapy.
In this context we present the development of a point-of-care detection system, based on a DNA-chip for the molecular sub-classification of KRAS and BRAF mutations in circulating tumour cells (CTCs). The system based on the affinity interaction of biotin-labeled hybridization products with streptavidin-conjugated horseradish peroxidase, which catalysed the deposition of silver between two micro-structured electrodes on the DNA-chip. Subsequently, the resulting gap-bridging is detected by either electrical or optical readout modes [1, 2].
Pivotal advantages of our biochip approach are firstly the rapid and parallel analysis of samples and secondly the feasibility of implementation in a miniaturized microfluidic platform for point-of-care applications.

Figure 1



blood sample with CTCs | CTC enrichment with magnetic beads | multiplex analysis of cancer-specific mutation

ACKNOWLEDGMENT
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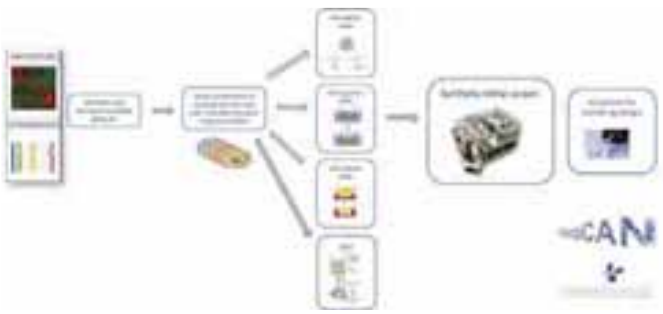
REFERENCES
[1] Seise, B., Brinker, A., Kretschmer, R., "Chip based detection system for the on-site analysis of animal diseases" Eng. Life Sci. 2011, 11, No. 2: 148–156
[2] Schüler, T., Kretschmer, R., Jessing, S., Urban, M., Fritzsche, W., Moeller, R., Popp, J., "A disposable and cost efficient microfluidic device for the rapid chip-based electrical detection of DNA", Biosensors & Bioelectronics, 2009, 25(1): 15-21

TARGETED FUNCTIONAL GENOMICS SCREEN FOR METASTASIS AND EMT MODULATORS IN BREAST CANCER

ANGELA RIEDEL[1,2], Monica Marie Blomstrøm[1,2], Steffen Schmidt[1,2], Mads Thomassen[1,3], Ines Block[1,2], Carolin Müller[1,2], Sidsel Bering Olsen[1,2], Pernille Lund Hansen[1,2], Aleksandra Jaskot[1,2], Jakub Trojnar[1,2], Helle Christiansen[1,2], Ann Knoop[5,6], Anne Bruun Krøigaard[3], Anne-Vibeke Lænkholm[7], Martin Bak[8], Jeanette Dupont Jensen[5,6], Petra Kioschis-Schneider[1,4], Torben A. Kruse[1,3], Jan Mollenhauer[1,2]
[1]Lundbeckfonden Center of Excellence in Nanomedicine NanoCAN, University of Southern Denmark, Odense, Denmark
[2]Molecular Oncology, University of Southern Denmark, Odense, DK
[3]Clinical Genetics, Odense University Hospital, Odense, Denmark
[4]Institute of Molecular Biology and Cell Culture Technology, University of Applied Sciences, Mannheim, Germany
[5]Department of Oncology, Odense University Hospital, Odense, DK
[6]Institute of Clinical Research, University of Southern Denmark, Odense, Denmark
[7]Department of Pathology, University Hospital of Copenhagen, Copenhagen, Denmark
[8]Department of Pathology, Odense University Hospital, Odense, DK

Metastatic spread still represents the major cause of death among women diagnosed with breast cancer, so that suppression of metastasis formation or causing selective lethality to metastatic breast cancer cells would represent a desirable goal. siRNAs can selectively address both corresponding target genes and miRNAs, thus represent a favorable option for the next generation of anti-cancer nanodrugs. The knowledge of targets to be addressed by this approach, however, is still limited.
To recover novel starting points for therapeutic approaches, we selected a set of 100 candidate genes and miRNAs from available molecular profiles of metastatic breast cancer and subjected these to systematic functional studies regarding their impact on breast cancer growth and invasion as well as their ability to cause the so-called epithelial-mesenchymal transition (EMT). According to current concepts EMT is tightly linked to both metastatic potential and the breast cancer stem cell (BCSC) phenotype. For this purpose, we utilize a unique system, which allows for the rapid creation of a large number of stable and standardized cancer cell lines. To this end, we identified numerous new modulators of breast cancer growth, invasion, and EMT, which may potentially qualify for the conception of siRNA-based nanodrugs. We further plan to schedule selected candidates from this panel for high-throughput screens to recover siRNAs causing synthetic lethality to metastatic breast cancer cells, using the robotic platform of the NanoCAN Center of Excellence.



DIRECT IN VIVO LABELING WITH SPION OF THE ENDOGENOUS CELLS IN THE ADULT RAT BRAIN

NINA ROGELIUS, Rene in ´t Zandt, Fredrik Olsson, Sarah Fredriksson.
Genovis AB, Lund, Sweden.

SUMMARY
Here, in this study we report the use of super paramagnetic iron oxide nanoparticles (SPION) to directly in vivo label the endogenous cells. In the adult rat brain, we have labeled the endogenous ventricular zone cells and demonstrate the ability to detect the labeled cells with MR, electron microscopy, and histology. For the detection of the labeled cell with histology both the fluorescence marker, Dy647 which is conjugated to the nanoparticle and also the iron within the particle have been used. The purpose of labeling the endogenous cells in vivo with SPION is to be able to study the cells fate or their migration over time in a live animal with MRI. At the injection site, the lateral ventricular, the labeled cells were detected in the wall during a 5 weeks' time period. The injection of nanoparticles in vivo didn't cause any change in the animals normal behavior and the surrounding tissue of the injection site were undamaged. Also, we have injected nanoparticles to skeletal muscle to directly label the cells and also these results confirm undamaged tissue and no change in behavior. The potential in using non-invasive imaging of endogenous cell using direct injection of nanoparticles could be useful in cell tracking studies over time and within the tissue.

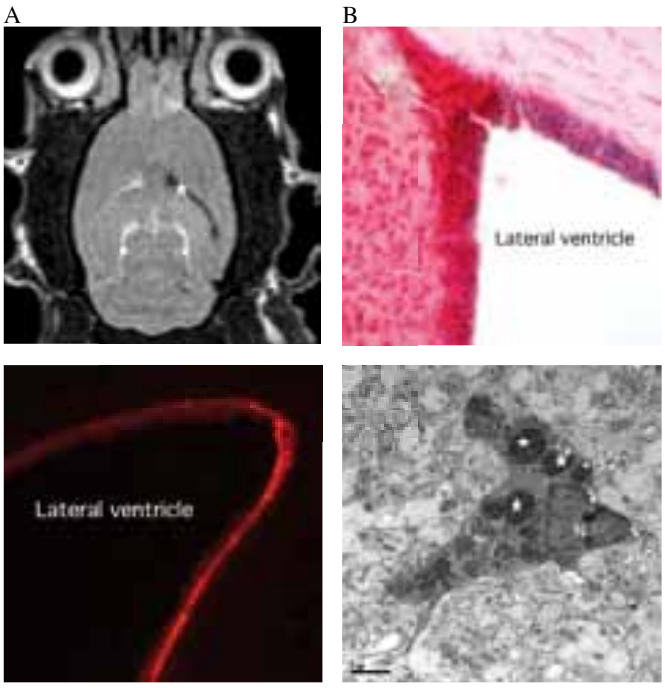


Figure text. Vizualisation of the cells in the adult brain by MRI, histology and TEM.The SPIOs were locally delivered to the lateral ventricle and imaged at 2.4T (figure A). The prescens of nanoparticels was confirmed with Prussian Blue staining (figure B) which stain the iron content. Also, the prescens of nanoparticles could be detected with fluorescens dye (Dy647) conjugated to the nanoparticles (figure C). Endosomal uptake of the SPIO was confirmed with TEM (figure D). Endosomes loaded with nanoparticles appear as dark islands, some of them highlighted with stars, N= nucleus.

RESULTS
MRI of the SPION labeled cells within the ventricular zone
One to five weeks post injections, SPION particles could be detected by MRI in the ventricular zone. The particle labeled cells are detected as dark area, in the horizontal view of brain by MR (figure A). At the injection time, 5 microliter was injected to the right lateral ventricle (marked with white star), 1 week post injection the entire ventricular wall was filled with particles. Still after five weeks, the labeled cells and the dark contrast from the SVZ, was clearly visible. In the contralateral side, no nanoparticles have been injected and serves as control side.

Histology analysis of the SPION labeled cells within the ventricular zone
Histology was performed on the brains to study the SPION labeled cells in the ventricular zone. Figure B shows an image from sections stained for iron with Prussian blue and counterstained with nuclear red, a stain for cell nuclei. The darker pink colored area comes from the high density of cells. In the ventricular area, nanoparticle labeled cells was present in the whole ventricular wall, which is illustrated by the dark blue color. Note that this in vivo labeling of the cells occurred spontaneously and without transfection agent. This labeling of the cells was still detectable 5 weeks post injection. The particle labeled cells could also be detected by the fluorescence from the dye (Dy647) conjugate to the particles. The figure C shows an image where the ventricular wall fluorescence in red from Dy647. This labeling overlap with the iron labeling, which indicate that the iron content in the cells and the fluorescence is at the same place.

EM of the SPION labeled cells in striatum
Electron microscopy of cells in the striatum of the rat brain directly labeled with nanoparticles. Endosomal uptake of the nanoparticles was confirmed with TEM. Endosomes loaded with nanoparticles appear as dark islands, some of them highlighted with stars, N = nucleus.

No adverse effects detected after injection of SPIONs
The particles have been injected to the skeletal muscles (musculus tibialis) and in two areas of the brain (the lateral ventricle and the striatum). The animals had normal behavior and curiosity. The fur was well-kept and no redness seen at the injection site. No visual weight loss and no decrease in the mobility could be seen. From the histology pictures no necrotic area around the nanoparticle labeled cells could be detected.

CONCLUSION
The findings in the current study show the ability that after direct injection of SPIONs in the lateral ventricle the labeled cells could be detected with MRI, histology, and electron microscopy. Cells were detectable for the time length of this study which was 5 weeks. The indication is that the nanoparticles injection didn't cause any toxicity. The aim to directly in vivo label cells with nanoparticles give the possibility to study the cells in live animal with MRI. This possibility to label and then follow the cells over time in live animals will be of great value in research of for example stroke and degenerative disorders such as Parkinson's disease. Not only the ability to follow over time but also the ability to spatially follow migrating cells is useful in cell fate mapping studies. For example, progenitor cells could maybe be identified and then follow the fate of a specific cell type spatially and over time.

METHODS
Adult Sprague-Dawley rats were stereotactically injected with 2-5 µl SPION (1 mg Fe/ml) to the lateral ventricle and the striatum. The iron oxide core of the SPIONs consists mainly of magnetite, Fe_3O_4, and the core diameter is approximately 11 nanometer. The cores are synthesized through a seed growth method by reducing FeO(OH) in 1-octadecene protected by oleic acid as described by (Yu et al). All three ingredients are mixed and then heated to 320˚C for 120 minutes under cooling and reflux of solvents. After 120 minutes the mixture is allowed to slowly cool down to room temperature. The hydrophobic cores are washed with diethyl ether and then coated with Distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000]. The SPION were conjugated with a fluorescent dye, Dy647 (Dy-647-NHS-Ester Dyomics, Gemany). The final coated nanostructures were washed with water before use. The MRI data were acquired on a Bruker Avance II system operating at 2.4T. A 3D volume was imaged using the turboRARE sequence (matrix size 128*128*128, field of view 40*40*40 mm3, 40 minutes scan time, fat suppression on). Following the MRI exam, animals were transcardially perfused with PBS followed by 4% PFA. The brains

were then aloud to sink in 30% sucrose for 1-3 days and then further processed for electron microscopy and histology analysis. The histological staining Prussian blue was used to detect the iron content in the labeled cells, cell nuclei are stained red (Shapiro et al).

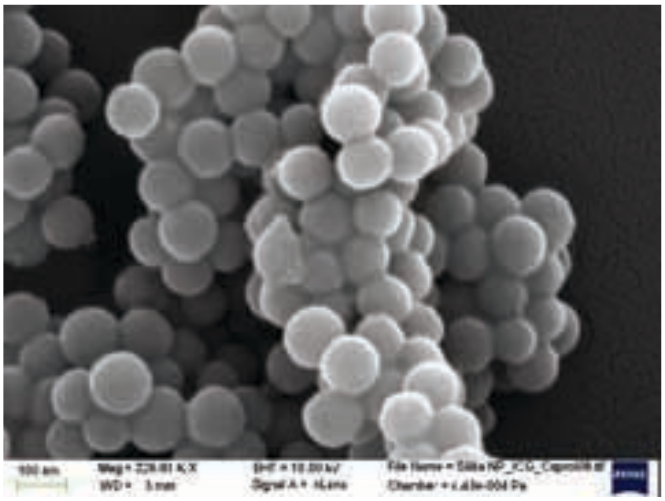
REFERENCE

Shapiro E. M. et al "Magnetic resonance imaging of the migration of neuronal precursors generated in the adult rodent brain". NeuroImage 2006, pages 1150-1157.
Yu W.W et al "Synthesis of monodiperse iron oxide nanocrystals by thermal decomposition of iron carboxylate salts" Chem. Commun. 2004, pages 2306-2307

BIOCOMPATIBLE SILICA NANOMATERIAL GRAFTED AMPHIPHILIC BLOCK COPOLYMER CONJUGATED WITH INDOCYANINE GREEN

ANDREA SCHÖNBÄCHLER[1], Olfa Glaied[1], Uwe Pieles[1], Martin Frenz[2], Michael Reinert[3], Meike Mevissen[4] and Jörg Huwyler[5]
[1]Institute of Chemistry and Bioanalytics, School for Life Sciences, University of Applied Sciences and Arts Northwestern Switzerland, Gründenstrasse 40, 4132 Muttenz, Switzerland.
[2]Department of Biomedical Photonics, Institute of Applied Physics, University of Bern, Sidlerstrasse 5, 3012 Bern, Switzerland.
[3]Department of Neurosurgery, Inselspital Bern, University of Bern, Freiburgstrasse 10, 3010 Bern, Switzerland.
[4]Institute of Veterinary Pharmacology & Toxicology, University of Berne, Länggass-Strasse 124, 3012 Berne, Switzerland.
[5]Division of Pharmaceutical Technology, Department of Pharmaceutical Sciences, University of Basel, Klingelbergstrasse 50, 4056 Basel, Switzerland.

Indocyanine green (ICG) has received increasing attention due to its wide spread application possibilities in various biomedical fields ranging from diagnostics to medical treatment. Most of these medical applications as for example laser-induced tissue soldering, where tissue is fused together by virtue of the ICG absorption, require a defined locally confined ICG concentration. One of the main drawbacks of ICG is its poor aqueous stability and its strong photo-degradation. One way to overcome these limitations can be achieved by incorporating ICG in a carrier delivery system.
Thus, the objective of this study was to develop a nanoparticle system for stabilisation of ICG in aqueous media to be used in biomedical applications. The stability of ICG can considerably be improved by encapsulating in a hydrophobic environment. Therefore we used Poly(ε-caprolactone), a semi-crystalline thermoplastic polyester obtained from ε-caprolactone monomer through anionic coordinated polymerization, being hydrophobic, biocompatible and biodegradable. Starting from a well defined silica core of approx. 80 nm, used as template for a 'grafting from' approach of ε –Caprolactone, ICG was embedded into the hydrophobic polymer coating. The use of a microwave reaction scheme gives well defined composite nanoparticles in high yield. The composite nanoparticles were extensively characterised by IR spectroscopy, DSC and SEM. Additionally, the polymer was studied with NMR and SEC. The change of the surface and the colloidal stability of the nanoparticles were followed by zeta potential.
The approach to synthesize ICG loaded particles demonstrates a new route to stabilize ICG by embedding in a hydrophobic biocompatible matrix. We obtained biocompatible nanoparticles containing a high ICG concentration that exhibit an excellent stability against photo-degradation and aqueous decomposition.



Picture 1: Poly(ε-caprolactone) coated silica nanoparticles with ICG embedded

ORIENTED NANOSTRUCTURES WITHIN THE HUMAN BRAIN UNCOVERED BY SCANNING SMALL ANGLE X-RAY SCATTERING

GEORG SCHULZ[1], Hans Deyhle[1,2], Oliver Bunk[2], and Bert Müller[1]
[1]Biomaterials Science Center University of Basel, Switzerland
[2]Paul Scherrer Institut, Villigen, Switzerland

INTRODUCTION

The human brain is one of the most fascinating structures in nature. Histology is so far the gold standard for the differentiation of the different structures on the cellular level with the limitation of the spatial resolution to a fraction of a micrometer.
Small-angle X-ray scattering (SAXS) 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. The 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.

METHODS

A human thalamus was extracted and fixed in formalin. 50 μm-thin slices were obtained by cryo-sectioning and mounted on glass slides, NISSL or myelin stained or transferred into polyimide sachets to prevent drying. The slices were measured at the cSAXS beamline using the photon energy of 11.2 keV and a specimen-detector distance of about 7.1 m. The X-ray beam was focused to 20 × 5 μm2 and scanned over the slice in 50 and 75 μm steps in the two directions perpendicular to the brain slice. The scattering signal was recorded using a PILATUS 2M detector.

RESULTS

The examination of the slices provides the local information on abundance, size, size distribution and preferential orientations of brain tissue nanostructures. The figure shows the results obtained from a non-stained human thalamus slice in the range between 8 and 9 nm. The color corresponds to the preferential orientations of the scattering signal perpendicular to the nanostructures (cp. color wheel), whereas the abundance of the latter is given by the brightness. The right image shows the signal obtained after the subtraction of the background signal. This corresponds to the second myelin peak shown in the radial intensity profile. Both images show no significant orientation inside the thalamus but in the adjacent tissues. The non-destructive method bridges the gap between the real space

optical techniques with micrometer resolution and large field of view and the reciprocal space scattering/diffraction techniques with nanometer resolution, but restricted field of view. In the future scanning SAXS and SAXS tomography will play a dominant role in the further development of nanomedicine and related fields.

REFERENCES

[1] M. De Felici, R. Felici, C. Ferrero, A. Tartari, M. Gambaccini, and S. Finet: Structural characterization of the human cerebral myelin sheath by small angle x-ray scattering. Physics in Medicine and Biology 53 (2008) 5675-5688.

[2] O. Bunk, M. Bech, T. H. Jensen, R. Feidenhans'l, T. Binderup, A. Menzel, and F. Pfeiffer: Multimodal x-ray scatter imaging. New Journal of Physics 11 (2009) 123016.

[3] B. Müller, H. Deyhle, D.A. Bradley, M. Farquharson, G. Schulz, M. Müller-Gerbl, and O. Bunk: Scanning X-ray scattering: Evaluating the nanostructure of human tissues. European Journal of Clinical Nanomedicine 3 (2010) 30-33.

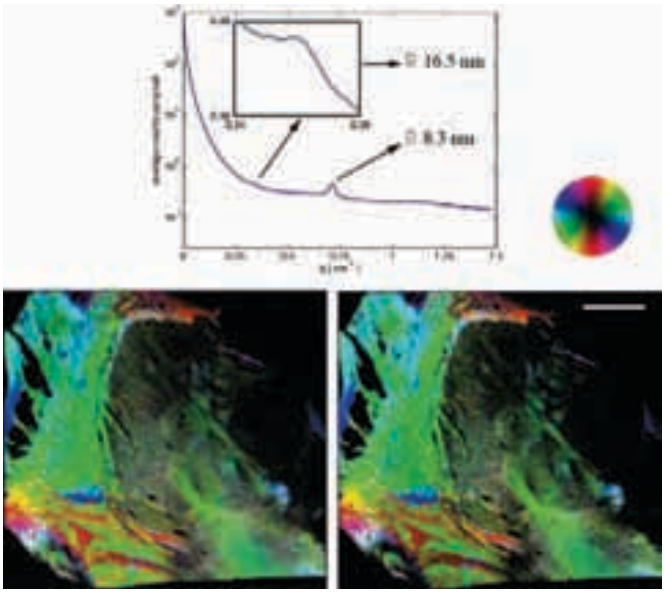


Figure: The images correspond to orientation of nanostructures in the ranges between 8 and 9 nm of a non-stained slice placed in a polyimide sachet. The bar corresponds to 5 mm. The radial intensity profile exhibits two peaks. The first peak corresponds to a typical myelin periodicity of 16.5 nm. The second peak corresponding to 8.3 nm is the second diffraction order signal. In the right image only the signal of the second myelin peak is shown.

FROM SYSTEMATIC PROFILING OF THE NANO-BIO INTERFACE TO AUTOMATED CELL AND TISSUE RESPONSE TESTS IN MICROFLUIDIC SYSTEMS

ROLAND H. STAUBER[1], Dominic Docter[1], Klaus Stefan Drese[2], Christoph Alexiou[3], Michael Maskos[2]

[1]Molecular and Cellular Oncology/Mainz Screening Center, University Hospital of Mainz, Germany

[2]Institut für Mikrotechnik Mainz GmbH, Mainz, Germany

[3]Else Kröner-Fresenius-Foundation-Professorship, Experimentelle Onkologie und Nanomedizin (SEON), Erlangen, Germany

Treating cancer by the application of (targeted) nanoparticle-based therapeutics is accepted as a highly promising approach in nanomedicine. At the same time, concerns about potential toxic effects of nanoparticles have been raised. Currently, (pre)clinical progress and public acceptance is hampered by the lack of standardized systematic platforms and point-of-care tests, allowing the rapid assessment of the anti-tumoral activity as well as potential adverse side-effects in nanomedicine. Consequently, we here present generalizable systematic approaches that provide evidence-based concepts for the assessment of nanomaterial-based anti-cancer drugs.

As in biological fluids, proteins associate with nanoparticles, leading to a protein "corona" defining the biological identity of the particle, a comprehensive knowledge of protein fingerprints and its dependence on nanomaterials properties is mandatory. First, we used label-free liquid chromatography mass spectrometry to present a comprehensive database of human blood plasma corona profiles of various nanoparticles, allowing a bioinformatic prediction of (patho)physiological effects in the blood system.

Second, to systematically explore the sheer number of potential nanoparticle variables that may influence their anti-cancer activities, we developed and employed a cell based high throughput screening platform to dissect structure-activity relationships of nanoparticles on several tumor cell models.

Finally, we present microfluidic concepts for the automated testing of cell and tissue response to nanoparticle-based therapeutics under standardized, robust and low-cost conditions.

A NOVEL MULTISTAGE NANOPARTICLE DELIVERY SYSTEM FOR OPTIMAL PENETRATION INTO SOLID TUMORS

TRIANTAFYLLOS STYLIANOPOULOS[1,2], Cliff R. Wong[3], Rakesh K. Jain[2], Moungi Bawendi[3], and Dai Fukumura[2]

[1]University of Cyprus, Nicosia, Cyprus

[2]Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

[3]Massachusetts Institute of Technology, Cambridge, MA, USA

The enhanced permeability and retention (EPR) effect has been a key rationale for the development of nano-scale carriers to solid tumors. As a consequence of EPR, nanotherapeutics are expected to improve drug and detection probe delivery, have less adverse effects than conventional chemotherapy, and thus, result in improved detection and treatment of tumors. Physiological barriers posed by the abnormal tumor microenvironment, however, can hinder the homogeneous delivery of nanomedicine in amounts sufficient to eradicate cancer. These barriers are likely to be responsible for the fact that only three nanoparticle formulations (i.e., Doxil®, DaunoXome® and Abraxane®) have been clinically approved to-date for the treatment of solid tumors, and the increase in overall survival is modest in many cases [1]. To effectively enhance the therapeutic outcome of cancer patients by nanotherapeutics, we have to find ways to overcome these barriers. One possibility is to exploit the abnormal tumor microenvironment for selective and improved delivery of therapeutic agents to tumors. We propose a multistage nanoparticle delivery system as a potential means to enable uniform delivery throughout the tumor and improve the efficacy of anticancer therapy.

The multistage nanoparticle delivery system has an initially size of ~100 nm, similar to the size of Doxil® and Abraxane®, which allows for preferential accumulation to tumor tissue through the EPR effect. Once the particle reaches the tumor, it shrinks to a size of ~10 nm that can more effectively diffuse throughout the tumor interstitial space. To achieve this size change in tumor tissues, the methodology makes use of matrix metalloproteinase enzymes (MMPs) that are overexpressed in the microenvironment of many tumors to degrade the core of a gelatin nanoparticle so that smaller diagnostic or therapeutic agents can be released from its surface [2] (Figure 1). Our delivery system is composed of the gelatin nanoparticle carrier, 100 nm in diameter, containing 10 nm quantum dots (QDs). These QDs are a stand-in for therapeutic or imaging nanocarriers. We optimized the size-changing capability of our multistage gelatin nanoparticle and characterized this change both in vitro and in vivo. The optimized structure was able to release cleaved QDs of only 10 nm in size, and we termed the resulting nanostructures multistage quantum dot gelatin nanoparticles (QDGelNPs). We investigated the ability of MMP-2 to change the size of QDGelNPs in vitro using gel filtration chromatography. We assessed that 50% of the QDs were released in ~1.5 hours and the percent of freed QDs saturated at ~90% (Figure 2A), regardless of longer incubation times or addition of more MMP-2.

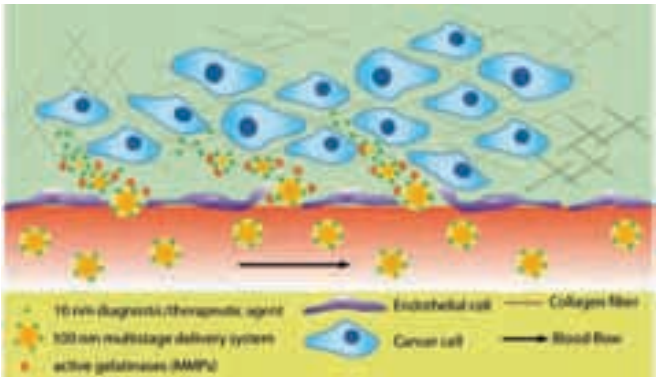


Figure 1. Schematic representation of the multistage nanoparticle delivery system.100 nm primary gelatin particles selectively accumulateto tumor tissue due to EPR effect. Enzymatic degradation of gelatin releases secondary 10 nm diagnostic or therapeutic particles.

We repeated this experiment with the incubation time kept constant at 12 hours but the amount of MMP-2 was varied (Figure 2B). Under this condition, only ~25 ng of MMP-2 was necessary to release 50% of the QDs. These results demonstrated the MMP-2 triggered size change occurred in an efficient manner.

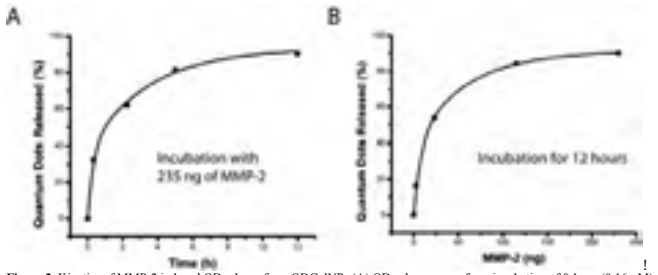


Figure 2. Kinetics of MMP-2 induced QD release from QDGelNPs (A) QD-release curve from incubation of 0.1 mg (0.16 μM) of QDGelNPs with 230 ng of MMP-2 (B) QD release from incubation of 0.1 mg of QDGelNPs for 12 hours with varying amounts of MMP-2.

We also evaluated the distribution of the multistage nanoparticles in the tumor milieu using a transparent window model and time-lapse multiphoton microscopy. We co-injected QDGelNPs and control silica quantum dots particles (SilicaQDs) intratumorally in the HT-1080 tumor, which is known to exhibit high MMP-2 activity. Multiphoton microscopy revealed a marked increase in QDGelNPs penetration into surrounding tumor tissue as compared with the non-cleavable SilicaQDs control, confirming a substantial enhancement in interstitial transport associated with size change (Figure 3). At 6 hours post-injection, the QDGelNPs had penetrated up to ~300 μm from the injection site while the SilicaQDs control exhibited little or no dissemination from its initial location.

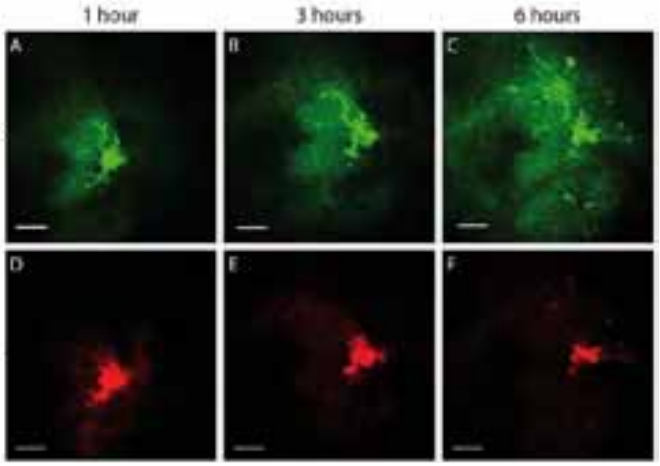


Figure 3. In Vivo comparison of multistage and single-stage nanoparticles: In vivo images of QDGeINP-multistage (green, A_C) and SilicaQD-single-stage (red, D-F) nanoparticles 1 (A,D), 3 B,E), and 6 h (C,F) after intratumoral co-injection into HT-1080 tumor. The multistage nanoparticles had penetrated up to ~300 μm from the injection site while the single-stage nanoparticles control exhibited little or no dissemination from its initial location. Scale bars, 100 μm.

Therefore, we have shown the proof of principle of this strategy by administering the multistage nanoparticles directly into the tumor. The gelatinase enzymatic activity in HT1080 soft tissue sarcomas was sufficient to degrade the gelatin and release 10 nm quantum dots that were conjugated to the nanoparticle. The next steps of the development of this multistage nanoparticle delivery system would be the optimization of the system via systemic administration, and, ultimately, the creation of drug-loading multistage nanoparticles, as well as the evaluation of their in vivo efficacy. These are necessary steps for the true evaluation of the hypothesis and for potentially moving toward clinical application.

[1] Jain RK and Stylianopoulos T, "Delivering Nanomedicine to Solid Tumors", Nature Reviews Clinical Oncology, 7, 653-664(2010).
[2] Wong C, Stylianopoulos T, et al., "Multistage Nanoparticle Delivery System for Deep Penetration into Tumor Tissue", PNAS, 108 (6), 2426-2431(2011).

IN-VITRO AND IN-VIVO CHARACTERISTICS OF FUNGAL CHITOSAN AS A NOVEL NANO-CARRIER FOR VIRUS DELIVERY SYSTEMS

FARZANEH TAJDINI[1], Mohammad Ali Amini[1], Ali Rezaei Mokarram[2], Morteza Taghizadeh[2], Hamideh Amini[3]
[1]Department of Microbiology, Faculty of Veterinary Medicine, Islamic Azad University, Karaj Branch, Karaj, Iran
[2]Department of Biotechnology, Razi Vaccine and Serum research Institute, Karaj, Iran
[3]Department of Microbiology, Faculty of Science, Islamic Azad University, North-Tehran Branch, Tehran, Iran

INTRODUCTION

During last two decades, soluble and particulate carriers prepared using chitosan have received particular interest for the delivery of proteins and antigens via mucosal routes. Recently, since commercially production of chitosan from shrimp, lobster and crab shell chitin deacetylation has limited potential for industrial acceptance because of seasonal and limited supply, several studies have investigated the capability of cell walls of fungi as a suitable alternative source. The fungal approach has benefit of easy handling, harvesting and control to produce high quality chitosan. However, the bio-capability of this fungi-originated chitosan has not been evaluated yet. The aim of this study was to investigate the ability of fungal CS as a new source to use as a nanocarrier. Besides, the potential of the polymer to encapsulate macrostructure proteins such as Food and Mouth Disease virus (FMDv) and stimulate both humoral and mucosal immune responses were studied and compared with those of low molecular weight chitosan (Commercial shrimp chitosan).

METHODS

Fungal chitosan extraction

The fungal biomasses obtained from cultivation of Rhizomucor miehei ATCC 26282 and a soil isolate of Mucor racemosus. Chitosan was extracted from dried mycelia based on Tajdini et al. method [1]. Chitosans elicited from R. miehei and M. racemosus were coded in the text as FCS1 and FCS2 ,respectively.

1H NMR analysis

The deacetylation degree (DD) of fungal CS were determined by the FT-NMR Varian Unity Plus spectrometer at 400 MHz. Samples were prepared by dissolving the CS in D2O/trifluoroacetic acid (1000:1; v/v) solution.

Preparation and in-vitro characterization of chitosan nanoparticles

CS nanoparticles were prepared according to the modified Vila et al. method [2], base on the ionotropic gelation of CS upon contact with the TPP anions. In order to avoid the denaturing of viruses in CS solutions, the pH was adjusted by NaOH 0.1 M up to 6.0. FMDv-loaded nanoparticles were prepared by adding 1ml of TPP solution containing (5×105 TCID50) of inactivated FMDv solution. The freeze dried samples were stored in tight closed vials at -20 °C for

more investigations.

The mean particle size and zeta Potential of nanoparticles were determined by Dynamic Light Scattering (DLS) technique, using a Zetasizer SZ3000 (Malvern instrument, Worcestershier, United Kingdom), and the zeta potential was determined by laser Doppler electrophoresis using the same apparatus.

The encapsulation efficiency of FMDv in all CS nanoparticles were determined by evaluation of free viruses in the supernatant after centrifugation at 16,000 g for 20 min at 4 °C using micro-complement fixation test (micro-CFT).

$$\%FMDv\ encapsulation\ effeciency = \frac{Total\ viruses - Free\ viruses}{Total\ viruses} \times 100$$

The in-vitro release behavior of fungal chitosans were determined by centrifugation of the FMDv loaded Fungal Chitosan nanoparticles at 10,000 g for 30 min at predetermined time. The supernatant was collected and the nanoparticles were re-suspended in fresh medium and agitated. The amount of released viruses was determined using micro-CFT.

In-vivo characterization of fungal chitosans

The in-vivo evaluation of the CS formulations was assessed in guinea pig following intranasal immunization at days 0, 7 and 14.

RESULTS AND DISCUSSION

Results showed that fungal chitosan nanoparticles with high loading efficacy (>94–97%), particle size within the range of 220–240 nm with positive charges (between 7.9 mV and11.5 mV) were obtained. The DD of CS1 and CS2 were calculated by the following equation:

$$DD = \left(1 - \left(\frac{1}{3}HAc / \frac{1}{6}H2 \rightarrow 6\right)\right) \times 100$$

Hence, H-Ac is represented to area under curve (AUC) of the peak at 1.8-2 ppm and H2→6 is represented to AUC of the H2→6 in glucose skeleton (The peak at 3.2-4 ppm) (Figure1). DD value calculated for FCS1 and FCS2, based on the equation, were 98.6% and 97.1%, respectively.

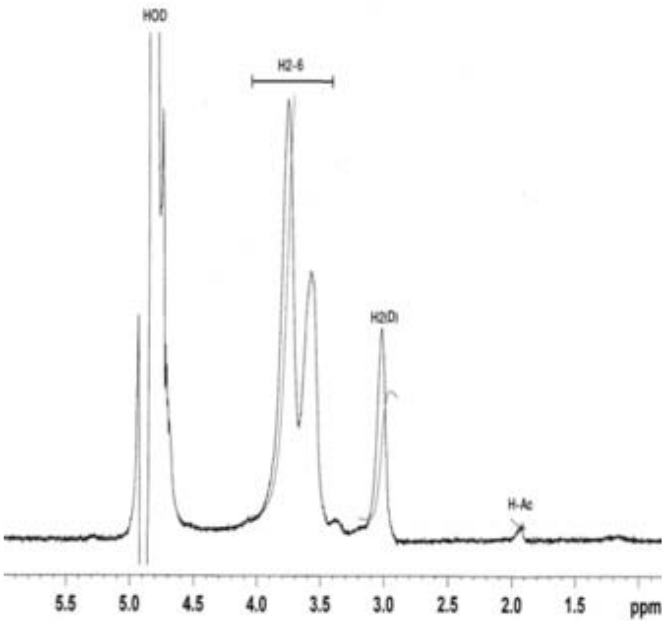


Figure 1. 1H NMR spectrum of chitosan from M. racemosus.

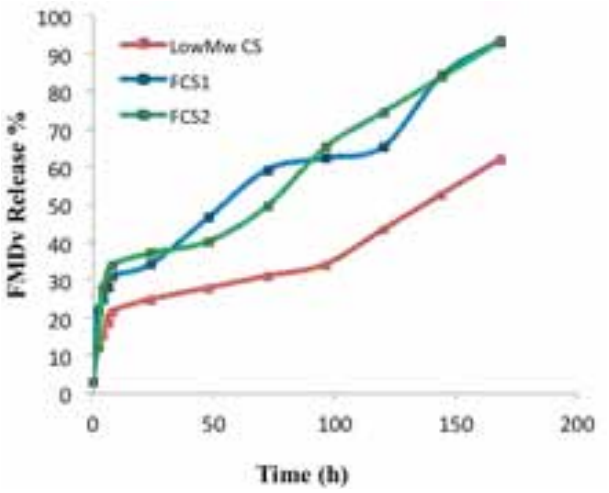


Figure 2. In vitro release of FMDv from fungal and low MW chitosans nanoparticles

All formulations (for FCS1, FCS2 and low Mw chitosan) exhibited a biphasic release profile (figure 2). A rapid release over the first 8 hrs, followed by a slow release for up to 7 days was noted. A faster release was observed for fungal chitosans which could be attributed to their smaller polymer chain length (lower MW). FMDv-loaded chitosan nanoparticles elicited an increasing and long-lasting humoral immune response (IgG concentrations) as compared to the fluid vaccine. As it could be observed from figure 3, the IgG titers obtained from chitosan nanoparticles were significantly higher compared to that from free-virus formulation. Besides, the total amount of IgG titer elicited from fungal chitosans were significantly higher to that of low Mw chitosan which could be based on their smaller particle size and consequently, their better penetration from mucosal barrier.

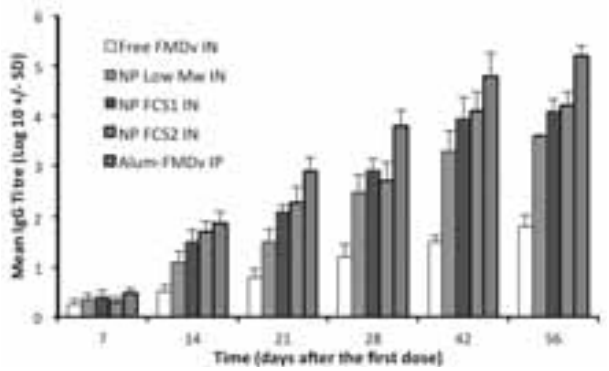


Figure 3. Anti FMDv IgG end-point titers in guinea pig serum after intranasal administration of 5×106 TCID50 incorporated in CS nanoparticles on days 0, 7 and 14

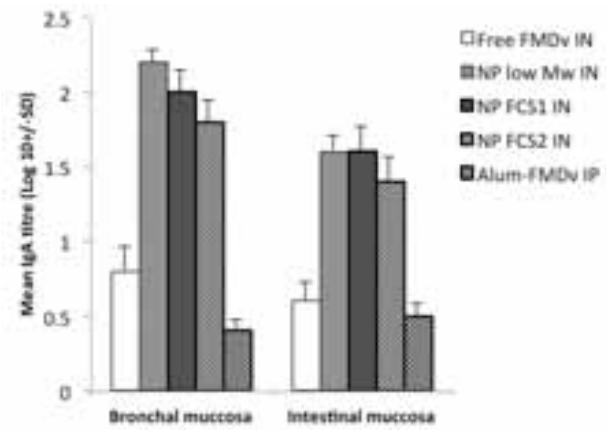


Figure 4. Anti FMDv IgA end-point titers in guinea pig tissues after intranasal administration of 5×106 TCID50 incorporated in CS nanoparticles on days 0, 7 and 14 (n=5).

Similarly, the mucosal response (IgA levels) at 2 months post-administration of FMDv-loaded chitosan nanoparticles was significantly higher than that obtained for the fluid vaccine and free-viruses (Figure 4). In conclusion, the fungal chitosan nanoparticles obtained from this study can be considered as a novel promising source of chitosan in vaccine delivery systems.

REFERENCES

1. Tajdini F et al. Int J Biolog Macromol. 2010; 47, 180–183.
2. Vila A et al. Eur J Pharm Biopharm. 2004; 57, 123–131.

REPORTER CELLS FOR CANCER STEM CELL-TARGETING NANODRUG SCREENS

JAKUB TROJNAR[1,3], Steffen Schmidt[1,2], Angela Riedel[1,2], Ines Block[1,2], Helle Christiansen[1,2], Aleksandra Jaskot[1,2], Carolin Müller[1,2],Markus List[1,2],Pernille Lund Hansen[1,2], Sidsel Bering Olsen[1,2], Monica Marie Blomstrøm[1,2], Marlene Lemvig Pedersen[1,2], Daniel Sdogati[1,2], Martin Røssel Larsen[3], Jan Mollenhauer[1,2]
[1]Lundbeckfonden Center of Excellence in Nanomedicine NanoCAN, University of Southern Denmark, Odense, Denmark
[2]Molecular Oncology, University of Southern Denmark, Odense, Denmark
[3]Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense, Denmark

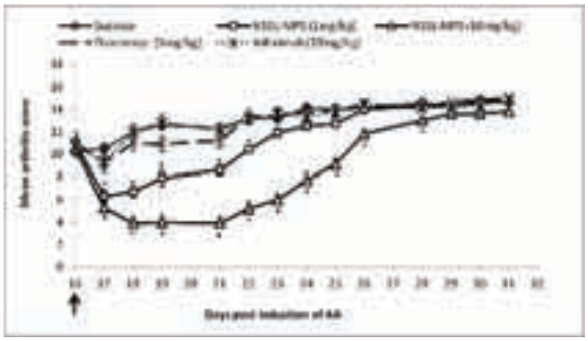
There is strong interest to target cancer stem cells (CSCs) for clinical treatment, since these cells are thought to be resistant against conventional chemotherapies and to cause tumor relapse and metastasis. Nucleic acid-based nanodrugs offer for targeting CSCs and simultaneously to achieve a personalization of cancer treatment. However, a challenging issue remains that CSCs represent a rare population of only less than one or few percent of the cells within a tumor. Moreover, even sorted CSCs differentiate rapidly while in culture due to asymmetric cell division, which in turn decelerates downstream studies and systematic screens for the identification of the new CSC-targeting agents. To facilitate analyses with breast CSCs (BCSCs), we aim at setting up different reporter systems using a targeted single integration into the cellular genome. Using these reporters, we can track the individual fate of BCSCs in vitro, e.g. conversion of normal (non-BCSC) cancer cells into BCSCs. Monitoring this process by fluorescence microscopy may assist the conception of nanodrugs that target BCSCs or that block the conversion events.

STERICALLY STABILIZIED NANO-LIPOSOMES HAVING FAVORABLE PHARMACOKINETICS AND CONTROLLED DRUG RELEASE RATE FOR MEDICAL APPLICATIONS

KEREN TURJEMAN*, Rina Ulmansky**, Pablo Kizelsztein*, Yuval Avnir*, Galia Katzavian**, Michal Harel**,
Yaakov Naparstek** and Yechezkel Barenholz*
*Laboratory of Membrane and Liposome Research, Hebrew University-Hadassah Medical School, Jerusalem, Israel
**Department of Medicine, Hadassah University Hospital, Hebrew University-Hadassah Medical School, Jerusalem, Israel

Twenty years ago the use of sterically stabilized nano-liposomes (nSSL) for intravenous drug delivery was introduced. The first such product approved by the FDA was the anticancer nano-medicine DoxilTM, developed in our laboratory. Our current research is focused on the use of nanotechnology to improve nSSL performance as a drug delivery system to treat diseases such as cancer and diseases having an inflammatory component, including multiple sclerosis (MS), rheumatoid arthritis (RA) and amyotrophic lateral sclerosis.
For this we are developing two different liposomal drugs: (1) liposomal glucocorticosteroids (nSSL-GCs) used systemically and subcutaneously as anti-inflammatory and anti-autoimmune therapy, and (2) a liposomal antioxidant, tempamine (nSSL-TMN).

Glucocorticosteroids are the drugs of choice in many diseases with inflammatory components. However, in many cases the efficacy of these drugs is not good enough and the drugs are highly toxic.
Natural antioxidants do not readily cross the blood-brain barrier. In addition, most antioxidants in their free form undergo fast clearance due to rapid chemical degradation in plasma and fast elimination via the urine. Therefore, they must be administered in very high doses.
To overcome some of these obstacles, we prepared ~80-nm pegylated nano-liposomes remote loaded with the "water-soluble" amphipathic weak acid steroid prodrugs methylprednisolone hemisuccinate sodium salt and betamethasone hemisuccinate sodium salt, or the amphipathic weak base nitroxide antioxidant TMN. Our results from 2 different murine models: experimental autoimmune encephalomyelitis, an accepted animal model for the neurodegenerative disease MS and adjuvant-induced arthritis, an accepted model for RA, clearly show that these formulations have therapeutic efficacy much superior to the free drugs and to most drugs currently used to treat these diseases. These nSSL selectively accumulate at sites of enhanced vascular permeability such as inflamed tissues. Accumulation of drug-loaded nSSL at these sites, followed by drug release there, explains the superior therapeutic efficacy of these nanomedicines.



(Ulmansky, Turjeman, et al. 2012, J.Control. Release, in press.)

The effect of a single injection of NSSL-MPS and TNF-α antagonists on AA. Rats were treated with NSSL-MPS (1 or 10 mg/kg), Etanercept (5 mg/kg), Infliximab (30 mg/kg) and sucrose-buffer (control). Etanercept was injected s.c.; NSSL-MPS, Infliximab, and sucrose-buffer were injected i.v..
Results are means ± SEM of n = 10. * $p < 0.05$ compared to control treatment.

EMULSOMES MODIFIED WITH AN S-LAYER FOR LIPOPHILIC DRUG DELIVERY

MEHMET H. ÜCISIK, Seta Küpcü, Uwe B. Sleytr, Andreas Breitwieser, Nicola Ilk and Bernhard Schuster
Department of NanoBiotechnology, University of Natural Resources and Life Sciences, Vienna
Muthgasse 11, 1190 Wien, Austria

Emulsome is a lipoidal vesicular system with an internal solid fat core surrounded by phospholipid bilayers. Due to its structural design, emulsomes embody the advantages of both polymeric nanoparticles and fat emulsions, thereby offering high loading capacity and prolonged drug release. Considered as a nanocarrier, emulsomes are especially suitable for the delivery of lipophilic therapeutic drugs, as well as lipophilic anti-cancer agents. Like liposomes, emulsomes are accessible for surface modifications and functionalization for controlled targeting and enhanced circulation in the body.

The current study constitutes a further development of emulsomes to design multi-functional nanocarrier systems which fulfill important features for pharmaceutical applications like stability, bioavailability, targeting and delivery, and ability to serve as imaging/contrast agents for tracking in-vivo. Essentially, the functionality of the nanocarriers is determined by the correct orientation and localization of the moieties in the nanometer scale on the surface. By mimick-

ing the nature's solution and design, crystalline bacterial cell surface (S-) layers are capable to fulfill this key requirement (Figure 1). Hence, the outermost shell of emulsomes comprising of a crystalline S-layer lattice provides functional groups in well-defined positions and orientation accessible for specific binding, e.g. of human immunoglobulin G (IgG).

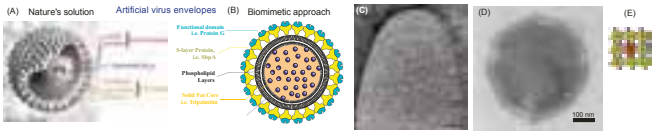


Figure 1. (A) Schematic drawing of virus envelope; (B) Schematic drawing of emulsome coated with S-layer fusion protein; (C) Electron micrograph of a freeze-etched preparation of whole cells of Lysinibacillus sphaericus CCM 2177 (SbpA) revealing a square S-layer lattice [3] (Bar: 100 nm); (D) Electron micrograph of a negative-stained emulsome sample coated with rSbpA-GG revealing the same square S-layer symmetry; (E) Schematic drawing of square (p4) lattice symmetry where four identical subunits compose one morphological unit.

The poster presentation illustrates the promising potential of emulsomes coated with an S-layer lattice and gives information on the recently achieved progress. In brief, a production methodology for emulsomes with a mean size of 300 nm and positive surface charge was developed. The recrystallization of S-layer proteins on emulsomes was tracked by dynamic light scattering (DLS) analysis indicating an alteration from a positive to a negative surface charge. Furthermore, the S-layer lattice was explored by transmission electron microscopy (TEM) where the lattice symmetry of the S-layer on the emulsomes was clearly displayed. For tracking purposes, emulsomes were coated with genetically modified S-layer fusion proteins to showcase fluorescent domains, e.g. enhanced fluorescent green proteins (EGFP). Furthermore, for targeting purposes, emulsomes were coated with S-layer fusion proteins presenting specific binding domains, e.g. protein G for binding IgG. The specific binding affinity of the designed nanocarriers was evaluated by using antibody assays like anti-IgG gold conjugates and fluorescent human IgG, separately. To sum up, S-emulsomes constitute multi-functional nanocarriers and are thus promising architectures with novel intrinsic features for drug targeting and delivery systems.

VARIOTHERM INJECTION MOLDED MICRO-CANTILEVER ARRAYS FOR SENSING

PRABITHA URWYLER[1,5], O. Haefeli[2], J. Köser3, H. Schift[1], J. Gobrecht[1,2], F. Battiston[4], B. Müller[5]
[1]Laboratory for Micro- and Nanotechnology, Paul Scherrer Institut, Villigen PSI, Switzerland
[2]Institute of Polymer Engineering, School of Engineering, University of Applied Sciences Northwestern Switzerland, Windisch, CH
[3]Institute of Chemistry and Bioanalytics, School of Engineering, University of Applied Sciences Northwestern Switzerland, Muttenz, CH
[4]Concentris GmbH, Basel, Switzerland.
[5]Biomaterials Science Center, University of Basel, Basel, CH.

OBJECTIVE

Micro-fabricated cantilevers, similar to those used in scanning probe microscopes, have become increasingly popular as transducers in biomedical sensors. Cantilever sensors detect surface stress created by the interaction of analytes with functional sensor surfaces. In the field of biomedicine, silicon-based micro-cantilevers are applied, but they are often too expensive for single use, due to their clean-room based micromachining processes. Polymer materials offer tailored physical and chemical properties, including biocompatibility that can be combined with low-cost mass production. Variotherm injection molding technique was employed to fabricate low-cost polypropylene micro-cantilever arrays with dimensions in the micrometer range with an aspect ratio as large as 10. Static deflection of the gold coated micro-cantilevers was characterised with heat cycling and self-assembled monolayer formation. With further functionalisation these micro-cantilevers were used for bio-chemical sensing.

METHODS

We have applied variotherm injection molding (IM) using metal molds made by laser ablation for the development and fabrication of disposable polymeric micro-cantilever (MC) arrays with 500 µm long and 100 µm wide cantilever beams. As polymer, an easy flow grade polypropylene (PP Metocene HM 648T) was used for all tests. Using variotherm IM process parameters, a complete filling of high-aspect-ratio micro-cavities was achieved for PP. Flash was observed for some shots as seen in Fig 1. The parts were manually removed from the mold and hence no mold releasing agent was used. To functionalize cantilevers with receptor molecules along with ensuring sufficient reflectivity of the laser signal, the PP MCs were gold coated on the flat smooth side using a thermal evaporator. The array of eight MCs each 500 µm long, 100 µm wide and 22 µm thick was functionalised by means of the Cantisens® FU-401 functionalization unit. The MCs 1, 2, 5, 6 were functionalised with a ss DNA oligonucleotide "Nl4-3" sequence, and MCs 3, 4, 7, 8 with "Sf162". All measurements were done using the Cantisens® Research platform. The experiments were conducted at a temperature of 30 °C, with a constant flow (0.42 µl/s) of a 1M NaCl buffer solution. The sample solution used in this experiment was 1 µM complementary Sf162 diluted in the 1M NaCl.

RESULTS

The thermal behavior of the MCs was supported via the heat tests. The heat tests included a temperature cycle from 25°C to 30°C and 25°C to 35°C. For a temperature difference of 5K and 10K, the maximum deflection for PP MCs in water correspond to (365 ± 20) nm and (800 ± 50 nm) respectively (Fig 2). Self-assembly of thiol monolayers on gold surface is a benchmark experiment for MCs. The thiols have a high affinity for the gold-coated surface of the MCs and bond to the gold forming a densely-packed self-assembled monolayer. The deflection caused by injection of 0.1µM mercaptohexanol (MCH) is shown in Fig 3. The difference of the deflection signals from the reference MCs (1, 2, 5, 6) and the signal MCs (3, 4, 7, 8) is shown in Fig 4. The first sample injection of the complementary Sf162 sequence gives a 7 nm signal, which is comparable to the signals achieved with Si cantilevers [1]. A second injection of the same complementary sequence was a control for saturation from the first injection and led to a 1.5 nm differential signal.

Fig. 1: Optical micrograph of a variotherm injection molded PP micro-cantilever array. Scale bar 100 µm.

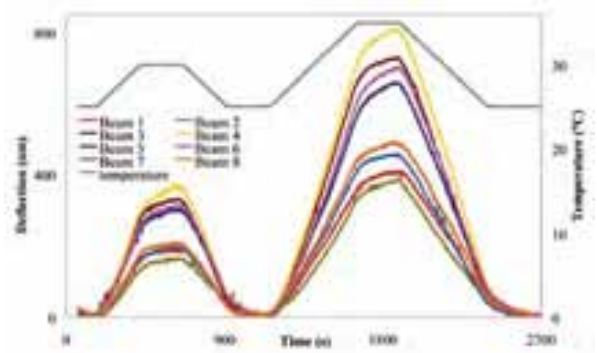


Fig. 2: Heat test of variotherm molded PP micro-cantilever array

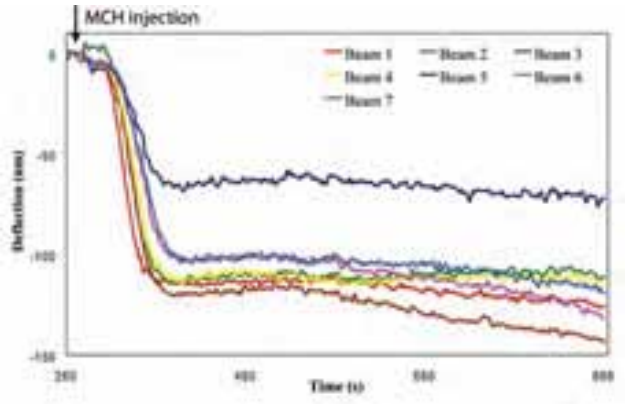


Fig. 3: Chemisorption of MCH on gold coated PP micro-cantilevers.

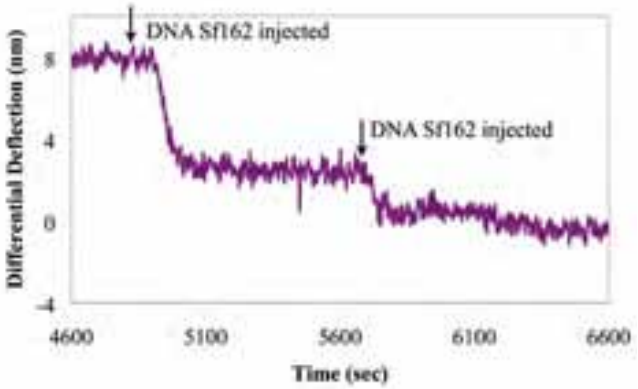


Fig.4: Deflection upon hybridisation of the complementary sequence.

CONCLUSIONS

The heat and functional tests imply that these polymeric MCs are mechanically compliant for use in biochemistry and biomedicine. The deflection signal with the variotherm injection molded MCs are relatively bigger as compared to their predecessors [2]. Polymer micro-cantilever arrays can be used to detect specific DNA sequences. These MCs can be surface structured using the hybrid technology described previously [2], which can enhance the amplitude of the deflection signal. Surface structuring also enhances cell adhesion and cell spreading, which is vital for further applications including measurement of contractile cell forces [3]. Thus, the disposable cantilever array sensors will support the selection of advanced surface-modified substrates and medical implant surfaces, along with opening more applications in the field of biomedicine.

REFERENCES

1. Köser J, Shahgaldian P, Bammerlin M, Battiston F, Pieles U. Journal of Physics, 2007.
2. Urwyler P, Schift H, Gobrecht J, Häfeli O, Altana M, Battiston F, Müller B. Sensors and Actuators A , 2011
3. Köser J, Gobrecht J, Pieles U, Müller B. European Cells and Materials 16, 2008.

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CELL MEMBRANE DISRUPTION AND CYTOSKELETON STRESS CONTRIBUTE TO THE CYTOTOXICITY OF CARBON NANOTUBES: A FOCUSED STUDY BY ATOMIC FORCE MICROSCOPY

LIJUN WANG, Dun Liu, Zhigang Wang and Alfred Cuschieri
Institute for Medical Science and Technology (IMSaT), Wilson House, 1 Wurzburg Loan, University of Dundee, DD2 1FD, UK

ABSTRACT

The interaction between multi-walled carbon nanotubes (MWCNTs) and human epithelial cancer cells (MCF-7) was investigated using advanced atomic force microscopy (AFM) techniques. This paper presents data which indicate that cell membrane damage and cytoskeleton stress induced by MWCNTs are closely related to MWCNT's cytotoxicity. The results should provide new information for better understanding of nanotoxicity of carbon nanotubes. Our reported methodology and AFM techniques could facilitate research on the dynamic cellular responses to nanomaterials.

INTRODUCTION

Carbon nanotubes (CNTs) are emerging nanotechnology materials that hold great promise in biomedical applications including drug delivery and cancer therapy [1, 2]. One of the key advantages that CNTs offer is their ability to cross biological barriers essential for the delivery of diagnostic and therapeutic molecules. As with any potential therapeutic or diagnostic nanomaterials, the toxicological impact and safety profile of CNTs has drawn considerable attention [1, 3, 4]. The interaction between CNTs and living organisms is still in the early stage of CNT nanotechnology and extensive research is needed before the clinical translation of these nanomaterials for the diagnosis and treatment of life-threatening disorders. CNT-cell contact could trigger stress responses in cell membrane and cytoskeletal network. We hypothesized that these responses may cause disruption of cell membrane and changes of cytoskeletal architecture and hence alteration in cell mechanical properties such as elasticity. We set out to explore the potential of the latest advances in atomic force microscopy (AFM) techniques to analyse the cell behaviour of MCF-7 cells during exposure to CNT. In this study we used 99% purified MWCNTs of two lengths: <1.3 μm as short- (S-MWCNTs) versus 3-8 μm as long-MWCNTs (L-MWCNTs). These have been shown in our previous study to demonstrate length-dependent cytotoxicity by end-point assays [5]. In contrast to traditional end-point cytotoxicity measurements in which assay components are often interfered by CNTs, AFM possesses unique versatile capacity in evaluating cell physiological status in a label-free, non-destructive way and therefore provides mechanistic analysis for cell dynamic responses to CNT exposure.

In this paper, we report the characterization by AFM of cell morphological and mechanical properties in response to MWCNTs. The characteristic properties observed by AFM are correlated with the barrier function of cell membrane.

RESULTS AND DISCUSSION

Cell membrane topography affected by MWCNT incubation: By virtue of AFM's high imaging resolution - nm scale, we were able to observe the subtle cell membrane topographic changes after MWCNT incubation. S-MWCNT bundles can be identified on the cell surface (Fig 1). Cells treated by S-MWCNTs generally remained intact with smooth surface comparable to control cells. However, when incubated with L-MWCNTs, significant changes in membrane topography were observed: the cell membrane became rougher compared to untreated cells, with some jagged structures and small pores; by 24 hr some line structures could be identified on the cell surface, which may be the result of stressed, rearranged cytoskeleton or MWCNT bundles (Fig 1). The latter is however unlikely as S-MWCNTs have been shown to have similar cellular uptake as L-MWCNTs [5]. This topographic change is also unlikely due to endocytosis for the same reason.

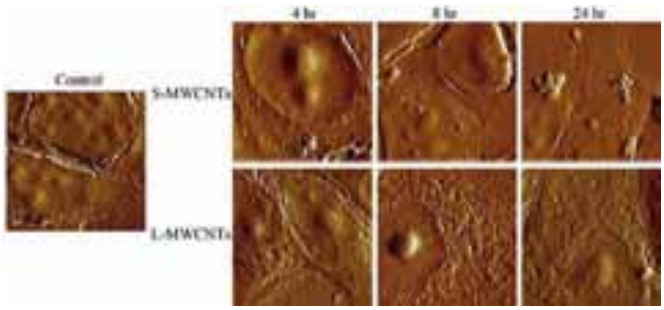


Figure1. Cell membrane topography by AFM. MCF-7 cells grown on glass cover slips were incubated with S- and L-MWCNTs at 50 μg/mL for 4, 8, and 24 hr, respectively. Cells were then washed by PBS and fixed by 2% (w/v) paraformaldehyde (PFA). Fixed samples were then scanned in air at room temperature by BioScope Catalyst (Bruker AXS, Cambridge, UK) in ScanAsyst imaging mode, at scan frequency of 0.2 Hz with 384 scan lines per image.

Disruption of cell membrane and intracellular LDH leakage by MWCNTs: As an indicator of cell membrane disruption and damage, LDH leakage to the culture media was measured to provide evidence for the observed membrane morphological change by AFM. The exposure of MCF-7 cells to both MWCNTs resulted in elevated LDH release from the cells, as shown in Fig 2, indicating disrupted cell membrane. S-MWCNTs induced 3-fold increase of LDH leakage compared to control cells; L-MWCNTs, on the other hand, had much more detrimental effect on cell membrane: with approximately 17-fold increase in LDH leakage, demonstrating that L-MWCNTs induced more severe membrane disruption in agreement with the topographic analysis by AFM (Fig1).

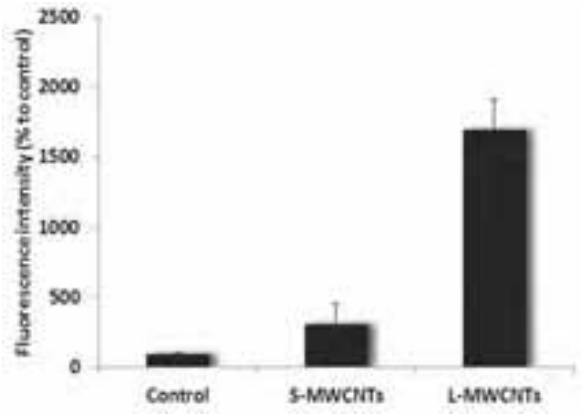


Figure2. Cell membrane integrity measured by LDH leakage. The CytoTox-ONE Homogeneous Membrane Integrity Assay kit (Promega) was employed for measuring lactate dehydrogenase (LDH) leakage of cells incubated with S- and L-MWCNTs (50 μg/mL) for 24 hr. Cell culture supernatants (50 μL) from each well were transferred to black plate and assay reagent at equal volume was added in each well. The plates were incubated for 10 min at 37°C and read at 560ex/590em.

Elasticity of living cells in response to MWCNTs: AFM offers the advantage of living cell scanning for mechanical properties without sample labelling and processing. Cell elasticity was determined by force measurements in PeakForce QNM. Data showed an overall trend of decreasing cell elasticity by MWCNTs (Fig 3). For the untreated MCF-7 cells the Young's modulus is 38.9±15.7 kPa, in agreement with the published data (20-30 kPa) [6]. Incubation with S- and L-MWCNTs (50 μg/mL) for 24 hr reduced cell elasticity to 24.8±13.2 and 16.1±10.2 kPa, respectively. Taking into account of membrane disruption evaluated by AFM topography and LDH leakage, we speculate that the reduced cell elasticity could be ascribed to the structural change of the cell membrane as well as reorganization of cell cytoskeleton network (work in progress).

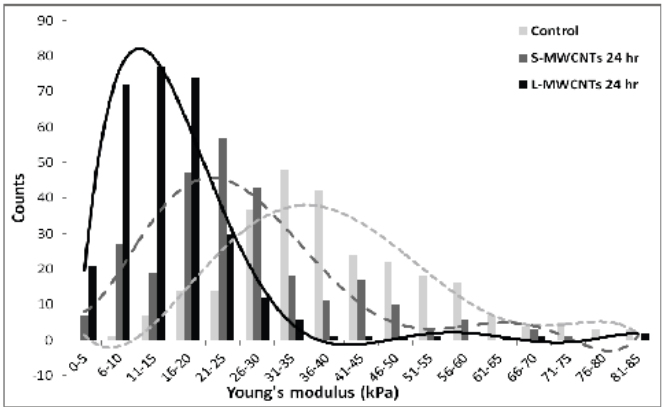


Figure3. Histogram of live cell Young's modulus of MCF-7 cells. PeakForce QNM (Quantitative NanoMechanics), an extension of Peak Force Tapping mode of AFM (Bruker) was used. Cell Derjaguin-Muller-Toropov (DMT) modulus was measured first and then converted to Young's modulus. 6-8 data points on DMT modulus map of each cell were randomly selected and up to 15 cells were scanned in each experiment. Totally 100 data points were selected in each treatment group. Experiments were conducted in triplicate.

CONCLUSIONS

Analysis by AFM of carbon nanotube-cell dynamic interaction as represented by alterations in cell membrane topography and mechanical properties was achieved in this study. The examined parameters are probably related to cellular oxidative stress-induced cytotoxicity.

REFERENCES

1. S. Beg, M. Rizwan, A. M. Sheikh et al. (2011) Advancement in carbon nanotubes: basics, biomedical applications and toxicity. J Pharm Pharmacol, 63:141.
2. S. Y. Madani, N. Naderi, O. Dissanayake et al. (2011)A new era of cancer treatment: canbon nanotubes as drug delivery tools. Int J of Nanomed, 6:2963.
3. M. v. d. Zande, R. Junker, X. F. Walboomers et al. (2011) Carbon nanotubes in animal models: a systematic review on toxic potential. Tissue Eng Part B: Reviews,17:57.
4. H.-F. Cui, S. K. Vashist, K. Al-Rubeaan et al. (2010) Interfacing carbon nanotubes with living mammalian cells and cytotoxicity issues. Chem Res Toxicol, 23:1131.
5. D. Liu, L. Wang, Z. Wang, and A. Cuschieri. (2012) Different cellular response mechanisms contribute to the length-dependent cytotoxicity of multi-walled carbon nanotubes. Nanoscale Res Lett, under review.
6. S. Leporatti, D. Vergara, A. Zacheo et al. (2009) Cytomechanical and topological investigation of MCF-7 cells by scanning force microscopy. Nanotechnol, 20:055103.

THE RELATIONSHIP BETWEEN IN VITRO COMPLEMENT ACTIVATION BY REACTOGENIC ANTICANCER DRUGS AND INFUSION HYPERSENSITIVITY IN CANCER PATIENTS

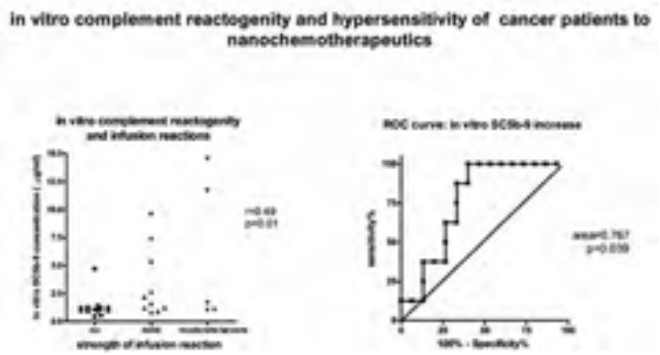
ZSÓKA WEISZHÁR[1,2], Rudolf Urbanics[1,2], Tünde Bián[1,2], Tamás Schneider[3], Zsolt Horváth[3], István Láng[3], Gergely Kiss[4], László Rosivall[1,2], János Szebeni [1,2,5,]

[1]Semmelweis University, Budapest, Hungary; [2]SeroScience Ltd, Budapest, Hungary; [3]National Institute of Oncology, Budapest, Hungary; [4]Diatron Ltd, Budapest, Hungary; [5]Nanomedicine Research and Education Center, Bay Zoltan Ltd, Budapest, Hungary

Complement (C) activation related pseudoallergy (CARPA) has been described as the underlying mechanism of many hypersensitivity reactions (HSRs) caused by micellar and liposomal nanomedicines. Although there are numerous studies addressing the C activating ability of these nanomedicines in human sera in vitro, the relationship between in vitro C reactogenity and symptoms of HSRs in can-

cer patients have not been studied to date in cancer patients.
We screened 29 cancer patients before chemotherapy for in vitro C activation by the monoclonal antibodies MabThera, Herceptin and Erbitux, and the taxanes Paclitaxel and Docetaxel, which are solubilized by the micellar emulsifiers Cremophor EL and polysorbate (Tween) 80, respectively. The in vitro C reaction was then correlated with the grade of clinical reactions during infusion chemotherapy. Sera for the in vitro studies were collected from the patients before the infusions and complement activation products were measured by Quidel SC5b-9, C3a and C5a ELISA kits, as well as from the samples collected during the infusion. Any kinds of hypersensitivity reactions were registered during the infusions, including brady/tachycardia, hypo/hypertension, dizziness, nausea, tremor or vertigo.
No reaction was present in 14 patients, mild reactions were observed in 10 patients, while significant hypersensitivity occurred in 5 patients. There was a mild, but significant correlation between the in vitro increase of SC5b-9 and the strength of hypersensitivity reactions (r=0.49, p=0.01, fig.1.), which was the most pronounced in case of Taxol and Taxotere. The area under ROC curve was 0.767 with p=0.04 (fig.1.). The pre-infusion and infusion sample C3a and C5a values did not correlate with the strength of hypersensitivity. MabThera, Herceptin and Erbitux did not cause major C activation in vitro, although mild to moderate HSRs were observed in 38% of patients.
It is concluded that in vitro C measurements may be predictive for in vivo HSRs only in the case of particulate or micellar nanomedicines. In the case of monoclonal antibody therapeutics this relationship is not notable in vitro, as these soluble antibodies do not activate C in solution, without target antigen.

Figure 1.



NEW NANOSTRUCTURES FROM ARTIFICIAL PHOSPHOLIPIDS

PIERRE-LÉONARD ZAFFALON

Natural and artificial phospholipids have long been a source of studies and inspiration for chemists.1 Our group has recently presented two approaches for the formation of 3D structures from artificial phospholipids: one involving polymerizable phospholipids and the other involving the Cu(I)-Huisgen-Sharpless click reaction discovered in 2002.
Since the work of Chapman and Ringsdorf in the 1980s with their diacetylene phospholipids, polymerization of phospholipids has been achieved with reactive groups at the end of the acyl chains, near the backbone and at the head group of phospholipids. So far no polymerization occurred from the backbone itself: with PanAc-PC-PanAc (see fig. 1A), acryl amide groups were introduced thank to the secondary amines in the chains. Vesicles of 100 nm diameter were formulated and polymerized with a 100 W UV lamp. Large structures (150 μm in diameter) were observed showing that individual vesicles co-polymerized into larger aggregates (fig. 1B).2

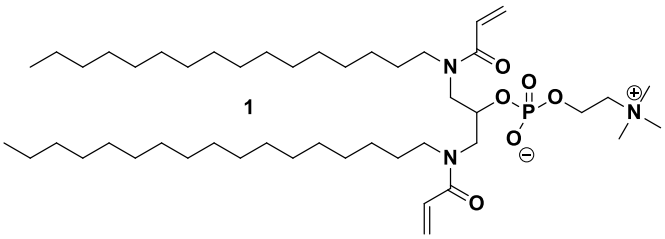


Fig. 1A: PanAc-PC-PanAc

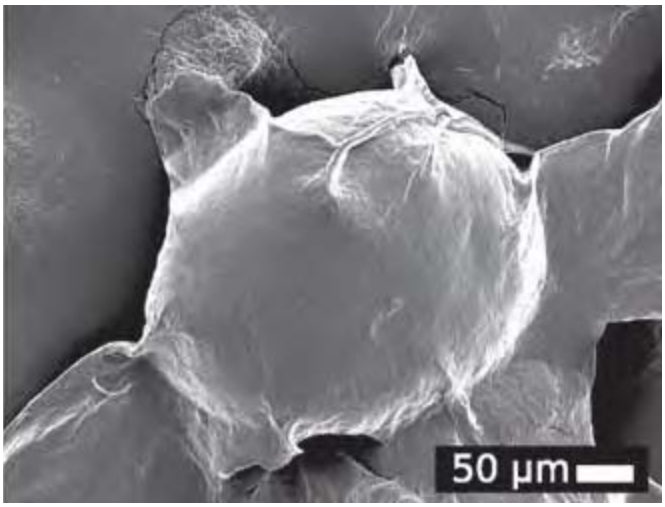


Fig. 1B: Polymerized PanAC-PC-PanAc vesicles.

In a second project, the 1,3 dipolar cycloaddition reaction was explored in order to link together several liposomes. Complementary phospholipids were introduced: one vesicle containing phospholipids with an azido moiety and the other vesicle containing an alkyno moiety. After a click reaction, the triazole linked lipid 2 was formed (fig. 2). Both phospholipids were incorporated in a 10 % ratio in egg-PC liposomes. The click reaction promoted the aggregation of the vesicles and the aggregates fused into giant unilamellar vesicles .3

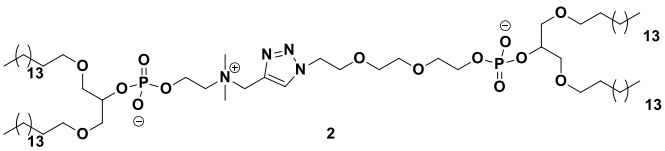


Fig. 2: Triazole phospholipid used as a vesicle connector.

(1) Zumbuehl, A. Chimia 2009, 63, 63–65.
(2) Zaffalon, P.-L.; Stalder, E.; Fedotenko, I. A.; Favarger, F.; Zumbuehl, A. Tetrahedron Let., 2011, 52, 4215–4217.
(3) Loosli, F.; Doval, D. A.; Grassi, D.; Zaffalon, P.-L.; Favarger, F.; Zumbuehl, A. Chem. Comm., 2012, 48, 1604-1606.

The European Summit for Clinical Nanomedicine

Basel, Switzerland, Monday, May 7, 09.00 am – Wednesday, May 9, 2012, 5.00 pm

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