CLINAM
European Foundation for Clinical Nanomedicine

nanomedicine
EUROPEAN TECHNOLOGY PLATFORM

EUROPEAN SCIENCE FOUNDATION
SETTING SCIENCE AGENDAS FOR EUROPE

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

European Society for NanoMedicine

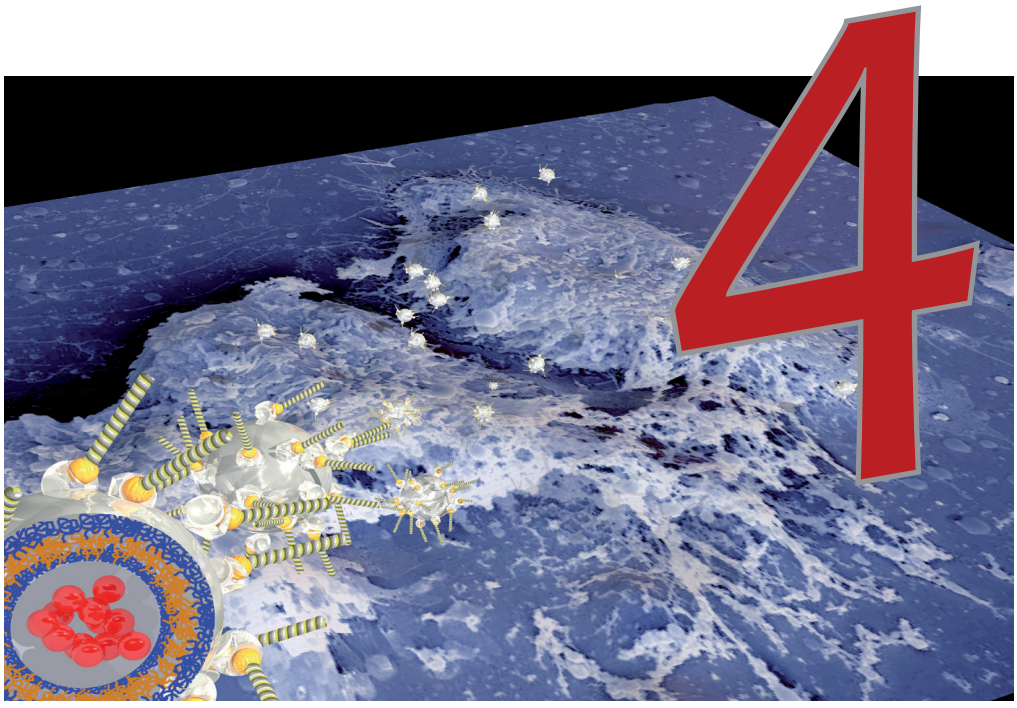
THE EUROPEAN JOINT CONFERENCE FOR NANOMEDICINE

4th European Conference for Clinical Nanomedicine

The Great Strides towards the Medicine of the Future

May 23-25, 2011 - Congress Center Basel, Basel, Switzerland

CONFERENCE PROCEEDINGS



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

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SNI
SWISS
NANOSCIENCE
INSTITUTE

Study nanoscience at the **Swiss Nanoscience Institute, Basel.**

Students are invited to apply to study for a BSc in Nanoscience, or for a Masters degree by thesis. PhD positions are also available to young researchers who want to work at the frontiers of nanoscience and nanotechnology in the internationally renowned Swiss Nanoscience Institute.

In Basel, we combine basic science with application-orientated research. Research projects can focus on nanoscale structures with the aim of providing new approaches to life sciences, the sustainable use of resources, and to the information and communication disciplines.

The University of Basel also coordinates the Swiss NCCR network of universities, federal research institutes, industrial partners and the Argovia-network (financed by the Swiss Canton of Aargau). Through these and other activities, the University continues to reinforce its internationally acknowledged position as a centre of excellence in nanoscale sciences.

See: www.nanoscience.ch, or contact Dr Katrein Spieler, katrein.spieler@unibas.ch for further information on our nanosciences courses, and how to apply.

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CONGRESS PLUS

Composition and Layout

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PART I

Curricula Vitae of the Speakers

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.

Pierre Attali

MD, MSc, has been working in academic hospitals in Paris for 11 years as specialist in liver diseases and gastroenterology before joining Synthelabo Research Clinical Department as study manager. He was rapidly promoted as project leader, group head, section head, and eventually in 1992 Head of the Clinical Research Department, managing 300-400 people in charge of clinical strategy and worldwide clinical operations. He personally brought 3 new chemical entities and several new formulations to the market, and through its management, many others. After the merger with Sanofi, he co-founded in 2000, and managed as CEO, OSMO, a CRO specialized in oncology. He then successively joined as CEO Molecular Engines Laboratories, a French biotech company dedicated to oncology, and later Urogene before joining BioAlliance Pharma as Chief Operating Officer, Strategy and Medical Affairs. He still has medical functions in Bicêtre and Paul Brousse hospitals (Assistance Publique-Hôpitaux de Paris) where he has consultations and acts as principal investigator in several clinical trials in liver diseases. He is cofounder and board member of several specialty pharma and biotech companies.

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

EDUCATION

1969-1975 M.S. in Chemistry, Kossuth L. University, Debrecen, Hungary 1980-1983 PhD. in Chemical Technology, Kossuth L. University, Debrecen, Hungary Postdoctoral: University of Massachusetts, Lowell (Profs. Rudi Faust and Alex Blumstein)

MAJOR HONORS

- Co-founder the American Society for Nanomedicine (2008)
- Session Chair and Organizer at the Annual World Congress on Nanocomposites (2000-2008)
- Fellow of the American Academy of Nanomedicine (2006-2008)
- Recipient of Excellence in Education and Teaching Award, Government of Hungary (1981)

NATIONAL COMMITTEE MEMBERSHIPS

NIH Small Business Initiative/Science and Technology Transfer on medical devices. NIH Nanotechnology Initiative and NANO Peer Review Panel. EPA NCER/STAR Nanotechnology Initiative. NIH Roadmap and COBRE (Centers of Biomedical Research Excellence) Peer Review Panels. NIH/NIBIB ZEB1 OSR-B(O1) Training grants and NIH/CSR F15, F33 Fellowship panel, etc.

INTERNATIONAL COMMITTEE MEMBERSHIPS

U.S. Civilian Research and Development Foundation for the Independent States of the Former Soviet Union (CRDF). US-Israel Binational Science Foundation (BSF). The Government of Hong Kong Innovation and Technology Commission Expert Panel. Networks of Centres of Excellence of Canada Expert Panel. Netherlands Organisation for Scientific Research (NWO)

MEMBERSHIPS IN OTHER EXPERT COMMITTEES

US Technical Advisory Group to ISO TC229 "Nanotechnology". American National Standard Institute (ANSI) Nanotechnology Steering Committee. ASTM E56 Nanotechnology Committee, Terminology and Nomenclature WG -Executive Board, American Society for Nanomedicine.

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 is on the faculty of the Hebrew University since 1968 and was promoted to a Professor on 1981. He was a visiting Professor at the Department of Biochemistry, University of Virginia School of Medicine, Charlottesville VA, USA from 1973 to 2005; a Donders Chair Professor at The Faculty of Pharmacy, University of Utrecht, The Netherlands, on 1992; a Visiting Professor at the University Kyoto University (Kyoto, Japan, 1998); at La Sapeinza University (Roma, Italy, 2006); Jaiotung University (Shanghai, China, 2006); Kings College (London, UK, 2006);and, Danish Technical University (DTU, Copenhagen, 2010). His current basic research focuses on composition, structure, function relationships of biological membranes with special focus and contributions related to sphingolipids. His applied research deals with development of drug delivery systems (DDS) and drugs based on such DDS including vaccines and nucleic acids' delivery systems. This is exemplified by DoxilTM development (together with Professor Alberto Gabizon and SEQUUS Pharmaceuticals, Menlo Park CA, USA).The anticancer drug DoxilTM (CaelyxTM 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 is having yearly sales exceeding half a Billion dollars. Professor Barenholz with help of others founded based on Barenholz inventions the following start up companies:1. NasVax Ltd (now a public company on the Israeli stock market), a vaccines' developing company now in clinical trials of Influenza vaccine which is based on VaxiSomeTM, which is based on Barenholz invented polycationic sphingolipid adjuvant; 2. Moebius medical which develops liposomes' based medical device for treatment of osteoarthritis; and 3. LipoCure Ltd for the development of liposomal nano drugs for treatment of cancer and inflammatory diseases [rheumatoid arthritis (RA) and multiple sclerosis (MS)]. Professor Barenholz is a coauthor in more than 350 scientific publications and a co-inventor in more than 30 approved patent families. He was an executive editor of Progress in Lipid Research and he is on the editorial board of 4 scientific journals. Professor Barenholz was awarded few prizes including twice the Kaye award (1995 & 1997), Alec D. Bangham (the Liposome field founder) award (1998), and Teva Founders Prize (2001). On 2003 Professor Barenholz founded (from Doxil royalties) the "Barenholz Prize" for Israeli Ph.D. students to encourage excellence and innovation in applied science of Israeli students.

François Berger

Francois Berger is professor of cell biology and oncology in Grenoble University hospital (France). After a MD, PhD; he spend 2 years at the Salk Institute in Fred Gage laboratory working on neuronal stem cells.

The specificity of his activity is to try to implement a dual clinical and research activity in the field of neuroscience, which goal is to translate at the bedside micro-nanotechnologies. He is now the head of the Cell Biology Department in Joseph Fourier University Medical School and of the clinico-biologal Neuro-oncology unit in Grenoble university hospital. He coordinates an INSERM research unit, "brain nanoMedicine" group in the INSERM Unity 836 including the Grenoble "transcriptomic and proteomic platform" and brain tissue bank. Ethical issues are crucial in the field of nanotechnology, which motivated is implication in this field, as member of the Ethical board of Nano2life excellence project and Ethentec European program.

Patrick Boisseau

M. Patrick Boisseau is graduate from the Institut National Agronomique in 1983 and from the Ecole Nationale du Génie Rural, des Eaux et des Forêts en 1985.He holds a MSc Degree in Human Nutrition.

He joined the French Atomic Energy Commission - CEA - in 1987 to work for 7 years as academic research fellow in plant biology. He then moved for 4 years to the Foresight & Strategy Division at the CEA headquarters as expert on strategy in life sciences and environment.

From 2001 to 2004, he is committed to the design, the organisation and the funding of the NanoBio innovation centre in Grenoble. The NanoBio innovation centre brings together engineers, physicists, chemists, biologists and medical doctors to develop new miniaturised tools for biological applications (130 people involved incl. SMEs). The Nanobio center has been established with the University of Grenoble. Fundamental and applied research in nanobiotech is performed by several institutes and universities in Grenoble in cooperation with leading industrial companies in the diagnostics and biochips sector. The NanoBio center is linked to the Minatec Innovation Center, the 1st European centre for micro- and nanotechnologies and will rely on its technological facilities

From 2004 to September 2008, he is the 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 in a comprehensive joint programme of activity. More than 400 scientists are participating to this network. Since 2006, he is Member of the Executive Board of the European Technology Platforms, and chairman of its working group on "nanotechnology based diagnostics and imaging". In December 2007, he founded the French Technology Platform on Nanomedi-

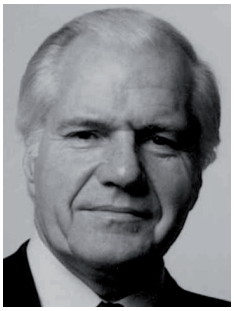
cine and he is member of its Executive Board. The recent European projects he coordinated are: FP7 Coordination & Support Action EuroNanoBio designing the European infrastructure in nanobiotechnology during year 2009. EuroNanomed TARGET-PDT project on targeted Photo Dynamic Therapy. He is partner of numerous other EU projects. Since 2008, he is in charge of the business development in NanoMedicine at CEA-Leti-MiNaTec, with emphasis on organic nanoparticles for diagnostics and therapy

MANDATES

- Editorial Board member of Nanomedicine: Nanotechnology, Biology and Medicine since June 2010
- Chairman re-elected in 2009 of the working group on "nanotechnology based diagnostics and imaging" at the European Technology Platform on Nanomedicine, and thus member of its Executive Board.
- Founder and Executive Board member at the French Technology Platform on Nanomedicine.(2007 onward)
- Expert for international organizations like European Science Foundation, the European Commission, Oesterreichische Forschungsfoerderungsgesellschaft GmbH (AT), Agence Nationale de la Recherche (FR)

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.

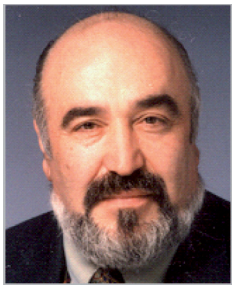


Fritz R. Bühler

Fritz R. Bühler is Professor of Pharmaceutical Medicine and Pathophysiology as well as of Internal Medicine and Cardiology at the Faculty of Medicine, University of Basel, Switzerland. He was Director of the Department of Research at the University Hospital in Basel, Switzerland. Fritz R. 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 in Pathophysiology. Fritz R. Bühler and his team were known for the contributions to cardiovascular pathophysiology. Fritz R. Bühler was Head of world-wide Clinical Research and Development at Hoffmann-La Roche from 1991 to 1995. For nineteen years, he is the (founding) director of the European Center of Pharmaceutical Medicine, ECPM at the Medical Faculty and Pharma-Center of the University of Basel. Prof Bühler is on the Board of the Center for Drug Development Science at UCSF Washington DC and was on the Executive Committee of the Swiss Academy of Medical Sciences. Fritz R. Bühler co-founded the International Biomedicine Management Partners Inc. in Basel and subsequently, he was a Managing Partner at Bear Stearns Health Innoventures in New York. He is also a co-founder and promoter of the tri-national BioValley at the Upper Rhine incl. in 2007 the BioValley Business Angels Clubs, BioBAC. In 2002 he received an honorary doctorate of the University Louis Pasteur of Strasbourg. He co-founded PharMida AG which develops Gold-Nano Partical-based new treatment opportunities.

Mateja Cegnar

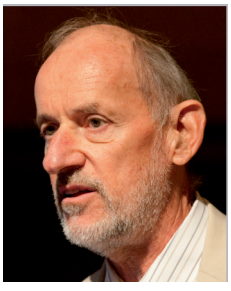
Mateja Cegnar received her B.Sc. in Pharmaceutical Science from University of Ljubljana, Faculty of Pharmacy in 2001, and her Ph.D. in Pharmaceutical Science from University of Ljubljana, Faculty of Pharmacy in 2005. During postgraduate study she worked at home faculty as an assistant in Pharmaceutical Technology and provided lectures on specific topics on nanotechnology. She attended several intensive postgraduate training under the Socrates Programme and Galenos network. In 2008 she joined pharmaceutical company Lek Pharmaceuticals d.d., Sandoz Development Center Slovenia, where she works as a researcher in the department New Delivery Systems. Her work was presented at numerous scientific and professional meetings home and abroad, and in scientific journals. Currently, her research interests include the development of nanosized systems for delivery of protein drugs (biopharmaceuticals) with special focus on non-invasive oral protein delivery.



Vincenzo Costigliola

graduated in Medicine from the University of Naples (Italy) in 1972 and with distinction, in Anaesthesiology and Intensive Care from the University of Pisa in 1978. Completed studies in Rheumatology, Dermatology, Proctology, Oncology, Surgery, Drugs Abuse, Emergency Treatment, Disaster Action, Hospital Organization, Medical Teaching Methodology, Computer and Audio-Visual Training for the Medical Profession. Professional experience ranges from senior military positions as Chief of Medical Services in the Italian Navy, responsibility of the outpatients clinic of the Shape Hospital in Belgium and since 1972 family practice in Italy and Belgium. Medical advisor: OTAN; W.E.U. Bruxelles.

- Member of the International Adisory Board at King Abdulaziz University J Djeddah Saudi Arabia
- President of EPMA (European Predictive, Preventive, Personalized Medicine http://www.epmanet.eu/
- President of E.M.A. (European Medical Association), http://www.emanet.org/
- President of E.D.A. (European Depression Association), http://www.eddas.org/
- President of I.R.M.A. (International Rescue Medicine Association), http://www.irmanet.eu/



Patrick Couvreur

UMR CNRS 8612, University of Paris-Sud XI - France. Professor Patrick Couvreur, PhD, is a full Professor of Pharmacy at the University of Paris South, France. Doctor Patrick COUVREUR received his degree in Pharmacy from the Catholic University of Louvain (Belgium) in 1972 and PhD from the same University in 1975. After a

posdoctoral position at the Federal Institute of Technology of Zurich (Switzerland), he became Associate Professor at the Catholic University of Louvain. He assumed his present position in 1984. Since 1998, he took the position of Director of the CNRS Unit «Physico-Chimie, Pharmacotechnie, Biopharmacie» (UMR CNRS 8612), a multidisciplinary research group (Department) of more than 120 scientists located in Chatenay-Malabry (France). He is also the founder of the Doctoral school «Therapeutic Innovation» which is a network of 92 laboratories located in the South suburb of Paris (University of Paris-Sud, Chatenay-Malabry, Kremlin Bicêtre and Orsay; CNRS, Gif-sur-Yvette; INRA, Jouy-en-Josas and the CEA, Saclay). Professor Patrick Couvreur has published over 350 peer-review articles in international journals and is the recipient of 45 patents (Europe, USA, Japan...); he was invited as a speaker in 185 international and national congresses.

He is the editor of the CRC Press Book «Polymeric Nanoparticles and Microspheres» and the co-editor of two books on liposomes: «Liposomes» (Lavoisier, France) and «Liposomes : new systems and new trends in their application» (Editions de Santé, France). Patrick Couvreur is also editor of a book on the «Pharmaceutical Aspects of Oligonucleotides» (Taylor and Francis). He received among others, the Prize 1984-1989 of Pharmaceutical and Therapeutical Sciences (Belgium), the Young Investigator Award 1990 of the Controlled Release Society (USA), the Forscheimer Fund 1990 (Israel), he is the recipient of the «1996 FIP Scientist of the Year» award and received in 1998 the International Glaxo-Wellcome Award from the Royal Pharmaceutical Society of Great Britain. In 1999, he received the «Trophé de l'Innovation-Paris-Ile de France Capitale Economique» from the «Chambre de Commerce de Paris». In 2002 he received the Prix Barré from the University of Montréal (Canada) and in 2004 the Pharmaceutical World Scientific Meeting Research Achievement Award in Kyoto (Japan). He is received the Host Madsen Medal in 2007 and is the 2008 Maurice Janot Lecture. Professor Patrick Couvreur is member of the editorial board of numerous international scientific journals. He was a member of the board of the governors of the Controlled Release Society (1990-1994) (USA) and elected in the BPS of the International Federation of Pharmacy (FIP) for the period 1994-2004. He is also a member of the board of the «Association de Pharmacie Galénique et Industrielle» (APGI) (France). He is the chair of the Academic Committee for Research Relations (CARR) of the European federation of Pharmaceutical Sciences (EUFEPS). Professor Patrick Couvreur is the President Founder of GTRV, a French Society concerned with Drug Targeting. In 1998, he was elected as a member of the Academy of Medicine in Belgium and in 2000 as a Member of the «Académie des Sciences et des Technologies» and of the «Académie de Pharmacie» in France. He was also sitting as a member of the French National Committee for Sciences (CNRS) (2000-2003).

The research of Patrick Couvreur has let to the fundation of two start-up companies (Bioalliance and Medsqual). Bioalliance entered the stock market in 2005. Patrick Couvreur is also Extraordinary Professor at the University of Louvain (Belgium) and was an invited Professor of the University of Parma (Italy), Jerusalem (Israel), Pavia (Italy), Modena (Italy) and Napoli (Italy). He was an invited researcher of the MITI (Japan). Patrick Couvreur research interest includes the development of new drug carriers and delivery systems, especially biodegradable nanoparticles, liposomes and prodrugs. Currently, his research focuses mainly on the conception of new nanotechnologies for the treatment of brain diseases, autoimmune diseases and cancer. He has also interest in the design of new nanoparticles for nucleic acids delivery.

Kenneth A. Dawson

Professor and Chair of Physical Chemistry
Year of appointment at University College Dublin: 1992. Academic degrees: (Institution and year of completion): BSc, (QUB) (1980); MSc Mathematics (QUB) (1981); DPhil (University of Oxford) (1984)
Positions held prior to University College Dublin: 1983-1984 Research Visitor, Institute Haute Etudes Scientific, Paris; 1983 Visiting Lecturer, Theoretical Chemistry, University of Ulm, West Germany; 1984 Lindemann Fellow; 1986-1987 Associate Fellow in Atomic & Solid State Physics; 1986-1987 Materials Science Postdoctoral Fellow Cornell University; 1985-1988 Strategic User at the Cornell National Supercomputer Centre; 1987-1990 Assistant Professor of Chemistry, University of California; 1989-1992 Adjunct Professor of Biophysics, University of California, Berkeley; 1989-1992, Chair of Physical Chemistry, University College Dublin; 1992-present Executive Board Member of SEAM, Director CBNI, Member RIA, ACS, APS.

PRIZES

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

RESEARCH INTERESTS AND KEY EXPERTISE:

- Quantitative bionanoscience, bionanointeractions, nanomedicine, nanosafety, nanodiagnostics
- Fundamentals (theoretical, simulation and experimental principles) of Soft Matter; Nanoparticle, colloidal and surface Science, particularly in relation to creation of organized structures and dynamically arrested systems.
- Interface between soft matter / dense colloidal system and biology, and biomaterials, bionanomaterials.
- Systems science, self-organized criticality, and advanced methods of computation

PRESENT RESEARCH ORIENTED INTERNATIONAL ACTIVITY

- Chair International Alliance for NanoEHS Harmonisation
- Member of The New York Academy of Sciences, 2009
- Member of DG SANCO Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR)
- OECD / ISO Nanotechnology standards working group member
- Coordinator of the EU FP7 Small Collaborative project Neuro-Nano
- Coordinator of the EU FP7 Infrastructure for Nanosafety (QNano) – final stages of negotiation.
- Executive Board of Centre of Excellence in La Sapienza, Complex Matter
- Editorial Board, Current Opinion in Colloid and Interface Science
- Associate Editor of Journal of Nanoparticle Research
- Editor in chief of Physica A.

RECENT ACTIVITIES ON BOARDS

- Member of EMEA Nanomedicines Expert Group, 2009-2010
- Member of Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), 2009-2010
- Irish Representative on OECD Working groups on Manufactured Nanomaterials
- Board of Review (External), Department of Energy, Board of Review (External), National Institute of Health, Maryland, U.S.A.
- National Committee for Chemistry, Royal Irish Academy
- Member of "The Open Colloid Science Journal" advisory Board.
- Founder Member of Council of Scientists of INTAS
- President of the European Colloid and Interface Society (2005-2008)
- Some Recent conference and seminar papers/talks:
- "Gordon Conference – Cancer Nanotechnology", Boston, USA, Jul 2011;"Gordon Conference – Biological Impacts in Nanotechnology", USA, May 2011; High level EU-US conference in nanosafety - "Bionanointeractions", Washington, Mar 2011; "2010 MRS Fall Meeting - Interdisciplinary Approaches to Safe Nanotechnologies", Boston MA, Nov 2010; "Nobel Mini-Symposium on Nanotoxicology: Understanding the Interactions of Engineered Nanomaterials with Biological Systems", Sweden, Oct 2010; "GENNESYS International Congress on Nanotechnology", Barcelona May 2010; "ISO TC229 meeting on nanomaterials", Maastricht May 2010 "8th International Conference and Workshop on Biological Barriers: in vitro tools, Nanotoxicology and Nanomedicine", Saarland University, Germany April 2010; "International Key Symposium on Nanomedicine", Stockholm September 2009;

"4th International Conference on Nanotechnology", Helsinki August 2009; "NIMS symposium on the social acceptance of nanomaterials", Japan July 2009; AAAS, Chicago Feb 2009; "NanoBioEurope2008", Barcelona 9-13 2008.

RESEARCH OUTPUTS

250 papers in theory, simulation, and experimental science in the field of biological materials, and bionanointeractions, theory, simulation, experiment

CURRENT RESEARCH GRANTS

- EU FP7 Large Infrastructure program, €7M, 2011-2015, Coordinator
- SFI Strategic Research Cluster, €7.5M, 2007-2011, PI. BioNanoInteract.
- EU FP7 Small Collaborative Research Program, 2009-2012 NeuroNano.
- HEA PRTLI NanoBio Centre 2007-20011 €1.5M recurrent, €24M capital (matched by UCD), and INSPIRE
- EPA Project Grant, €350,000, Dec 2008- Dec 1010, PI, Visualisation and Quantification of the interaction of fluorescent nanoparticles with ecotoxicologically relevant species.
- SFI Research Frontiers Grant, PI, €220,000; Spatio-temporal aspects of nanoparticle interactions with cells.
- EU – FP6, NMP Programme FP6-2004-NMP-TI-4, STREP NanoInteract, 2006-2009
- NSF-CEIN Centre in UCLA, $40M, 2009-2013, Named Partner.

Bruno De Geest

Ghent University, Laboratory of Pharmaceutical Sciences, Ghent.

Dr. Bruno De Geest studied chemical engineering at Ghent University and obtained his master degree in 2003. In 2006 he obtained his PhD entitled 'Polyelectrolyte Microcapsules for Pharmaceutical Applications' at the Faculty of Pharmaceutical Sciences, Ghent University, Belgium.

For his PhD work he was awarded the AAPS graduate student award for Pharmaceutical Technology and the Andreas De Leenheer award from Ghent University. Between November 2006 and October 2008 he worked as a post doctoral fellow at Utrecht University, The Netherlands. From October 2008 he was appointed as FWO post doc at the Laboratory of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Ghent University.

His expertise is situated on interface between material science, biology and drug delivery with a special emphasis on targeting the immune system. He has about 50 peer reviewed A1 publications and submitted several patent applications.

Mauro Dell'Ambrogio

State Secretary for Education and Research Mauro Dell'Ambrogio, the holder of a Doctorate in Law from the University of Zurich, held a number of public offices in canton Ticino from 1979 to 1999 after passing his bar exam: Judge, Chief of the Cantonal Police, Secretary-General for Education and Culture, project manager for the creation of the University of Lugano (USI), and Secretary-General of the USI.

After four years heading up a group of private clinics, he was made Director of the University of Applied Sciences of Southern Switzerland (SUPSI) in 2003. He has been mayor of Giubiasco, a member of the Ticino cantonal parliament and chairman of the Ticino electricity works. He took up the post of State Secretary for Education and Research in January 2008.

Neil P. Desai

Neil P. Desai, Ph.D. has more than 20 years of experience in the research and development of novel therapeutics, drug delivery systems and biocompatible polymers.

He is currently Vice President, Strategic Platforms at Celgene Corp. Prior to the acquisition by Celgene in Oct 2010, he was SVP, Global Research and Development, for Abraxis BioScience (ABI) where he was responsible for the development of ABI's proprietary product pipeline and intellectual property portfolio. This included the development of products from the early discovery phase through preclinical testing, late stage clinical studies and development for commercial manufacturing.

Dr. Desai is a co-inventor of ABI's *nab*® tumor targeting nanotechnology platform which led to the first protein-based nanotechnology product (Abraxane®) to be approved by the FDA and regulatory agencies worldwide, for treatment of Metastatic Breast Cancer. At predecessor companies of ABI, VivoRx, Inc and VivoRx Pharmaceuticals, Inc., he worked on the early discovery and development of 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 is an inventor on over 100 patents, has authored or co-authored over 35 peer-reviewed publications, has made over 150 presentations at scientific meetings and has served on FDA, USP and European Union task forces and panels. He holds an M.S and Ph.D. in Chemical Engineering from the University of Texas at Austin and a B.S. in Chemical Engineering from the University of Bombay.

Mike Eaton

Professor Mike Eaton has worked in research in the Pharma industry for more than 35 years. Initially at GD Searle, where he built the first synthetic gene for Urogastrone and sequenced human fibroblast interferon.

He was a founding member of Celltech in 1980; later acquired by UCB. He has worked on a number of marketed drugs - Mylotarg in 2000, the first Antibody drug conjugate and certolizumab pegol in 2009, the first PEGylated antibody. Unusually he has worked with both small molecules and large molecules, including DNA. He built the first automated DNA synthesiser in Europe, which is now owned by the Science Museum in London. This machine was used for the first cloning of pre-prochymosin, a key ingredient in cheesemaking.

He has worked on low molecular weight drugs including the first non-emetic PDEIV inhibitor. Mike is a special professor at Nottingham and has been an executive board member of the European Technology Platform for Nanomedicine, since its inception in 2005. He left UCB in February 2010 and is now a strategic and technical adviser to a number of companies. His particular interest is commercial translation of nanotechnology research to nanomedicines – medicines to help patients.

Falk Ehmann

MD, PhD, MSc. Falk Ehmann is currently working in the Scientific Support and Project Section of the European Medicines Agency (EMA) were his main responsibilities include holding the Scientific Secretariat of the Pharmacogenomics Working Party and the Innovation Task Force promoting Innovation and new methodologies in drug development and being involved in the development of policies, guidelines and the annual working program in these areas. He

held various positions and responsibilities at the EMA since 2004, including Scientific Secretariat of the Vaccine Working Party and Biosimilar Working Party (BMWP).
Prior to joining the EMA Dr. Ehmann was a Public Health Researcher at the Robert Koch Institute in Berlin and Medical Intern at different University Hospitals including Bordeaux, Munich, Berlin, Geneva and Tanzania.
Falk Ehmann wrote his PhD thesis in the department for Cellular Signal Transduction at the University Hospital Hamburg-Eppendorf in the Centre of Experimental Medicine of the Institute of Biochemistry and Molecular Biology.

Bernice Elger

Prof. Dr. med. dipl. theol., Centre universitaire romand de medicine légale, University of Geneva. Prof. Elger studied medicine and theology in Germany, the US, France and the French speaking part of Switzerland. She obtained her medical diploma as well as a 6 year university degree in protestant theology in Germany and her FMH in internal medicine in Switzerland. For the past 16 years she has been teaching ethics and health law at the University of Geneva and has recently accepted a full professorship as head of the Institute of bio- and medical ethics at the University of Basel, starting in May 2011. In 2004, she obtained a grant for advanced researchers of the Swiss National Science Foundation for research in the US (University of Pennsylvania Center for Bioethics, Kennedy Institute of Ethics and the Department of Clinical Bioethics at the NIH). In 2010, she was awarded the "Swiss Award for research in primary care" for her work on medical confidentiality, and in 2005 the "Prix Bizot" for her work on ethical issues of research involving biobanks (Habilitation, University of Geneva). In 1999 she obtained the "Award of the Medical Faculty" for her doctoral thesis about medical paternalism and in 1997 the "Prix Arditi en éthique" for her work on predictive medicine. She has widely published in medical and ethical journals about biomedical ethics and human rights, including research ethics and clinical ethics, in particular about topics related to the new biotechnologies, genetics, biobanks and the use human tissue.

Rutledge G. Ellis-Behnke

Dr. 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 as well as Honorary Associate Professor in the Faculty of Medicine at the University of Hong Kong. His primary research interest is using nanotechnology to reconnect the disconnected parts of the brain in order to restore function. Through additional discoveries, his work in Nanomedicine has broadened to include hemostasis without clotting; preserving stem cells; preservation and restoration of vision and immobilizing cancer stem cells.
Ellis-Behnke received his PhD from MIT in Neuroscience, BSci from Rutgers University and graduated from Harvard Business School's International Senior Manager's Program (AMP/ISMP).
Prior to returning to school to pursue his PhD, Ellis-Behnke held various management positions including Senior Vice President of Huntingdon, a public company for testing and consulting services and Co-founder/CEO in 1995 of one of the first internet companies to do online commerce.
Ellis-Behnke is Associate Editor/Neurology for the journal Nanomedicine: Nanotechnology, Biology and Medicine; member of both the Executive and Scientific Advisory Boards for the Glaucoma Foundation; member of the Executive Board of the Asia Foundation for Cancer Research; member of the China Spinal Cord Clinical Trial Network, Society for Neuroscience, American Chemical Society, Association for Research in Vision and Ophthalmology and Sigma Xi, the scientific research society.
Technology Review named his "Nanohealing" discoveries one of the "Top 10 Emerging Technologies of 2007." His "Nano Neuro Knitting" and "Immediate Hemostasis" technologies have each been licensed for translation to humans.
In addition to his work in neuroscience and nanomedicine Ellis-Behnke introduced the TabletPC to MIT and the University of Hong Kong as part of the migration to the paperless classroom to deliver all course material and texts to the students digitally.

Martin Erdmann

Dr. Martin Erdmann was the chairman of the New Testament Department of the Staatsunabhängige Theologische Hochschule Basel for several years before he took up the position of Senior Scientist at the University Hospital in Basel. At the latter institution he was involved in a research project (Ethics of Clinical Nanomedicine) for five years. From 2003 to 2010 he was Professor of Biblical Studies at Patrick Henry College, Virginia (distance education). In 2005 he became the chairman of the New Testament Studies Department at the Academy for Reformation Theology in Hannover, Germany while also directing the affairs of the Verax Institute.

Bengt Fadeel

Fadeel holds M.D. and Ph.D. degrees from Karolinska Institutet, Stockholm, Sweden, and he is currently Professor of Medical Inflammation Research. He is also Head of the Division of Molecular Toxicology and Vice Chairman of the Institute of Environmental Medicine at Karolinska Institutet. He acts as project coordinator of FP7-NANOMMUNE, a research consortium funded by the European Commission, focusing on studies of the hazardous effects of engineered nanomaterials on the immune system. Fadeel organized the 1st (2006) and 2nd (2010) Nobel Forum Mini-Symposium on Nanotoxicology, at Karolinska Institutet, and co-organized the 1st (2009) and 2nd (2010) Autumn School on Nanosafety in Venice, Italy. He is a member of the Nanomedicines Expert Group at the European Medicines Agency (EMA) in London (2009-present) and a member of the Nanosafety Working Group in the multi-sectorial platform, NanoFUTURES of the European Commission (2010-present). He is also an active member of the Editorial Board of the international journals, APOPTOSIS and NANOMEDICINE.

Sergej Fatikow

studied computer science and electrical engineering at the Ufa Aviation Technical University in Russia, where he received his doctoral degree in 1988 with work on fuzzy control of complex non-linear systems. After that he worked until 1990 as a lecturer at the same university. During his work in Russia he published over 30 papers and successfully applied for over 50 patents in the area of intelligent control. In 1990 he moved to the Institute for Process Control and Robotics at the University of Karlsruhe in Germany, where he worked as a postdoctoral scientific researcher and since 1994 as Head of the research group "Microrobotics and Micromechatronics". He became an assistant professor in 1996. In 2000 he accepted an associate professor position at the University of Kassel, Germany. A year later, he was invited to establish a new Division for

Microrobotics and Control Engineering at the University of Oldenburg, Germany. Since 2001 he is a full professor in the Department of Computing Science and Head of this Division. He is also Head of Technology Cluster Automated Nanohandling at the Research Institute for Information Technology (OFFIS) in Germany. His research interests include micro- and nanorobotics, automated robot-based nanohandling in SEM, micro- and nanoassembly, AFM-based nanohand-ling, sensor feedback on the nanoscale, and neuro-fuzzy robot control. He is author of three books on micro-system technology, microrobotics, microassembly, and nanohandling automation, published by Springer in 1997, Teubner in 2000, and Springer in 2008. He also published since 1990 over 70 book chapters and journal papers and over 190 conference papers on micro- and nanorobotics, nanohandling automation and control. Since 2000 he has supervised more than 40 MSc students and more than 20 PhD candidates in his Division.

PROFESSIONAL ACTIVITIES

Acquisition and coordination of numerous joint projects in various R&D programs of the European Union, German Research Foundation, Federal Ministry of Education and Research, and Federal Ministry of Economics and Technology. The fund for the research in Oldenburg since 2001 adds up to over 12 million Euro.

Reviewer/Evaluator for the EU, the German Research Foundation (DFG), Swiss National Science Foundation (SNF), American National Science Foundation (NSF), French National Research Agency (ANR), Swiss Federal Institute of Technology, Danish National Research Foundation (DNRF), Nanyang Technological University, Singapore, and others. Member of a professor search committee at many international universities.

Member of the Editorial Board of the Int. Jour. of Optomechatronics (Taylor & Francis, USA), the Jour. of Systems and Control Engineering: Proc. of the Institution of Mechanical Engineers (PEP, UK), the IEEE-ASME. Trans. on Mechatronics (USA), the IEEE Trans. on Automation Science & Engineering (USA), and the Int. Journal of Intelligent Mechatronics and Robotics (ICI-Global, USA)

Founding Chair of Int. Conf. on Manipulation, Manufacturing and Measurement on the Nanoscale (3M-NANO), Conference Chair of 2007 SPIE Int. Conf. on Optomechatronic Systems Control, Program Chair of 2010 IEEE/ASME Int. Conf. on Advanced Intelligent Mechatronics and of 2010 Int. Symp. on Opto-mechatronic Technologies, Honorary Chair of 2008 WSEAS Int. Conf. on Automation and Information, Bucharest, Romania, Tutorials & Workshop Chair of 2009 IEEE/ASME Int. Conf. on Advanced Intelligent Mechatronics. Plenary speaker at numerous conferences.

Koh Young Best Paper Award 2007 from the International Journal of Optomechatronics (Taylor & Francis, USA). Best paper awards at 2010 IEEE Int. Conference on Automation Science and Engineering, Toronto, Canada, 2010 IEEE ECTI-CON, Chiang-Mai, Thailand, , 2006 SPIE Optics East, Boston, MA, U.S.A., 2005 Int. Conf. on Automation, Robotics and Autonomous Systems, Cairo, Egypt. Various award nominations. Honorary professor at Zhejiang University, Hangzhou, and at Changchun University of Science and Tech-nology, China. Distinguished Visiting Fellowship of the Royal Academy of Engineering at the Manufacturing Engineering Centre, Cardiff University, UK. Visiting Fellowships and Invited lectures at universities in Canada, China, Finland, Greece, Japan, Singapore, South Korea, UK, the U.S.A., and others.

Memberships: IEEE, IEEE TC on Micro/Nano Robotics and Automation, IEEE Nanotechnology Council: TC on Nanorobotics and Nanomanufacturing, German Academia Association (DHV)

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. SMFS studies of proteoglycan interactions. 2. Application of nanotechnology to the study of functional amyloids. 3. Nanotechnology against malaria: Strategies for the identification of new drugs and their targeted delivery.

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: “Enantioselective reactions in organic medium using immobilized enzymes.” 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, area of Biochemistry and Molecular Biology: “Functional dynamics of the interaction between histones, chromatin, and singlestranded DNA. Applications of the cloning of nucleosomal DNA.” 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 & July-December 1986: Trainee student. Zentrale Forschungslaboratorien, Ciba-Geigy AG, Basel, Switzerland.
- December 1985-June 1986: Trainee student. Fundamental Biology Institute, Universitat Autònoma de Barcelona, Spain.

PEER-REVIEWED PUBLICATIONS: 49 , Conference Contributions: 88

Marc Fournelle

Marc Fournelle, born in Luxemburg, 1982. Studied physics at Saarland University and Universidad de Barcelona. Member of the Biomedical Ultrasound Group of the Fraunhofer IBMT in Sankt Ingbert since 2005, PhD in the field of \”Optoacoustic Molecular Imaging\” in 2010.

Research topics: Ultrasound and optoacoustic imaging, synthesis of contrast agents, Image reconstruction and signal processing algorithms.

Alberto (Abraham) Gabizon

(born 1951 in Tetuan, Morocco), received his medical degree 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 residency in Oncology at the Hadassah Medical Center in Jerusalem, and obtained the Israeli board

certification in Radiation and Medical Oncology in 1985. Between 1985-1988, he spent 3 years on a research fellowship at the Cancer Research Institute of the University of California in San Francisco, 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. Dr. Gabizon returned to Israel in 1989 as Senior Staff Physician and Investigator at the Sharet Institute of Oncology of Hadassah Medical Center where he continued his research and clinical activity until 2001. In 2002, Dr. Gabizon 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 appointment.

Dr. Gabizon has received the Spanish National Prize of Medicine Graduation (1975), the Career Research Award (1989) and Professorship Award (2008) of the Israel Cancer Research Fund, the Hebrew University Kaye Innovation Award (1997) for the invention: Liposomal Doxorubicin for Cancer Treatment, the Tel Aviv University Sarnat Lectureship (2000), and the Alec Bangham Life Time Achievement Award of the International Liposome Research Society (2010).

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 of drugs, and experimental cancer therapy, and has published around 120 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 pharmacologic and safety 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.

Dr. Gabizon is a resident of Jerusalem, married and father of 4 children.

Jim Gallivan

Health Canada, Ottawa, ON. Dr. Gallivan has a Ph.D. in physiology, with post-doctoral research in pathology and epidemiology. After his post-doctoral training, he taught university and consulted on data analysis and research design. He joined Health Canada as a toxicologist in the Pest Management Regulatory Agency. He then moved to the clinical trials unit in the Therapeutic Products Directorate, and later to the Biologics and Genetic Therapies Directorate, as a senior evaluator with responsibility for early development studies. He is currently the manager of the vaccine safety unit in the Marketed Biotechnology Products Section of the Marketed Health Products Directorate.

Rogério Gaspar

obtained his PhD in Pharmaceutical Sciences from the Catholic University of Louvain (Belgium) in 1991, after a graduation as pharmacist from the University of Coimbra (Portugal) in 1985. During his PhD he developed nanoparticulate formulations of bioerodible polymers for experimental chemotherapy of Leishmaniasis. Back to his home University (Coimbra, Portugal) he started in 1993 a new research group (Colloidal Drug Carriers Unit) within the Centre for Neurosciences and Cell Biology at Coimbra (Department of Molecular Biology and Biotechnology). His main research areas where then concentrated in nanoparticle-macrophage interactions, imaging systems for MRI, nanoparticulate ocular drug delivery, brain delivery, cancer targeted therapeutics using stealth-targeted liposomes and cytosolic delivery of nucleic acids using different cationic or pH sensitive systems. For years he was involved in the development of European research networks looking at advanced training (e.g. as founder and first coordinator of the now Galenos network, started in 1993).

More relevant aspects concerned the nanoparticle-macrophage interactions having established that polyalkylcyanoacrylate (PACA) nanoparticles but not PLA or PLGA nanoparticles, can trigger under appropriate environment a reactive oxygen intermediates burst following endocytic uptake by macrophages, a structure-relation to that in the case of PACA nanoparticles and that under certain circumstances NO can also be associated to it. Essentially in cytosolic delivery a number of different strategies were established using pH sensitive liposomes and cationic lipoplexes to perform gene transfer and essentially antisense oligonucleotide cytosolic delivery. In cancer therapy a strategy was established using targeted stealth nanosystems for lung cancer, using surface modification of liposomes, modifying the pharmacokinetics and anti-cancer activity in SCLC, as well as using lactide nanoparticles for paclitaxel delivery in cancer cells.

In summary published work points to the possibility of using stealth-targeted nanosystems for cytosolic delivery in cancer. That is the basis for the development of current research projects in oncology looking at cytosolic delivery of pharmacological agents. Additionally capacity for coordination and technological transfer was developed under collaborations established within more than 7 years experience within the European regulatory system for medicinal products and additional 6 years of experience in pharmaceutical R&D in industry.

In the period 1995 to 2002 in parallel with his research activities at academia, he was involved at different levels on the European Regulatory System for Medicinal Products. From 1995 to 2000 as expert and member of the national evaluation board and of the CPMP (now CHMP, at the European Medicines Agency, EMA). From January 2000 to July 2002 he was Vice-chairman of the Management Board of INFARMED (Portuguese medicines regulator) and member of the Management Board of the European Medicines Agency (EMA), as well as member of several committees and expert groups of the European Council of Ministers and European Commission, including participation in the initial steps of the mutual recognition between Japan and EU in the pharmaceutical sector (2002). In 2000 he was the coordinator of the working group that concluded the approval at Council of Ministers level for the European Directive in Clinical Trials, finalised one year later at the European Parliament.

From 2002 to 2008 he was acting as external consultant with a role of coordination and overview on the research activities of a pharmaceutical company (Tecnimede, Portugal). Also acted as a consultant to ASEAN countries (2005-2006 in the context of a EU cooperation programme) and in 2007 as consultant to Cell Therapeutics Inc. (a US based pharmaceutical company from Seattle, USA). Since 2008 he is back to the regulatory system as an expert to the Portuguese national regulatory authority in medicines (INFARMED), and recently (since April 2009) as a member of the newly formed Ad-hoc expert group in Nanomedicines of the European Medicines Agency.

Rogério Gaspar has been involved in the Nanomedicine coordination activities of the European Science Foundation (ESF) as expert or group member. His particular committement to advanced training of young PhD students and post-docs has involved him as co-chair of the European Research Conference in Nanomedicine 2006 (St. Feliu de Guixols, Catalonia, Spain) and of the European Summer School in Nanomedicine 2007 (Cardiff, Wales, UK) and also as Chair of the European Research Conference in Nanomedicine 2008 (St. Feliu de Guixols, Catalonia, Spain) and of the European Summer School in Nanomedicine 2009 (Quinta da Marinha-Cascais, Lisboa, Portugal). Currently (after leaving the University of Coimbra in 2006) he is a Full Professor at the Faculty of Pharmacy University of Lisbon (FFUL) where he is the coordinator for the Pharmaceutical Technology sector, and also the coordinator of the Nanomedicine & Drug Delivery Systems research group (Nano&DDS) within the Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL) which he co-founded in 2007. At Faculty he was elected for the Scientific Council and to the Faculty Assembly governing bodies for the current 4 year terms (2009-2013). He serves also as member of the General Council of the University of Lisbon (overview body of government) and until recently supported the technology transfer unit of the University of Lisbon (UL INOVAR). Rogério Gaspar was recently elected (June 2009) as a member of the Executive Com-

mittee of the European Federation for Pharmaceutical Sciences (EUFEPS). He was member of SAB at EPSRC (UK) Platform in Nanomedicines (2007 and 2008), has been member of the EAB of EuroNanoMed (ERA-NET) (2009) and he is member of the SAB at CIBBER-BBN (2010). Current research interests of his group (~30 researchers) regards several research objectives including the development of new therapeutic strategies using liposomes, polymeric biodegradable nanoparticles, and polymer therapeutics. His main focus in research is currently oriented towards cytosolic delivery of nucleic acids and use of targeted delivery systems for combination therapy in cancer. Current more relevant projects: ♦ OncoNanoMed-Target, Iberian Platform in Nanotechnology (2009-2011) ♦ NanoStem, EuroNanoMed, ERA-NET (2010-2013).

Ehud Gazit

Prof., Ph.D., Vice President for Research and Development, Chair for Nano-Biology, Professor of Biotechnology, Chairman of the Board, Ramot Ltd.

Prof. Ehud Gazit is TAU Vice President for Research and Development and the incumbent of the Chair for Nano-Biology. Gazit received his B.Sc. summa cum laude after completing the Special Program for Outstanding Students at Tel Aviv University, and his Ph.D. (with highest distinction) from the 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 post-doctoral studies at the Massachusetts Institute of Technology (MIT) where he has held a visiting appointment since 2002.

Gazit's research is directed toward the study of protein folding, misfolding, and self-assembly. His work has resulted in the identification of elements that facilitate the assembly of amyloid fibrils, associated with Alzheimer's disease, and he has identified novel ways to inhibit this process. His laboratory was the first to discover aromatic dipeptides that form nanotubes and nanospheres with unique mechanical and chemical properties. Applications of these nano-assemblies include ultra-sensitive biosensors, energy-storage devices, and metallic nanowires. His work has been published in prestigious academic journals such as Science, Nature Nanotechnology, and Proceedings of the US National Academy of Science, among others.

Gazit served as one of the Strategic Research Program (SRP) Leaders of the European Union's Nano2Life Network of Excellence; as an expert of the European Observatory of Nanobiotechnology (EoN); and as a resident expert in the field of nano-biology for Science At Stake. He is on the editorial board of several journals including Journal of Bionanoscience, Nanomedicine, PLoS ONE, Amyloid and Current Chemical Biology.

Gazit had received numerous awards and honors including the Landau Research Award, Dan David Scholarship Award, and TAU Research Council Prize for excellence in research. His technology transfer achievements were acknowledged by inclusion in the 40/40 list of the The Marker journal in 2007 and the 2008 list of "100 Innovations from Academic Research to Real-World Application" by the Association of University Technology Managers (AUTM). He is currently serving as the chairman of the board of Ramot Ltd, the Technology Transfer company of Tel Aviv University.

Blaise Genton

Date of birth: 15.06.1956. Nationality: Swiss. Language: Fluent in French, English and Melanesian Pidgin, understanding German. Family status: Married, 4 children (1989, 1991, 1994, 2006). Prof. address: Swiss Tropical and Public Health Institute, Socinstrasse 57, 4002 Basel and Policlinique Médicale Universitaire, Bugnon 44, 1011 Lausanne, Switzerland

ACADEMIC TITLES

2010: University of Lausanne, Switzerland - Full Professor Tropical/Trav Med, Epidemiology. 1999: University of Lausanne, Switzerland - Privat-docent Tropical Med/Epidemiology. 1997: University of Basel, Switzerland - PhD Epidemiology. 1991: University of London, UK - MSc - Clinical Tropical Medicine. 1988: University of Lausanne, Switzerland - Doctorate - Medicine. 1981: University of Lausanne, Switzerland - Diploma - Medicine

PROFESSIONAL TITLES

2003: International Society Travel Medicine - Certificate in Travel HealthTM. 1997: Federatio Medicus Helvaeticorum - FMH Specialist in Tropical Medicine. 1991: Royal college of Physicians - Diploma of Tropical Medicine and Hygiene (DTM&H). 1990: Federatio Medicus Helvaeticorum - FMH Specialist in Internal Medicine

AWARDS

- Laureate of the 1996 'Prix Claude Perrier' of the foundation for the promotion of the teaching and research in clinical pharmacology for the work in the field of anti-malarial vaccinology.
- Senior author of the best oral presentation awarded the Novartis Price at the 2001 Annual meeting of the Swiss Society of Internal Medicine (D'Acremont V, Landry P, Müller I, Pécoud A, Genton B. Clinical and laboratory predictors of imported malaria : a case-control study)
- Senior author of the best poster awarded at the 2003 Joint Annual Meeting of the Swiss Society of Microbiology, Infectious Diseases, Tropical Medicine and Parasitology (Marfurt J, Felger I, Beck H-P, Genton B. DNA chip technology: a new epidemiological tool to monitor drug resistance in malaria) resistance in malaria)
- Senior author of the best oral presentation awarded at the 2009 11th Conference of the International Society of Travel Medicine (V. D'Acremont, J. Kahama-Maro, D. Mtasiwa, C. Lengeler, B. Genton. Low quality of routine microscopy for malaria in Dar es Salaam, Tanzania: implications for the sick traveler)
- First author of the best poster awarded at the 2010 Annual Meeting of the Swiss Society of Infectious Diseases (B. Genton et al. Virosome-formulated peptidomimetics as vaccine against falciparum malaria: randomized double-blind controlled phase 1 trials in healthy non-immune adults in Switzerland and in semi-immune adults and children in Tanzania).

CARRIER OBJECTIVES: To develop excellence in translational research – medicine with a special focus on improving health in developing countries

AREAS OF EXPERTISE

- Tropical and travel medicine – Evidence-based medicine - Public health and epidemiology in developing countries – Clinical Trials
- Intervention effectiveness and impact assessment in developing countries. - Clinical epidemiologist, project leader in the Department of Epidemiology and Public Health, Swiss Tropical and Public Health Institute, Basel, Switzerland (50% position) - Head of the travel clinic and consultant of tropical diseases, Division of Infectious Diseases, Department of Ambulatory care and Community Medicine, University Hospital, Lausanne, Switzerland (50% position) - Tanzania: Project leader at the Ifakara Health Institute, Dar Es Salaam 2006 – 2008 - Papua New Guinea: Senior research fellow at the Papua New Guinea Institute of Medical Research, Madang, 1991 – 1996 - Uganda: Medical Coordinator at the International Committee of the Red Cross 1985-1986 and 1987 - Lebanon: Physician at the International Committee of the Red Cross 1985 - Consultant: Tropical/Travel Medicine & epidemiology Oct.1997- Senior registrar: Internal & Tropical/Travel Medicine Jan.1997-Sep.1997. Registrar: Internal Medicine Apr.1990-Sep.1990. Senior house officer: Internal Medicine Apr.1988-Mar.1989. Infectious diseases Sep.1989-Apr.1990. Senior house officer: Psychiatry Apr.1986-Mar.1987. Gynecology Apr.1987-Sep.1987. Medical delegate ICRC: Lebanon Uganda Apr.1985-Dec.1985. Senior house officer: Surgery Oct.1982-Sep.1983. Internal Medicine Oct.1983-Mar.1985. House officer Anesthesiology Apr.1982-Sep.1982. Project leader in the Intervention Unit, Swiss Tropical and Public Health Institute in Basel, Switzerland (2001-2006) working mainly on i) the evaluation of the impact of the introduction of artemether/lumefantrine as first

line antimalarial treatment on child mortality and morbidity in two districts of Tanzania, ii) the implementation and evaluation of Rapid Diagnostic Tests for malaria in urban and rural Tanzania, iii) the clinical development of malaria vaccines in Switzerland and endemic areas, iv) the development and implementation of molecular methods (microarrays) to assess treatment effectiveness through the investigation of single nucleotide polymorphisms of the Plasmodium parasite (molecular markers of parasite resistance to antimalarials drugs) and of the human host for drug metabolism (pharmacogenetics) in Africa, South East Asia and Oceania, including technology transfer.. Head of the research (1997-) in Travel Medicine and Vaccinology, Department of Ambulatory Care and Community Medicine, University of Lausanne, Switzerland, with a special focus on i) the development and validation of evidence based recommendations for the management of fever in returning travelers and migrants, and ii) phase I-III clinical trials of new vaccines. Previously research fellow (1991-1994) and senior research fellow (1995-1996) in Clinical Epidemiology, Malariology, in charge of the Malaria Vaccine Epidemiology and Evaluation Project in Madang and Maprik, Papua New Guinea Institute of Medical Research, Papua New Guinea, working mainly on the establishment of demography and morbidity surveillance systems to conduct malaria vaccine trials and monitoring disease trends as well as parasite resistance

MAJOR RECENT ACHIEVEMENTS

- Impact assessments on mortality, morbidity and drug consumption of i) the implementation of rapid diagnostic tests for malaria and ii) the introduction of new first line treatment policy (artemether/lumefantrine) in Tanzania.
- Conduct of 11 clinical trials, 8 on malaria vaccines and 3 on new anti-malarial drugs, both in Switzerland and malaria endemic countries
- Development and field implementation of new molecular tools (microarray technology) to assess and monitor malaria parasite resistance to drugs and pharmacogenetics in Africa and Asia
- Development, implementation and evaluation of web-based guidelines through a global internet study in primary care settings
- Data and safety monitoring boards Malaria
- Chairman of the Safety Monitoring Committee for the `Phase Ib safety and immunognicity study of the recombinant lactococcus lactis hybrid GMZ2 malaria vaccine in children' in Gabon
- Chairman of the Safety Monitoring Committee for the `Phase Ib safety and immunognicity study of the recombinant lactococcus lactis hybrid GMZ2 malaria vaccine in adults ' in Gabon
- Chairman of the Independent Safety Monitoring board for the 'Phase Ia-IIa safety, immunogenicity and pilot efficacy of of the candidate malaria vaccine PfLSA-3' in Holland
- Chairman of the Independent Safety Monitoring Committee for the `Phase I and IIa studies of ICC-1132 malaria vaccine in healthy adults' in Germany
- Chairman of the Data Safety Monitoring Board for the Phase I of 'Wanxing Bio-Pharmaceuticals' AMA-1/MSP-1 recombinant malaria vaccine (PfCP-2.9) adjuvanted with Montanide ISA 720 compared to Montanide ISA 720 alone in healthy adult volunteers' in China
- Member of the Independent Safety Monitoring Committee for the Phase I and IIa studies of 'MVA-CSO and RTS,S/ASO2A malaria vaccine in healthy adults using a prime-boost delivery schedule' in Oxford
- Chairman of the Data and Safety Monitoring Committee for the `Phase Ib trial of GMZ2 vaccine candidate in Gabonese children' in Gabon
- Chairman of the Data and Safety Monitoring Board for the ACT consortium for the studies 'An equity and cost-effectiveness analysis of alternative strategies for the deployment of artemisinin-based combination therapy (ACT) at the community level' and 'Effects of restricting the use of Artesunate plus amodiaquine combination therapy to malaria cases confirmed by a dipstick test: A cluster randomised control trial'Trypanosomiasis
- Chairman of the Data and Safety Monitoring Board for the studies 'Improvement of the treatment of human sleeping sickness using the trypanocidal drug melarsoprol ; clinical, pharmacological and biological investigations' (Impamel I & II) Meningitis
- Chairman of the Independent Data Monitoring Committee for the `Phase II studies of GlaxoSmithKline (GSK) Biologicals' Hib-MenAC vaccine - Globorix' in Ghana, Thailand, The Philippines, Nicaragua and South Africa
- Member of the Independent Data Monitoring Committee for the 'Phase II safety, immunogenicity and reactogenicity trial of Mencevax ACW135 polysaccharide vaccine' in Ethiopia

Subrata Ghosh

was born in 1978 in Howrah, India. He received his bachelor's degree in 2000 from Burdwan University, India and Post graduated from the same University in 2002. He received his Ph.D. degree in the year 2010 from Jadavpur University and IACS, India for the Synthesis of Bioactive Natural Products. During his Ph.D he explored the total synthesis of allelopathic sesquiterpenoid compounds. In 2009 he joined the ANCC group in NIMS, Japan as a postdoctoral fellow. His current research interests include theoretical simulation of artificially intelligent molecular nano brain and practical synthesis of supramolecular architectures for showing in-vivo and in-vitro application of nano brain in human cell as a anti-cancer and anti-aging drug and synthesis of conjugated dendritic nano brain for gene therapy.

Christophe Ginestier

PhD, Professional address: Centre de Recherche en Cancérologie de Marseille, U891-Inserm-Institut Paoli-Calmettes, 27, Bd Lei Roure, BP 30059, 13273 Marseille Cedex 09, France. Phone: (33) 4 91 22 35 09, christophe.ginestier@inserm.fr

EDUCATION: University of méditerranée, Aix-Marseille II, France. 2002-2005: Ph.D. in Oncology : Pharmacology and Therapeutics. 2000-2002: Master (MS) in biochemistry (Oncology). 1997-2000: Bachelor degree (BS) in Biology.

WORK EXPERIENCE

- Since 2009: Centre de Recherche en Cancérologie de Marseille, INSERM, France, Research investigator, Molecular Oncology Lab. Research project : Characterization of normal and malignant human mammary stem cells. Breast primary tumor xenobank establishment
- 2006-2009: University of Michigan, Comprehensive Cancer Center, Ann Abor, MI. Postdoctoral Research Fellow in Max Wicha's Lab. Postdoc research project : Characterization of normal and malignant human mammary stem cells. ▪ Identification of ALDH1 as a normal and malignant human mammary stem cells marker. ▪ Role of BRCA1 in Regulation of Human Mammary Stem/Progenitor Cell Fate.
- 2002-2005: INSERM - U519, Marseille, France : Molecular Oncology Laboratory. PhD supervisor : Dr Daniel Birnbaum. PhD fellowship from French Ministry of Research. PhD research project : Molecular taxonomy of breast cancers. ▪ Identification of Subclasses of Breast Cancer by Protein. ▪ Expression Profiling. ▪ ERBB2 target: Gene Expression Signature and Herceptin resistance. ▪ Genome Alterations (amplifications, fragile sites).
- 2001-2002: Institut Paoli-Calemettes, Marseille, France : Molecular Pathology Laboratory. Master degree Supervisor: Dr Jocelyne Jacquemier. Project : Distinct and Complementary Information Provided by Use of Tissue and DNA Microarrays in the Study of Breast Tumor Markers.

MEMBERSHIPS IN PROFESSIONAL SOCIETIES: Since 2009 ISSCR associate member, Since 2008 AACR associate member

SCIENTIFIC ADVISOR: Since 2009, Oncostemcell, Inc.

AWARD: 2008 AACR-Bristol-Myers Squibb Oncology Award

BOOK CHAPTER: Ginestier C, Wicha M.S. Stem cells in breast development and carcinogenesis: Concepts and Clinical perspectives. Disease of the Breast, Fourth Edition by Jay R. Harris, Marc E. Lippman, Monica Morrow and C. Kent Osborne.

PATENTS: Targeting CSC through CXCR1 blockade, US PA 61/113,458 ALDH1 – a marker of normal and cancer stem cells, US PA 10181 Identification of an ERBB2 gene expression signature in breast cancer, WO/2005/021788.

Gershon Golomb

Personal Details: Date and Place of Birth: 5 January 1952, Jaffa (Tel-Aviv), Israel. Army Service: 1971-1974, Lt. Col. (reserve forces). Marital Status: Married, three children.

ACADEMIC EDUCATION

1978, B.Sc.Pharm.; 1980, M.Sc. with Distinction; 1984, Ph.D. in Pharmaceutical Sciences – The Hebrew University of Jerusalem, Israel.

ACADEMIC CAREER

- 1984–1986 Postdoctoral Fellow, the Children's Hosp., Harvard Medical School, Boston, MA; and Visiting Scientist, Chemical Engineering, MIT, Cambridge, MA, USA.
- 1987–1991 Lecturer, Dept. of Pharmaceutics, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Israel.
- 1991–1992 Visiting Prof., Dept. of Cardiology and College of Pharmacy, Univ. of Michigan, USA.
- 1992–1997 Senior Lecturer (with tenure) in Pharmaceutics, Faculty of Medicine, HUJI.
- 1997–2000 Assoc. Prof. of Pharmaceutics, Faculty of Medicine, HUJI.
- 2000 Full Prof. of Pharmaceutics, Faculty of Medicine, HUJI.
- 2008 Visiting Prof., Dept. Chemical Engineering, MIT, USA.

APPOINTMENTS

1997–2000 Chairman, Department of Pharmaceutics, School of Pharmacy, HUJI. 2000–2006 Chairman, School of Pharmacy, Faculty of Medicine, HUJI (2 available terms). 2009–2012 Elected member, Senate of HUJI.

AWARDS AND HONORS (SELECTED)

1988 Principal co-author of paper awarded "The 1988 Ebert Prize" of the Am. Pharm. Assoc. for the most outstanding paper of the year in J. Pharm. Sci.. 1989 Outstanding Lecturer, Faculty of Medicine, HUJI. 1991 The J. Schmerler Faculty Prize for excellence in research in the year 90-91. 1999 The Juludan Prize for Excellence in Biomedical Research (Technion, Israel). 1999 The "Ben Schendar Prize" in "Pharmacology and Drug Development". 1999–2004 The "Harry W. and Charlotte Ullman Labov Chair in Cancer Studies". 2000 Elected Fellow of the "American Institute of Medical and Biological Engineering". 2002 Kaye prize for innovations, HUJI. 2003 Best paper of the year award, The D. Bloom Center for Pharmacy, HUJI. 2008 Distinguished Service Award, The Pharmaceutical Society of Israel.

PROFESSIONAL ACTIVITIES (SELECTED)

Organizer, chairman/co-chairman of international conferences. Member of 3 editorial boards, and referee. Member & chairman of several HUJI and national committees. Over 150 worldwide invited lectures in academia and industry (not including contributed presentations in meetings/conferences).

TEACHING (2001): Undergraduate courses in Pharmacy (Introduction to Pharmaceutics, Advanced Drug Delivery Systems); Medicine (part of Pharmacology); Graduate course (Implantable and Injectable Drug Delivery Systems); Supervisor of 3 MSc & 5 PhD students.

RESEARCH (KEY WORDS): Controlled / Sustained release drug delivery systems; Dosage forms; Nanoparticles; Liposomes; Gene Delivery and Therapy; Immunomodulation; Bisphosphonates; Pathophysiology & therapy of cardiovascular (restenosis, MI) and bone disorders; Mammary carcinoma; Endometriosis; BBB. Basic-research grants and industrial projects.

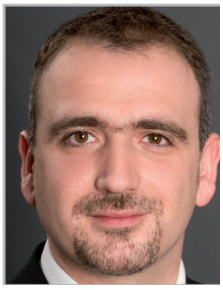
PUBLICATIONS: 128 full publications (113 papers & 15 chapters in books), >29 patents, and >150 abstracts & proceedings.

Enrique Gómez-Barrena

47 years old, Full Professor in Orthopaedic Surgery and Traumatology at La Paz University Hospital, and Chair at the Universidad Autónoma de Madrid, Madrid, Spain, since 2010. Trained as an Orthopaedic Surgeon in Madrid, until 1992, completed his Ph.D. degree and attended The Hospital for Special Surgery in New York at the Hip and Knee Service and the Biomechanics and Biomaterials Department in 1994. Back in Spain, he became Attending Orthopaedic Surgeon at the Hospital "Nuestra Señora de Gracia" in Zaragoza (1996-2000), and then Associate Professor of Orthopaedic Surgery and Traumatology at Fundación Jiménez Díaz Hospital in Madrid (2000-2010).

He has been involved or lead 15 competitive funded research projects and is now coordinating the Orthopaedics Working Package at the 7th FP-EU funded project REBORNE. He is currently the Vice-President of the European Orthopaedic Research Society.

He has authored about 90 peer-reviewed articles on Orthopaedic Surgery clinical and basic research, significantly on biomaterials in bone and joints.



Nicolas Gouze

has an engineer's degree in opto-electronics from the University Paris XI and studied Innovation Management at the University of Valenciennes (France).

Since 2004 he is working with the Department "Innovation Europe" of VDI/VDE-IT in Berlin. 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 at VDI/VDE-IT on the 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.

He is involved in the management of the ETP Nanomedicine as well by offering support to the members and being responsible for the platform's events.

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

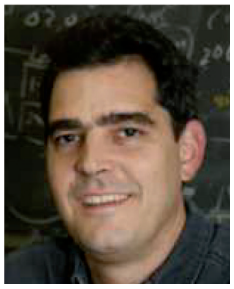
- 2004 member of the Executive Board of IUVISTA; head of Nanometer Structure division
- 2005 Doctor honoris causa of University of Neuchâtel
- 2006 Honorary President of NCCR Nanoscale Science and Swiss Nanoscience Institute (SNI)

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



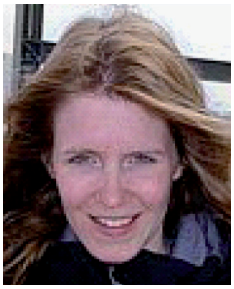
Martin Hegner

Trinity College Dublin, Physics / CRA, Dublin. Martin Hegner is appointed associate professor in the School of Physics at the Trinity College Dublin and PI at the Nanocenter CRANN (since '07).

MS degree in Biochemistry and Molecular Biology ('89) and PhD ('94, Biological Scanning Probe Microscopy) Swiss Federal Institute of Technology, Zürich, Switzerland. Postdoctoral research in the US ('96 –'99) Oregon & Berkeley with focus on optical tweezers manipulation of single biomolecules. PI in the field of Nanobiotechnology at the newly founded National Competence Center for Nanoscale Science, University of Basel, Switzerland ('99-'07) • Fellow of Trinity College Dublin 4/2011.

Martin Hegner's scientific interests are focused on interdisciplinary research in the fields of single molecule manipulation, biophysics, bio-diagnostics and development and application of biological sensing devices. Current interests are centred on biological nanomechanics, which is explored firstly with single bio-molecule manipulation techniques such as optical tweezers or scanning probe microscopy. Here transport of polypeptides through biological membranes and motor protein action of the ribosome are investigated. Secondly his team is developing novel cantilever array devices to enable label free diagnostics. Taylor made bio-engineered nanomechanical biosensors for genomics, proteomics, microbio (environmental) and chemical sensing applications are engineered. Collaborations span around the globe (e.g. CalTech (US), McGill (CAN) European and local Irish research partners) and involve also industrial partners such as Hoffmann La-Roche Ltd. and Novartis AG.

Nanobiotechnology will have a deep impact on our daily lives translating laboratory research into products that will revolutionise healthcare. www: http://www.tcd.ie/Physics/People/Martin.Hegner/index.html



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 in the group of Professor Wendelin Stark at the ETH Zurich, she focused on the development of a nanomagnet-based blood purification technology in collaboration with the University Hospital Zurich. In 2010, she finished her PhD studies. 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 infarction, stroke).

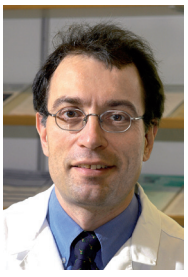
Heinrich Hofmann

Professor for Materials Science. Director of the ERU Surface, Coating, and Particle Engineering (SPERU). Hofmann Heinrich, Prof. Dr.-Ing. Studied first foundry engineering at the Applied University of Duisburg followed by a study of Material Science and Engineering at the Technical University of Berlin. 1983 he got his PhD in Material Science with a thesis prepared at the Powder Metallurgy Laboratory at the Max Planck Institute in Stuttgart.

Between 1983 and 1985, he was senior scientist at the same Max Planck Institute working on novel hard metals and composites. In 1985 he joined the R&D center of Alusuisse-Lonza Services AG, at Neuhausen-am-Rheinfall. He was first involved in the development of new alumina powders for ceramic application. In a second part, he developed a new titania stabilized zirconia powder as well as a pilot plant for a first fabrication of such powders in industrial quantities. In parallel, he also developed carbothermic processes for the fabrication of silicon nitride powders.

In 1993 he joined the Swiss Federal Institute of Technology as Professor and Director of the Powder Technology Laboratory at the Department of Materials Science and Engineering. His research area includes the synthesis of nanostructured materials based on nanoparticles and the modification of surfaces with nanoparticles using colloidal methods. The application of such materials are in the medical and biological field (drug delivery, hyperthermia, cell separation, biosensors), electronics and sensors as well as coating of medical devices, turbine blades and paper. He is member of several professional organizations as well as of the "Europäische Akademie für Technikfolgen Abschätzung" (technology assessment) and Member of the Swiss Federal working group "Nanoregulation".

Since 2006 he is director of the research unit "Surface, Coating and Particle Engineering" SPERU of the Competence Centre of Material Science. He is member of various scientific advisory boards in Japan, China and Thailand. Since 2008 he is a cofounder of a company developing nanocomposites for cancer treatments (ANTIA Therapeutics). His publication list comprises over 100 Publications in reviewed journals, 33 publications in proceedings, co-author of 4 books and co-editor of 2 MRS proceedings and he is co-inventor of 15 patents or patent applications. Additionally he has given more than 20 invited and key note lectures on particles synthesis, modification and nanoparticles in biomedical applications in EU, US Australia and Asia. He supervised 25 PhD students in the period 1994 – 2009. More information about the Powder Technology Lab: see: http://ltp.epfl.ch



Patrick Hunziker

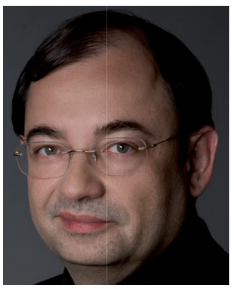
Patrick Hunziker has studied Medicine the University of Zurich, Switzerland. He received a doctoral decree based on thesis work in experimental immunology from the University of Zurich and did further research in experimental haematology at University Hospital in Zurich, Switzerland. He earned specialist 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

Nadja Jessel

Dr. Nadia Jessel is the Leader of the "Active Biomaterials and Tissue Engineering" team at INSERM U595, 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. 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 (Chargé de Recherches, CR1) in the INSERM 595 laboratory in 2004 and currently 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. Jessel is a co-author of 40 peer-reviewed publications in high impact factor journals (Proc. Nat. Acad. Sci. USA; Adv. Mater.; Adv. Funct. Mater.; Small; Nanoletters, Biomaterials), 5 chapters reviews and 3 international patents, she is a regular referee for a number of scientific journals..



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.

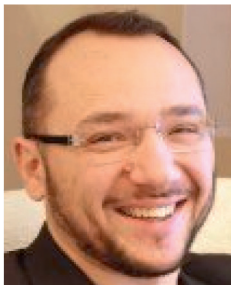
Varvara Karagkiozaki

Med, MSc, Specialist Cardiologist, MSc in Nanosciences & Nanotechnologies, PhD in Nanomedicine. Department of Physics, Lab for "Thin Films -Nanosystems & Nanometrology", Aristotle University of Thessaloniki, Greece. AHEPA University Hospital, 1st Cardiology Department, Lab for "Cardiovascular Engineering & Atherosclerosis", 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 a 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 Honored 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.
She is a Member of Nanomedicine – Nanobiotechnology team of LTFN, member of Lab for Cardiovascular Engineering & Atherosclerosis, Ahepa Hospital, Medical School AUTH, member of European Society of Clinical Nanomedicine, a founding member of International Society of Nanomedicine, member of American Society of Nanomedicine, member of Greek Pediatric Cardiology Association, of Greek Medical association for Obesity. 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 Nano-medical, 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 and has been active in Nanomedicine field with papers on Nanocardiology and especially on Nanotechnology's contribution on stent coating technology. She gave lectures at Nanomedicine Summer Schools (ISSON9, ISSON10) and at N&N Postgraduate Program of Auth, during the period of 2010-2011.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 also a member of the Organizing Committee of the Nanomedicine and Nanobiotechnology Workshop during the 7th International Conference on Nanosciences & Nanotechnologies NN10 held last year. She is a member of the Organizing Committee of the Nanomedicine Workshop during the upcoming 8th International Conference on Nanosciences & Nanotechnologies NN11, at 12-15 July 2011, Thessaloniki Greece. More information can be found at the website: http://nanotex4.com

Costas Kiparissides

Dr. Costas Kiparissides is a Professor at the Chemical Engineering Department of Aristotle University of Thessaloniki since 1981. During the period 2001-2006, he was Director of Chemical Process Engineering Research Institute (CPERI) at CERTH and in the period 2005-2010 Director of Centre for Research & Technology Hellas (CERTH). He is member of the Expert Advisory Group (EAG) of the NMP programme, member of the Mirror Group of the European Technology Platform for Sustainable Chemistry, member of the Ad-hoc Advisory Group on Industrial Nanotechnologies, vice-chair of the working group: Nanopharmaceutics of the European Nanomedicine Technology Platform, etc.

He has supervised more than fifty Ph.D. graduate students, 160 diploma theses and has presented more than three hundred invited seminars and lectures at international scientific conferences, industrial research centres, institutes and universities in Europe and North America. In addition he has published 200 papers in refereed journals, 350 conference papers and 6 books. His published work has received more than 3000 citations. His current research interests are in the areas of functional materials, molecular and morphological characterization of polymers, novel micro- and nano-encapsulation technologies, functional micro- and nano-particles for health, food and environmental applications, molecularly imprinted polymers (MIPs) for selective recognition and separation of biological molecules, microbial production of functional biopolymers from renewable sources and advanced multi-scale modelling of chemical and biological systems.



Kostas Kostarelos

Professor, Chair of Nanomedicine, Head, Centre for Drug Delivery Research, The School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX, United Kingdom, e-mail: kostas.kostarelos@pharmacy.ac.uk, website: http://www.nanomedicinelab.org

Professor Kostarelos is the Chair of Nanomedicine & Head of the Centre for Drug Delivery Research at The School of Pharmacy of the University of London. In 2010 he was awarded the Japanese Society for the Promotion of Science (JSPS) Professorial Fellowship with the National Institute of Advanced Industrial Science and Technology (AIST) in Tsukuba, Japan. He has been invited Fellow of the Royal Society of Medicine (FRSM), Fellow of the Institute of Nanotechnology (FIoN) and Fellow of the Royal Society of Arts (FRSA) all in the United Kingdom.

He has co-author the 'Strategic Research Agenda for Nanomedicine' published in November 2006 by the European Commission on behalf of the European Technology Platform in Nanomedicine (Brussels, Belgium) and was invited to co-author the 'GENNESYS White Paper - A New European Partnership between Nanomaterials Science & Nanotechnology' published in 2009 by the Max-Plank Institute (Stuttgart, Germany).

He is past Treasurer and Board Member of the International Liposome Society and Advisory Board Member of the European Foundation for Clinical Nanomedicine. He obtained his Diploma in Chemical Engineering (DIC) and PhD from the Department of Chemical Engineering, Imperial College London. Previous appointments include: Assistant Professor of Genetic Medicine and Chemical Engineering in Medicine at Cornell University Weill Medical College, NY, USA; Deputy Director of Imperial College Genetic Therapies Centre, London, UK; Research Associate, University of California at San Francisco (UCSF) and Memorial Sloan-Kettering Cancer Center in New York, USA.

Prof. Kostarelos is the Founding and Senior Editor of the journal Nanomedicine (ISI® 2009 Impact Factor: 5.98) and sits on the Editorial Board of The Journal of Liposome Research, The International Journal of Nanomedicine, Nanomedicine: Nanotechnology, Biology and Medicine, and Frontiers in Neuroengineering.

Felix Kratz

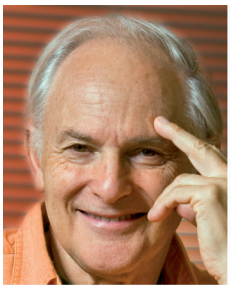
Dr. rer. nat. Felix Kratz, Head of the Division of Macromolecular Prodrugs; Clinical Research, Tumor Biology Center at the Albert-Ludwigs-University of Freiburg, Breisacher Straße 117, 79106 Freiburg i.Br. Tel.: 0049-761-2062930, Telefax.: 0049-761-2062905; E-Mail: kratz@tumorbio.uni-freiburg.de. The Division Macromolecular Prodrugs was founded in 1994 by Dr. Felix Kratz in the Clinical Research Department at the Tumor Biology Center, Freiburg, Germany. The Tumor Biology Center at the University of Freiburg is a leading cancer clinic and research institution focusing on the development of novel approaches for the effective treatment of cancer patients (http://www.tumorbio.uni-freiburg.de). The primary goal of his research team is the development of novel drug delivery concepts for improving the efficacy and toxicity of anticancer agents. Felix Kratz graduated in Chemistry from the University of Heidelberg in 1991. He then carried out postdoctoral research at the University of Florence in the Bioinorganic Institute of Professor Ivano Bertini and developed tumor-specific carrier systems with ruthenium(III) complexes. Since 1994 he has been Head of the Division of Macromolecular Prodrugs at the Tumor Biology Center, where he is in charge of preclinical drug development and organizing and managing translational research from the laboratory to the clinic. His research areas are drug targeting, drug delivery systems in oncology, prodrugs, receptor targeting, bioconjugate chemistry, polymer therapeutics and nanocarriers.

He has over 20 years of experience in the preclinical development of anticancer drugs and profound knowledge of translational research from the laboratory to the clinic and has successfully transferred a first albumin-binding prodrug (DOXO-EMCH, INNO-206) into the clinic which is scheduled to enter phase II clinical trials in 2011 (see http//:www.cytrx.com). He is also the inventor of an albumin-binding prodrug of methotrexate for the treatment of rheumatoid arthritis which is being further developed by medac GmbH for clinical evaluation in patients with rheumatoid arthritis. He serves on the Editorial Board for Bioconjugate Chemistry, Current Medicinal Chemistry, Current Bioactive Compounds, and Pharmacology & Pharmacy. He has authored approximately 210 scientific publications and proceedings and is the inventor of 25 patents and patent applications.

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.



Harold Kroto

Sir Harold (Harry) Kroto is currently a Francis Eppes professor of Chemistry at Florida State University, where he is carrying out research in nanoscience and cluster chemistry as well as developing exciting new Internet approaches to STEM educational outreach. In 1996 he was knighted for his contributions to chemistry and later that year was one of three recipients of the Nobel Prize for Chemistry in 1996. He is a Fellow of the Royal Society of London, and holds an emeritus professorship at the University of Sussex in Brighton, United Kingdom. The research program focuses on the complex range of molecular constituents in carbon vapour; the development of novel 2 and 3D metal-cluster/organic frameworks as well as peptides; the stabilization of small fullerenes; and carbon nanotube based devices behaviour. He has also initiated the Global Educational Outreach for Science, Engineering, and Technology programme (GEOSET - www.geoset.info and www.geoset.fsu.edu). GEOSET seeks to exploit the revolutionary creative dynamics the Internet (which he calls it the GooYouWiki-World) to improve the general level of science teaching worldwide.
Harry obtained a first class BSc honours degree in chemistry (1961) and a PhD, in molecular spectroscopy in 1964 at the University of Sheffield (UK). After post-doctoral positions at the National Research Council in Ottawa, Canada (1964-66) and at the Murray Hill Bell Laboratories (NJ, US) in 1966-67 he started his independent academic career at the University of Sussex. In 1970 his research group conducted laboratory and radio astronomy studies on long linear carbon chain molecules, and with Canadian astronomers discovered that they existed in interstellar space. In 1985 together with Robert Curl, Richard Smalley and research students Jim Heath, Sean O'Brien and Yuan Liu at Rice University (Texas) he carried out laboratory experiments which simulated the chemical reactions in the atmosphere of red giant stars. These experiments uncovered the existence of C60 Buckminsterfullerene, a new form of carbon for which he together with Curl and Smalley received the 1996 Nobel Prize in Chemistry. In 1995, he launched the Vega Science Trust (www.vega.org.uk) to create science films of sufficiently high quality for broadcast on UK network television. He has several other awards including the Copley Medal and Faraday Lectureship of the Royal Society and the Longstaff Medal of the Royal Society of Chemistry. He holds some 30 honorary degrees from universities all over the world. From 2004 he has been on the Board of Scientific Governors at Scripps Institute. He was elected a Foreign Associate of the National Academy of Sciences in 2007.

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'). Since 2007, he has been appointed as a postdoctoral fellow at the Department of Pharmaceutics at Utrecht University, where he predominantly works on the MediTrans project (FP6: 'Targeted delivery of nanomedicines'). Since 2009, he has also been appointed as a group leader at the Department of Experimental Molecular Imaging at the University of Aachen (RWTH) and the Helmholtz Institute for Biomedical Engineering. In 2010, he edited a theme issue on HPMA copolymers for Advanced Drug Delivery Reviews, and he was elected to the editorial boards of Theranostics, the Journal of Nanomedicine and Biotherapeutic Discovery and 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 head of the department "Drug Delivery" at the recently established Helmholtz Institute for Pharmaceutical Research Saarland (HIPS). HIPS belongs to the Helmholtz Centre for Infectious Diseases (www.helmholtz-hzi.de), Braunschweig, and is the first public research institute in Germany explicitly dedicated to the Pharmaceutical Sciences.
The research of Prof. Lehr's team focuses on biological barriers to drug delivery, in particular the gastro-intestinal tract, the skin and the lungs. This also includes the design of appropriate carriers capable to cross these epithelial barriers and to deliver the active molecule to the target. The expansion of this approach by developing new in vitro models of higher sophistication and relevance, based on the epithelial cells and tissues concerned, is in advanced progress. In parallel, the nanotechnology approach is improving and broadened in terms of formulating multifunctional nanocarriers that allow in vivo tracking of the system, targeting to the site of action, release the payload in a controllable manner and last but not least being safely biodegraded and excreted from the body.
Prof. Lehr was the recipient of the CRS Young Investigator Award (2001), the APV Research Award 2006 for outstanding achievements in the Pharmaceutical Sciences and the biannual International Price 2008 of the Belgian Society for Pharmaceutical Sciences. In 2010 he became „Fellow" oft the American Association of Pharmaceutical Scientists (AAPS).

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).

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.

Ian MacLachlan

3331 Sylvester Road, Mission, BC, Canada, V2V-0B1, Tel No.: 604-820-8130, E-mail: imaclachlan@tekmirapharm.com

EDUCATION: May 1988-Jun 1994: Ph.D. (Biochemistry) University of Alberta, Edmonton, Canada, & Department of Molecular Genetics, University of Vienna, Austria. Sep 1985-May 1988: B.Sc. (Biochemistry) University of Alberta, Edmonton, Canada. Sep 1982-May 1984: Biological Sciences University of Calgary, Calgary, Canada.

EXPERIENCE

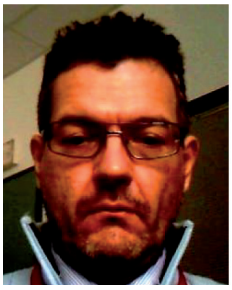
- May 2008-Present: Executive Vice President, Chief Scientific Officer, Tekmira Pharmaceuticals, Corp.,Burnaby, BC, Canada. Development of siRNA Based Therapeutics for Cancer, Inflammatory, Metabolic and Infectious Disease.
- Sep 2000-Present: Chief Scientific Officer, Protiva Biotherapeutics, Inc.,Burnaby, BC, Canada. Development of Non-Viral Nucleic Acid Delivery Systems and siRNA Based Therapeutics for Cancer, Inflammatory, Metabolic and Infectious Disease.
- Jul 1996-Aug 2000: Team Leader / Research Scientist, Inex Pharmaceuticals Corporation, Burnaby, BC, Canada. Non-Viral Cancer Gene Therapy. Suicide Gene Therapy, Pharmacology, Vector Development, Tumor Modeling, Inducible Expression.
- Jul 1994-Jun 1996: Research Fellow, Howard Hughes Medical Institute Dept. of Internal Medicine, University of Michigan, USA.
- Supervisor: Dr. G.J. Nabel, TNF Mediated Activation of NF-κB and the HIV LTR Adenoviral Gene Therapy for Restenosis. The Role of NF-κB in Vertebrate Development.
- May 1988-Jun 1994: Graduate Student, Lipid and Lipoprotein Research Group University of Alberta, AB, Canada. & Dept. of Molecular Genetics University of Vienna, Austria. Supervisor: Dr. Wolfgang Schneider, Molecular Genetics of the Lipoprotein Receptor Family. Characterization of the Lipoprotein Receptor Mediated Uptake of Riboflavin Binding Protein Including Cloning and Characterization of the rd Mutant.
- Jan 1988-Apr 1988: Undergraduate Research, University of Alberta, AB, Canada. Supervisor: Dr. Wayne Anderson, Computerized Sequence Analysis of Lipoproteins, Crystallography of Membrane Proteins.
- Sep 1987-Dec 1987: Undergraduate Research, University of Alberta, AB, Canada. Supervisor: Dr. Wolfgang Schneider, Purification and Characterization of Apolipoprotein VLDL-II, an Inhibitor of Lipoprotein Lipase.
- Summer 1987: Undergraduate Research, Bamfield Marine Station, Canada. Supervisor: Dr. Ron Ydenberg, Behavioral Analysis of the Polychaete, Eudystilia vancouveri.
- May 1983-Dec 1986: Computer Programmer, Canadian Hunter Exploration Ltd., Calgary, Alberta, AB, Canada. Programming of Oil and Gas Reservoir Simulations and Data Analysis Tools Used to Guide the Exploration Efforts of an Oil and Gas Company.

TRAINING

- June 2004: American Society of Gene Therapy/ USFDA, Long Term Follow-up of Participants in Human Gene Transfer Research
- March 2003: American Society of Gene Therapy/USFDA, Non-Clinical Toxicology in Support of Licensure of Gene Therapies
- Sept 2002: Protiva Biotherapeutics, WHMIS and Chemical Safety Retraining
- Sept 2002: TLM Consulting, Basic GMP Training
- June 2002: American Society of Gene Therapy/USFDA, Clinical Gene Transfer Comprehensive Review Course
- Apr 2002: TLM Consulting, Introduction to Gene Therapy Clinical Trials and GLP/GMP
- Jul 2001: Protiva Biotherapeutics, Cytotoxic Drug Training
- May 2001: American Society of Gene Therapy / USFDA Clinical Gene Transfer Training Course
- Jun-Sep 1998: Leadership Edge Consulting, Lab-to-Leader Training Program, Project Management, Coaching, Team Management
- Oct 1997: Pape Management Consulting, Project Management Training II
- May 1997: University of British Columbia, Radionuclide Safety and Methodology
- Feb 1997: Pape Management Consulting, Project Management Training I

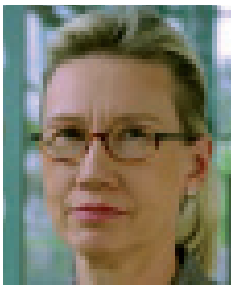
AWARDS AND DISTINCTIONS

- 1995-1998: Medical Research Council of Canada Fellowship
- 1993: Mary Louise Imrie Graduate Award, Faculty of Graduate Studies and Research, University of Alberta
- 1992-1994: Austrian Fonds zur Förderung der Wissenschaftlichen Forschung (Austrian Ministry of Science Scholarship)
- 1989-1993: Heart&Stroke Foundation of Canada Research Trainee
- 1982: Rutherford Scholarship



Alessandro Maiocchi

Dr. A. Maiocchi is the Research Projects Manager at the italian Bracco Research Centre. In this position he conceptualise and supervise projects of early discovery carried out in collaboration of universities and research institutes. He has almost 20 years of experience in the field of design and physico-chemical characterization of contrast agents for clinical and preclinical diagnosis. He is currently leading the activities of the nanotechnology unit at the Bracco Research Centre which are mainly focused on the design of nanostructured MRI contrast agent based on self-assembled systems suitable for targeting physiopathological tissues.



Marja Makarow

Marja Makarow is Chief Executive of the European Science Foundation since January 2008 and Professor of biochemistry and molecular biology at the University of Helsinki, where she was Vice-Rector for Research from 2003-2007. She is an advisor to the Finnish Government in her capacity as member of the Research and Innovation Council, and to the EU Commissioner for Research in the European Research Area Board. She has been a member of many committees dealing with research, assessments, doctoral training, researchers' careers, infrastructure, innovation and technology transfer in Finland and at the European level, and has served on boards and scientific councils of several universities. She was member and chair of the jury of the Millennium Technology Prize. Her responsibilities have included the presidency of the EMBC/EMBO and membership of the Council of the EMBL. She is an evaluator of the Starting Grants scheme of the European Research Council as well as programmes in Europe and Canada.

Philippe Martin

Dr., MBA, DEA, MS, PhD. Dr. Philippe Martin manages emerging issues at the science/policy interface for the Health and Consumers Directorate-General of the European Commission (SANCO) and, specifically, nanotechnologies. His team supports the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR [1]). The SCENIHR areas of activity include antimicrobial resistance, new technologies (e.g., nanotechnologies), medical devices including those incorporating substances of animal/human origin, physical hazards (e.g., noise, electromagnetic fields), tissue engineering, blood products, fertility reduction, cancer of endocrine organs, interaction of risk factors, synergic effects, cumulative effects, and methodologies for assessing new risks.

Before joining SANCO, Dr. Martin held international appointments in research policy, innovation financing (cf., creation of new financial vehicles to finance innovation with the European Investment Bank), climate research, and energy planning.

A decision analyst, energy economist, and environmental physicist by training and trade, Dr. Martin earned an MBA from the ESSEC Graduate School of Business in Paris (joint program with the Graduate School of Business of the University of Chicago), a DEA in Energy Systems Economics from the University of Paris-Dauphine and the French Nuclear Commission, and an MS (as a Fulbright Scholar) and a PhD in Energy & Resources from the University of California at Berkeley (collaborative thesis in climatology at the US National Science Foundation Advanced Study Program at the National Center for Atmospheric Research, Boulder, CO). [1] http://ec.europa.eu/health/scientific_committees/emerging/index_en.htm

Fabio Martinon

Fabio Martinon received his PhD in 2003 from the University of Lausanne (Switzerland) for his work on the characterization of the Inflammasome in the laboratory of Jürg Tschopp. After a short post-doctoral fellowship in Jürg Tschopp's laboratory, he moved in 2006 to the laboratory of Laurie Glimcher at the Harvard School of Public Health, where he investigated the link between inflammatory programs and the endoplasmic reticulum stress response. In August 2010 he joined Department of Biochemistry of the University of Lausanne as Assistant Professor. His current research focuses on signaling pathways emerging from the endoplasmic reticulum and on the characterization of the molecular mechanisms of inflammation

AWARDS

- 2011 Human Frontier Science Program career development award
- 2008 Pfizer award in Rheumatology and Clinical Immunology

REPRESENTATIVE PUBLICATIONS:

- Martinon F, Chen X, Lee A-H, Glimcher LH. 2010. TLR activation of the transcription factor XBP1 regulates innate immune responses in macrophages Nat Immunol 11: 411-8.
- Martinon F, Mayor A, Tschopp J. 2009. The Inflammasomes: Guardians of the Body. Ann Rev Immunol 27:229-65
- Martinon F, Petrilli V, Mayor A, Tardivel A, Tschopp J. 2006. Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature 440:237-41.
- Martinon F, Burns K, Tschopp J. 2002 The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-1beta. Mol Cell 10:417-26.

Yasuhiro Matsumura

Director, Investigative Treatment Division, Telephone: 81-4-7134-6857, National Cancer Center Hospital East, Fax: 81-4-7134-6866, 6-5-1 Kashiwanoha, Kashiwa, E-mail: yhmatsum@east.ncc.go.jp, 277-8577, Japan.

1975-1981 : MD student, Kumamoto University Medical School. 1981-1982: Trainee, First Department of Surgery, Kumamoto University Medical School. 1982-1983 : Trainee, Department of Surgery, Saiseikai Kumamoto Hospital. 1983-1984 : Trainee, Department of Surgery, Kyushyu Memorial Hospital. 1984-1988 : Postgraduate student, Department of Microbiology, Kumamoto University Medical School 1988-1989 : Assistant Professor, Department of Microbiology, Kumamoto University Medical School. 1989-1990 : Postdoctoral Fellow, Department of Neoplastic Diseases, Maunt Sinai Medical Center. 1990-1993 : Postdoctoral Fellow, Nuffield Department of Pathology, John Radcliffe Hospital, Oxford University. 1993-1994 : Senior Clinical Research Scientist, Nuffield Department of Pathology John Radcliffe Hospital, Oxford University. 1994-1999 : Staff Physician, Department of Medicine, National Cancer Center Hospital. 1999-2002 : Head, Department of Medicine, National Cancer Center Hospital. 2002-present : Chief, Investigative Treatment Division, National Cancer Center Research Institute East.

AWARDS

- 2005: The 5th Nagai Award (Japan Soc. of Drug Delivery System)
- 2006: Tamiya Memorial Award (National Cancer Center)

RESEARCH INTEREST

According to his leadership, a phase 2 study of a PTX incorporated micelle, NK105, has been completed against stomach cancer as a second line therapy. A phase 1/2 study of a CDDP incorporated micelle, NC-6004, in combination gemcitabine against pancreatic cancer is now underway in Taiwan and phase 2 studies of an SN-38 incorporated micelle, NK012, are underway in patients with colorectal cancer in Japan, and in patients with triple negative breast cancer and SCLC in the USA (Adv. Drug Delivery Rev. 2008). A phase 1 study of a DACHpt incorporated micelle, NC-4016 is now underway in Europe.

His current research focuses on delivering cytotoxic immunoconjugates to cancer stroma. Namely, conventional low molecular weight (LMW) anticancer agents including molecular targeting agents can easily extravasate from normal blood vessels and are distributed throughout the whole body leading to adverse side effects of the drugs. In order to overcome such off-target effects caused by LMW anticancer agents, a so-called missile therapy was developed in which an anti cancer drug or toxin is conjugated to a cancer cell-specific monoclonal antibody. Such macromolecules are too large to pass through a normal vessel wall but can extravasate from leaky tumor vessels and accumulate selectively in tumor tissue. At first, this strategy of "missile" therapy was expected to be highly successful. However, most human solid tumors, possess abundant stroma that hinders the distribution of macromolecules, including anti-cancer agent-conjugated antibodies. This tissue consequently becomes a barrier preventing immunoconjugates from attacking cancer cells. We therefore showed the successful development of a new strategy that overcomes this drawback and achieves highly localized concentration of a LMW and time-dependent cytotoxic agent by conjugating it to a specially raised antibody to the tumor stroma. These newly developed immunoconjugates selectively extravasated from leaky tumor vessels, bound to the tumor stromal component ensleeving tumor vessels and created a scaffold, from which effective sustained release of a time-dependent anti-cancer agent occurred. This released anti-cancer agent subsequently diffused throughout the tumor tissue causing marked arrest of tumor growth associated with damage to tumor vessels and death of cancer cells. Cancer stromal targeting (CAST) therapy, utilizing a cytotoxic agent conjugated to a monoclonal Ab directed at a specific inert constituent of the tumor stroma is thus validated as a highly effective new modality of oncological therapy. The CAST therapy may give the anti-tumor stromal Ab a chance to eradicate solid tumors, especially for refractory, stromal-rich cancers.

Peter J. Meier-Abt

Prof. PJ Meier-Abt received his MD from the University of Basel in 1974. After training in Internal Medicine and Clinical Pharmacology at the University Hospitals of Basel and Zurich, he completed a two year research fellowship in hepatology at Yale University School of Medicine, New Haven Ct USA. In 1984 he became chief of the Division of Clinical Pharmacology and Toxicology at the University Hospital Zurich. In 1992 he was promoted to full professor for Clinical Pharmacology and Toxicology.

He served also as medical director of the Swiss Toxicological Information Center, Zurich (1989-2003) and as first director of the Center of Clinical Research of the Medical Faculty Zurich (2001-2004). His research interests focus around the molecular physiology of bile formation, hepatobiliary bile acid and drug transport, pathophysiology of cholestatic liver disease, drug and toxin induced liver damage, pharmakogenetics/-genomics of adverse drug reactions and individualisation of drug therapy and drug safety. His research resulted in > 350 publications and many honours and research awards.

Prof. Meier-Abt served at numerous boards of national and international associations and research organisations including the Swiss National Science Foundation (Council member 1993-2004; president of Div. Biology and Medicine 2003/04) and the Swiss Academy of Medical Sciences (Vicepresident since 2004). He coordinates the national MD-PhD Programme (1998-2008) and is especially committed to the further development of clinical research in Switzerland (Swiss Trial Organisation). Since 2005 Prof. Meier-Abt is vice rector for research at the University of Basel.

Marie-Edith Meyre

Born July, 4th, 1981. 65, boulevard Soult, 75012 Paris, Phone number: +33(0)140262684, marie-edith.meyre@nanobiotix.com

EDUCATION: 2004-2007: PhD - Paul Pascal Research Center, Bordeaux 1 University, France. 2004: Master 2 in Colloids, Bordeaux 1 University, France. Interface - Formulation - Inorganic synthesis - Physico-chemical characterization of colloids. 2002-2003: Licence (3-year university degree) and Master 1 in Chemistry, Bordeaux 1 University, France. Organic synthesis - Analytical chemistry - Polymers

WORK EXPERIENCE

- 2008-2011: Chemistry Researcher (Nanobiotix, France): EU project Sonodrugs, Image-controlled ultrasound-induced drug delivery, Thermosensitive magnetoliposomes; superparamagnetic nanoparticles; monitoring of drug release thanks to temperature-sensitive Magnetic Resonance Imaging (MRI) contrast agents
- 2004-2007: PhD (Paul Pascal Research Center, France): Incorporation of inorganic nanoparticles in « onion-type » multilamellar lipidic vesicles. Intravesicular synthesis of gold (radiolysis, photoreduction, oxidoreduction) and iron oxide (precipitation) nanoparticles; encapsulation of superparamagnetic nanoparticles inside "onion-type" vesicles and characterization; magnetic properties as MRI contrast agents; magnetic hyperthermia
- 2003-2004: Research training (2 months and 5 months - Paul Pascal Research Center, France): Synthesis of inorganic-organic hybrid nanoparticles (gold/onions) Formulation; nanoparticles synthesis; characterization of synthesis mechanism by Fourier-transform Infrared (FTIR)

Lucian Mocan

28.05.1978. 2003: Surgical University Hospital no.3,MD. General Surgery, Associate Researcher (2009), Surgical Activity, Teaching Activity, Research Activity. 2007: Surgical University Hospital no.3, Associate Researcher University of Medcine and Pharmacy Cluj Napoca Vicepresident of Romanian Society of Nanomedicine, Research Member of Nanomedicine Department at Iuliu Hatieganu University of Medicine and pharmacy,Clinical ActivityTeaching ActivityResearch Activity

ACADEMIC TITLES: MD in General Surgery, (main expertise:colon, liver and pancreatic cancer surgery) Ph D in Medical Sciences

FOREIGN LANGUAGES: French, English

SHORT PUBLICATION LIST

- Iancu C, Mocan L, Bele C, Orza AI, Tabaran FA, Catoi C, Stiufiuc R, Stir A, Matea C, Iancu D, Agoston-Coldea L, Zaharie F, Mocan T. Enhanced laser thermal ablation for the in vitro treatment of liver cancer by specific delivery of multiwalled carbon nanotubes functionalized with human serum albumin. Int J Nanomedicine. 2011 Jan 17;6:129-41.
- Alokita Karmakar, Cornel Iancu, Lucian Mocan, Dana Todea Iancu, Teodora Mocan, Ashley Fejleh, Philip Fejleh, Yang Xu, Enkeleda Dervishi, Samuel.L.Collom, Thikra Mustafa, Fumiya Watanabe, Zhongrui Li, Alexandru R. Biris, Mariya Khodakovskaya, Dan Casciano, Alexandru.S. Biris "Carbon Nanotubes Conjugated with EGF for In Vitro Specific Targeting and Enhanced Destruction of Pancreatic Cancer Cells by Photo-Thermal Ablation" (Nanoletters 2011, under external review)
- Mahmood M, Karmakar A, Fejleh A, Mocan T, Iancu C, Mocan L, Iancu DT, Xu Y, Dervishi E, Li Z, Biris AR, Agarwal R, Ali N, Galanzha EI, Biris AS, Zharov VP."Synergistic enhancement of cancer therapy using a combination of carbon nanotubes and antitumor drug" *Nanomedicine* 2009 Dec;4(8):883-93
- Mahmood M., Casciano D.A., Mocan T., Iancu C., Xu Y, Mocan L, Todea-Iancu D., Dervishi E., Li Z., Biris AR, Abdalmuhsen M., Ali N., Biris AS. "Cytotoxicity and Biological Effects of Functional Nanomaterials Delivered to Various Cell Lines": *Journal of Toxicology and Applied Pharmacology*, 2010 Jan;30(1):74-83.
- Iancu C, Ilie IR., Georgescu C, Ilie R, Biris AR, Mocan T, Mocan C, Zaharie F, Todea-Iancu D., Susman S, Rus Ciuca D., Biris AS. "Applications of Nanomaterials in Cell Stem Therapies and the Onset of Nanomedicine". *Particulate Science and Technology*, 2009, 27(6) , 562 – 574
- Osian G, Procopciuc L, Vlad L, Iancu C, Mocan T, Mocan L. C677T and A1298C mutations in the MTHFR gene and survival in colorectal cancer. J Gastrointestin Liver Dis. 2009 Dec;18(4):455-60.

S. Moein Moghimi

Moein Moghimi is Professor of Nanomedicine and Head of the Nanomedicine Group at the Department of Pharmaceutics and Analytical Chemistry (Faculty of Pharmaceutical Sciences, University of Copenhagen, Denmark). He further serves as the Director of the Centre for Pharmaceutical Nanotechnology and Nanotoxicology (CPNN) and Group Leader in Pharmaceutical Nanotechnology at the NanoScience Center (University of Copenhagen). He is also the Guest Professor of Nanomedicine at 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 Systems at the Department of Pharmaceutical Sciences, University of Nottingham (UK). His research activities are focused on experimental nanomedicines, 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 Faculty of Pharmaceutical Sciences Research Achievement Award (Copenhagen University). His contributions to peer-reviewed high impact international journals include over 90 original full research papers and invited critical reviews 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 in nanomedicine and bionanotechnology and act as principal investigator of numerous nanomedicine research projects and partnering European Commission FP-7 programmes.

Professor Moghimi has previously served as invited Theme Editor for a number of Theme Issues of the prestigious Advanced Drug Delivery Reviews (Elsevier, The Netherlands) and currently act as the European Editor of NanoMed Journal (Pan Stanford, Singapore), Associate Editor for both Nanomedicine: Nanotechnology, Biology and Medicine (Elsevier) and Journal of Biomedical Nanotechnology (American Scientific Publishers, USA) as well as being a member of editorial/advisory board of 12 other 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. 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).

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.

Guy Morin

Dr. med., President of the Executive Council 2009. Born 1956, married to Christa Züger Morin, 2 children, Member of the Green Party

PROFESSIONAL CAREER: 1978-1984: Read medicine at the University of Basel. 1986-1993: Further training as General Practitioner, FMH, at various hospitals within the Canton of Basel-Stadt. 1993-2004: General Practitioner in St. Johann, Basel. 1996-2004: President of the HMO Association of General Practitioners (VIPA)

POLITICAL ACTIVITIES

- 1985-1990: Secretary of the Swiss Section of Doctors for Social Responsibility
- since 1988: Member of the Doctors for Environmental Protection Association
- 1988-2001: Member of the Great Council of the Canton of Basel-Stadt
- 1997-2001: President of the Health Commission of the Great Council of the Canton of Basel-Stadt
- 2004: Election to the Executive Council of the Canton of Basel-Stadt
- 2008: Election as President of the Executive Council of the Canton of Basel-Stadt

Jan Mous

PD Dr., Born in St.Niklaas (Belgium) on July 25th 1950; Belgian nationality; married, three adult children.

EDUCATION

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

PROFESSIONAL CAREER

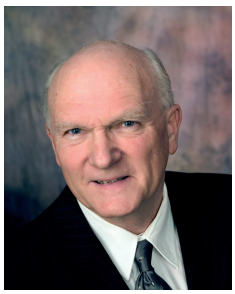
- 1985-2000: R&D manager at Hoffmann-La Roche's Pharma division in Basel (Switzerland), last position: VP Genomics;
- 2000-2002: CSO of LION bioscience AG in Heidelberg (DE) and non-executive director of LION bioscience Inc.(Boston, MA);
- 2003-2007: President & CEO of IntegraGen SA in Evry (France); 2008-2009: independent consultant, owner of MRM Consulting GmbH, in Giebenach (Switzerland);
- 2010-: CEO of PharMida AG in Basel (Switzerland) and COO of Midatech Ltd. In Oxford (UK)

Bert Müller

Bert Müller holds the Thomas Straumann-Chair for Materials Science in Medicine at the University of Basel, Switzerland. After receiving a diploma in mechanical engineering (1982), he worked as electrical fitter building large transformers in Berlin, Germany until 1984. Subsequently he studied physics and received 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 performed research in leading positions at the Physics Departments of the Paderborn University, Germany, of the EPF Lausanne, Switzerland and of the ETH Zurich, at the Swiss Institute for Materials Testing and Research and at the Materials Department of the ETH Zurich.

Dr. Müller became a Faculty member of the Physics Department at ETH Zurich (venia legendi) in April 2001. From 2001 to 2006 he was the General Manager of the National Center of Competence 'Computer-aided and image-guided medical interventions' (www.co-me.ch) at the Department of Electrical Engineering of the ETH Zurich. After his election as Associate Professor at the Medical Faculty of the University of Basel and his appointment at the Surgery Department of the University Hospital Basel in September 2006, he founded the Biomaterials Science Center (www.bmc.unibas.ch).

Since February 2008 he also heads the Materials Science Institute of the Dental School of the University of Basel. Currently, Dr. Müller is teaching courses on physics and materials science at the ETH Zurich and the Universities of Bern and Basel.



Hans O. Myhre

M.D., Fallanveien 30B, Leil. 47, 0495 Oslo, Norway, e-mail: hans.myhre@ntnu.no. Born: 19 October 1939. Professor of Vascular Surgery, St. Olavs Hospital, University Hospital of Trondheim, Norway.

Specialist in general-, thoracic-, cardiovascular and vascular surgery. Supervisor for 20 PhD candidates. Honorary member The Norwegian Surgical Society and the European Society for Vascular Surgery. Knight Royal Order of St. Olav, 2005. Chairman of the Scientific Board for the project "Operating Room of the Future". This is a cooperation between St. Olavs Hospital, the Norwegian University of Science and Technology and SINTEF. Research projects on deformation and treatment of aortic aneurysms.

Bradley Nelson

Brad Nelson is the Professor of Robotics and Intelligent Systems at ETH-Zürich and is the founder of the Institute of Robotics and Intelligent Systems where he leads the Multi-Scale Robotics Lab. His primary research direction lies in extending robotics research into emerging areas of science and engineering. His current research is in microrobotics, biomicrorobotics, and nanorobotics, including efforts in robotic micromanipulation, microassembly, MEMS (sensors and actuators), mechanical manipulation of biological cells and tissue, nanofabrication and NanoElectroMechanical Systems (NEMS).

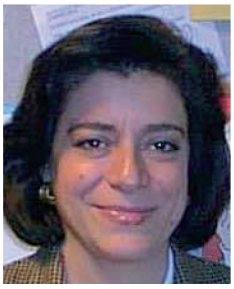
Prof. Nelson received a B.S. (Mechanical Engineering) from the University of Illinois at Urbana-Champaign in 1984, an M.S. (Mechanical Engineering) from the University of Minnesota in 1987, and the Ph.D. degree in Robotics (School of Computer Science) from Carnegie Mellon University in 1995. During these years he also worked as an engineer at Honeywell and Motorola, and served as a United States Peace Corps Volunteer in Botswana, Africa. In 1995 he became Assistant Professor at the University of Illinois at Chicago, Associate Professor at the University of Minnesota in 1998, and Professor at ETH in 2002.

He has received a number of major awards and honors was named to the Scientific American 50, Scientific American magazine\'s annual list recognizing fifty outstanding acts of leadership in science and technology. He is a Fellow of the IEEE and the ASME. He has won over a dozen best paper awards at major robotics conferences and journals. Professor Nelson serves on or has been a member of more than ten editorial boards, has chaired several international workshops and conferences, has served as the head of the Department of Mechanical and Process Engineering, the Chairman of the ETH Electron Microscopy Center (EMEZ), and is a member of the Research Council of the Swiss National Science Foundation.

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.



Marisa Papaluca-Amati

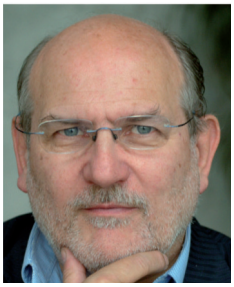
MD specialist Internal Medicine. Medical Director of the Italian Authority Operations Center for Community Procedures in early 90's. Former member of the CPMP (now known as the CHMP) until 1994.

She joined the EMEA in late 1994 and occupied various positions as scientific secretary of the Biotech Working Party, as deputy head of the Sector Biologicals and Biotech Products.

Since 2000 she is the Deputy Head of Sector for Safety and Efficacy Sector in the Pre-Authorisation Human Unit. She is currently the EMEA Leader of the Task Force on Innovation. Scientific co-ordinator to the CHMP Working Parties on Gene Therapy, Pharmacogenetics and to the Working Party on Similar Biological Medicinal Products. Head of Section for Scientific Support and Projects in Sector Human Medicines Special Areas, Unit Human Medicines Development and Evaluation.

The Section is in charge of provide scientific input and support across the Agency on statistics and clinical trials methodology, non-clinical development and Environmental Risk Assessment, Nanotechnology, Pharmacogenomics and Business Pipeline.

Chair of the Agency's Innovation Task Force Prof Papaluca has been in charge for the agency at EU and international level of regulatory and scientific activities in the field of innovative pharmaceuticals development with special scientific interest on Advanced Therapies and genomics. She also holds the position of Professor on Pharmacogenomics at the Faculty of Medicine in Rome – Tor Vergata University.



Didier Payen de la Garanderie

Department of Anesthesiology & Critical Care & Samu, Lariboisiere University Hospital, AP-HP, Université Diderot Paris, 2 rue Ambroise Paré, F-75010 Paris. dpayen1234@aol.com

DIPLOMAS & TITLES: Medical Doctor thesis and certification September 1982, Nantes University of Medicine, France. Masters in Physiology 1982. D.E.R.B.H of Physiology 1983. Ultrasound & Echocardiography Certificate, 1983. PhD thesis in Human Biology; Option Physiology. January 1988 "Circulatory consequences of positive pressure breathing" 162 pages. Mention: "Très Honorable". Full Professor in Anesthesiology & Intensive Care, March 1997

HOSPITAL & ACADEMIC & INSITUTIONAL POSITIONS

- Professor of Anesthesiology & Surgical Critical Care, July 1990, Lariboisière University Hospital.
- Expert at the French Agency for Drug Delivery from 1994-1999.
- Member of the Council of European Society of Intensive Care Medicine from 1996-01.
- Chairman of the Department of Anesthesiology & Intensive Care, Lariboisiere Hospital since 1996 till now
- Member of the Scientific Committee of the UFR Lariboisiere-Saint Louis 1997-1999, Université Paris 7
- President of the committee" Intensive Care Medicine" of the National Society of Anaesthesiology and critical
- care medicine 1997-1999
- Treasurer of the European Society of Intensive Care Medicine from 1997-2001
- Full Professor, First Class, in Anesthesiology & Surgical Critical Care 1998
- Associate Professor at McGill University; Anesthesiology & Critical Care Sept 1999-sept 2001

RESEARCH POSITIONS

Director of the research program in Anesthesiology & Intensive Care, Lariboisière University Hospital., 1986-present; Expert for research grant program for AP-HP, INSERM, and Ministry of Research & Technology. From 1986 till now; Director of the quadriennal programme certified by the Ministry of Research & technology from 1988-92, 1993-97, 1998-03, and 2004-08. Chief of the Certified Research Group as Equipe d'Accueil, University Paris 7 from 1988 ; Chief Investigator for Clinical Research and Director of Research Laboratory of Anesthesiology & Surgical Critical Care, Lariboisière University Hospital form 1989 till now; Founder and President of the Interface committee between National Society of Anaesthesiology and Critical Care Medicine and INSERM1992-96 "Post Doc" for 6 months at Institut Pasteur, Paris France 1994 to be trained in molecular biology; In charge of Interface Commission between French Society of Anesthesiology & Surgical Critical Care and INSERM 1997-00; Member of the National commission for scientific évaluation department of INSERM 2002– 07 (specialized scientific commission n° 1 : microbiology and infectious diseases) Member of scientific committee of national society of anaesthesiology and critical care medicine 2003-07

PARTICIPATIONS IN CLINICAL TRIALS

- Principal Investigator of Nitric Oxide trial in ARDS 1996-99
- Co-principal investigator of Hemofiltration in sepsis 1998-99
- Investigator of "Enhance Study" on activated protein C 2000-01
- Investigator in ICOS protocol in sepsis 2001-till now
- Principal investigator of regional clinical research program – promoter Assistance Publique – Hôpitaux de Paris on: "GENOMIC PROFILE AND PRONOSTIC MARKERS IN HUMAN SEPTIC SHOCK. 2002- on going
- Investigator of the study "Address" on activated protein C. 2003-04
- Principal investigator of ACCESS study on TLR 4 blocker in sepsis from 2007- on going
- Principal Investigator of national RCT on PMX device: "PREVENTION OF SEPTIC SHOCK IN PERITONITIS" 2007-10
- French Principal Investigator for the world wide RCT PROWESSHOCK "Activated Protein C in septic shock". 2007-2010

PUBLICATIONS

Critical Care Med. 2004-05-06-07, Chest 2005, Anesthesiology 2002-04-05-07, Circulation 2004, Eur H Jl: 2007, Anesthesia-Analgesia 2003-04, Surgery 2006-07, Journal of Thoracic & Cardiovascular Surgery 2003, Shock 2004-07, Cytokines 2000, Biochemical 2002, Intensive Care Medicine 2003-05-06-08, J Thoracic Cardiovascular Surgery 2003, Stroke 2008, New England 2008

PRINCIPAL CONTRIBUTIONS

1) Creation "de novo"of a new intensive care unit in 1990 – 2) Creation of a central Recovery Room functioning 24 Hrs/24 – 3) Creation of an experimental Research Laboratory in 1990, Accreditation DRED since 1990 until now – 4) Opening of two new sectors of anaesthesia: gastro-intestinal endoscopy, orthopedic surgery and traumatology 5) creation of the national intensive care training programme (110 hrs).- 6) Management of the Department of Anesthesiology& Crit Care Medicine: board committee.

Richard Peck

Dr Richard Peck FRCP, FFPM is Global Head of Clinical Pharmacology for Roche. He trained in pharmacology and medicine at Cambridge University and has twenty years experience in the pharmaceutical industry, working in Clinical Pharmacology, Experimental Medicine and Drug Discovery for GlaxoWellcome, SmithKline Beecham and Eli Lilly, before joining Roche.
He is a member of the UK Medical Research Council (MRC) Population and Systems Medicine Board and several MRC grant review panels, was a member of the UK Government\'s Translational Medicine Board and former chair of the ABPI Experimental Medicine Group. Recently he was an industry member of the review groups for the UK Government Office of Life Sciences Capability Clusters Initiative and is a keen supporter of improved collaboration between the pharmaceutical industry and academia to enhance drug development and clinical use.
His research interests include improving the productivity of early clinical drug development, understanding and utilising variability in drug response to improve drug dosing and clinical benefit in different patient groups, applying clinical pharmacology to enable the development of personalised/stratified medicines, and the use of model-based drug development strategies in which experimental data and quantitative models are used in an iterative learn/confirm cycle to increase the level of knowledge obtained from experimental data and thereby increase development and clinical success rates.

Matthias Emil Pfisterer

Born September 14, 1945. Citizen of Basel, Switzerland. Married, 3 children.
Medical school: University of Basel/Switzerland.
-1971; MD degree 1972. Residency 1972-1976, University Hospital Basel and Kantonsspital Lucerne, Switzerland. Registrar. 1976-1977, Dept. of Internal Medicine, University Hospital Basel. Research Fellow. 1977-1978 Div. of Nuclear Medicine and Cardiology, UC San Diego, USA. Senior Registrar. 1978-1988 Division of Cardiology, University Hospital Basel. Venia docendi (Ass.Professor) Internal Medicine/ Cardiology, University Hospital Basel (1981). Head Nuclear and Interventional Cardiology Sections, Division of Cardiology, University Hospital of Basel (since 1988). Assoc. Professor of Cardiology, Faculty of Medicine, University Hospital, Basel (since 1990). Full Professor of Cardiology, Faculty of Medicine, University Hospital, Basel (since 1997). Head Division of Cardiology, University Hospital Basel (since 1997). Chairman Department of Internal Medicine, University of Basel (since 2004).

IMPORTANT PRIZES: Swiss Society of Cardiology 1982. Theodor Naegeli prize awarded 2004/ 2005.

MEMBERSHIPS: Swiss Society of Cardiology - Member of the Board (1990-1998, President 1996-1998). Working Group on PTCA and Thrombolysis (chairman 1991 -1993). Working Group Heart Failure. European Society of Cardiology, Fellow (since 1984). Working Group "Nuclear Cardiology" of ESC (chairman 1986-1990). Working Group "Heart Failure". Swiss Society of Cardiothoracic Surgery (since 1988). American College of Cardiology, Fellow (since 1985). American Heart Association, Fellow, Member of the Council on Clinical Cardiology (since 1992). American Society of Nuclear Cardiology, Founding Member (since 1993). Swiss Heart Foundation, Vice President/President of the Scientific Council (since 1995).

PUBLICATION/ SCIENTIFIC WORK: Author and coauthor of more than 400 published scientific papers (>250 peer-reviewed papers, >150 invited papers and >300 abstracts). Member of the Steering Committee of Large international trials: ECSG, GUSTOI, II, IV, PARAGON A, B, SYMPHONY I, II, ASSENT I, ACTIVE, SENIOR PAMI, APEX, OAT and others
Principal Investigator of important trials: TIME (Lancet, JAMA, Circulation, EHJ), BASKET (Lancet, JACC, EHJ), TIME-CHF (AmHJ design).

John Pickup

John Pickup is Professor of Diabetes and Metabolism at King's College London School of Medicine, Guy's Hospital, London. He received both his PhD and medical training at the University of Oxford. After a fellowship at the Endocrine Unit, Massachusetts General Hospital, Boston, and an appointment at the Hammersmith Hospital in London he moved to Guy's Hospital, London. He is also currently Visiting Professor in the Dept of Physics at the University of Strathclyde, Glasgow.
He has a long-standing interest in the development and clinical application of novel technology for the improved management of diabetes, starting from the development of insulin pump therapy through to in vivo glucose sensors. His current research focuses on insulin pump therapy and continuous glucose monitoring in clinical practice, developing novel fluorescence approaches to glucose sensing, applications of nanomedicine in diabetes, and activation of the innate immune system and inflammation as a cause of type 2 diabetes and its complications.
John Pickup is the recipient of several research awards, including the R D Lawrence Lectureship of Diabetes UK, the Gotch Prize, the Boehringer Mannheim Fellowship of the Biochemical Society, the BUPA Foundation Research Award and the Diabetes Leadership Award of the Diabetes Technology Society, and book awards from the Charlesworth Foundation, BMA, Royal Society of Medicine and Society of Authors.

Christian Plank

Christian Plank graduated in biochemstry from the University of Vienna (Austria). 1990-1994 Ph.D. thesis at the Research Institute for Molecular Pathology,Vienna, research group of Prof. Ernst Wagner. 1994-1997 postdoctoral fellowship University of California, San Francisco, research group of Prof. Francis C. Szoka at the School of Pharmacy. Since 1997 head of a research group at the Institute of Experimental Oncology, Technische Universität München, Munich, Germany. Co-founder of OZB Biosciences, Marseille, France, and ethris GmbH, Munich, Germany. Research topics: Nucleic acid delivery, gene therapy, nanomagnetic drug targeting, nanomedicine. Member of CeNS (Center of Nanoscience) and \"Nanosystems Initiative Munich\" (NIM). Deputy coordinator of the DFG Research Unit "Nanoparticle-based targeting of gene- and cell-based therapies" (Nanoguide/FOR 917).

Michael Reinert

Michael Reinert studied medicine from 1986 to 1992 in Basel, Switzerland and started his medical career with general surgery and in- ternal medicine. In 1995 his neurosurgical education began in Bern, Switzerland and Richmond Virginia, USA. He board certified in 2002. Thereafter, his further specialization was neurovascular surgery to the present time. His current clinical position is vice-chairman of the Department of Neurosurgery at the University of Bern, Switzerland.

Wolfgang A. Renner

PhD in biotechnology, is the founder and Chief Executive Officer of Cytos Biotechnology. He obtained his PhD in 1995 from the Swiss Federal Institute of Technology (ETH), Zurich. It was his thesis that provided the basis for the foundation of Cytos Biotechnology GmbH in 1995. He is a member of the Committee of Science and Technology of the Swiss Federation of Commerce and Industry (Economiesuisse) and of the Foundation Council of the Swiss National Science Foundation.

Bernd Riebesehl

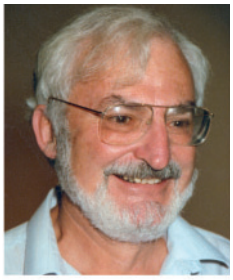
Dr. Bernd Riebesehl joined Novartis as Principal Fellow, Technical Research & Development, Novartis Pharma AG, Basel in 2007, acting as Formulation Expert and Technical Project Leader. The scope of his responsibilities includes the development of drug substances and parenteral drug formulations from 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.

Cristianne Rijcken

(1979, The Netherlands) studied Pharmacy at the University of Utrecht with a specialisation in Pharmaceutical Technology and participation in the Honours Programme. From 2003 till 2007, she worked as a PhD student at the Department of Pharmaceutics (University of Utrecht) under supervision of Prof. Dr. Ir. Wim Hennink. This resulted in a thesis entitled 'Tuneable & degradable polymeric nanoparticles for drug delivery: from synthesis to feasibility *in vivo'*, for which she received the Thesis Award 2008 of the Dutch Association for Pharmaceutical Sciences and the Simon Stevin Gezel Award 2008. She is (co-)author of approximately 15 scientific publications and co-inventor of 3 patents.

After interim projects, including lecturing and working as a formulation scientist for Enceladus B.V., Cristianne acquired STW valorisation grants phase I and II, and a European grant that enable her continuation with the preclinical development of the nanoparticulate drug delivery technology. Parallel to pursuing scientific progress, she attended several business courses, including the Masterclass BioBusiness, to facilitate the commercial exploitation of the developed technologies in her own spinoff company. Cristal Delivery B.V. is recently founded with Cristianne as CEO. Its mission is to develop first-in-class polymeric drug delivery products and technologies for optimal therapeutic responses. The company is her main hobby, but she occasionally finds time to do some sport and to relax with music and good food & drinks.



Heinrich Rohrer

Heinrich Rohrer, born in Switzerland in 1933, received his PhD in experimental physics in 1960 from the Swiss Federal Institute of Technology (ETH-Zurich) with a thesis on superconductivity.

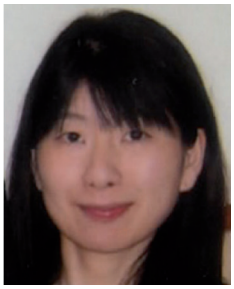
After a two-year post-doctorate at Rutgers University, New Jersey (USA), he joined the IBM Zurich Research Laboratory in 1963 as a research staff member. In 1974/75 he spent a sabbatical at the University of California, Santa Barbara. His research interests included, in chronological order, Kondo systems, phase transitions, multicritical phenomena, scanning tunnelling microscopy, and, most recently, nanomechanics.

He retired from IBM in 1997. For the invention of the scanning tunnelling microscope, Gerd Binnig and Heinrich Rohrer were co-recipient of both the King Faisal Prize and the Hewlett Packard Europhysics Prize in 1984, of the Nobel Prize in Physics of 1986, and of the Cresson Medal of the Franklin Institute in Philadelphia (USA) in 1987. He is a member or honorary member of various academies and professional societies. He has also received honorary degrees from several universities.

Eder Lilia Romero

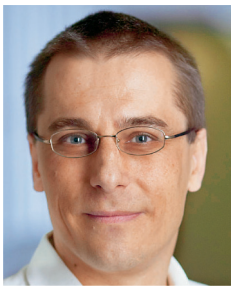
Eder L. Romero was educated at University of La Plata, Argentina where she obtained her M.A. Biochemistry and PhD in Exact Sciences (1996). Following a post-doctoral research in Groningen University, The Netherlands under the supervision of Prof. Gerrit Sherphof (1997-1998), she returned to Argentina 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).



Kumiko Sakai-Kato

received her B.S. and M.S. degrees from the University of Tokyo. She developed her carrier as a research scientist at a pharmaceutical company. She received her Ph.D. degree in analytical chemistry at the University of Tokyo in 2004. After postdoctoral work of the Japan Society for Promotion of Science, she became an assistant professor at Musashino University. In 2008, she became a section head of Division of Drugs at the National Institute of Health Sciences. Her research fields are analytical science and nanomaterial science for drug evaluation.



Bernhard Sauter

Prof. Dr. med. Partner, Digestive Disease Center, The Hirslanden Private Clinic Group, Zurich. Adjunct Assistant Professor of Medicine, Mount Sinai School of Medicine, New York.
2006– Partner, GastroZentrum Hirslanden, Zurich, Switzerland
2006– Adjunct Assistant Professor of Medicine, Mount Sinai School of Medicine, New York

- 2001–2006: Internal Medicine, Gastroenterology, Hepatology, Liver- and Intestinal-Transplantation, Gene and Cell Medicine, Mount Sinai Hospital and Mount Sinai School of Medicine, New York: Assistant Professor of Medicine 2002, Assistant Professor of Gene and Cell Medicine 2002, Medical Director, Intestinal Transplantation and Rehabilitation 2003.
- 1998–2001: Research Associate, Institute for Gene and Cell Medicine, Mount Sinai School of Medicine, New York
- 1995–1998: Fellow, Gastroenterology und Hepatology, Mount Sinai Hospital, New York
- 1994–1995: Intern, Internal Medicine, Mount Sinai Hospital, New York
- 1991–1994: Resident, Internal Medicine, University Hospital of Zurich
- 1990–1991: Resident, Pathology, University Hospital of Zurich
- 1990: Dissertation: “Monoclonal antiidiotypic antibodies specific for a paraprotein of a human myeloma”
- 1982–1989: Medical School, University of Zurich

Gottfried Schatz

Gottfried Schatz was born on August 18, 1936 in a little Austrian village near the Hungarian border. He grew up in Graz, but spent one year as a high school student in Rochester, NY. After receiving his Ph.D. in Chemistry from the University of Graz, he joined the Biochemistry Department of the University of Vienna where he began his studies on the biogenesis of mitochondria and participated in the discovery of mitochondrial DNA. After his postdoctoral work with Efraim Racker at the Public Health Research Institute of the City of New York on the mechanism of oxidative phosphorylation and a brief interlude in Vienna, he accepted a professorship at the Biochemistry Department at Cornell University in Ithaca, NY. Six years later, he moved to the newly created Biozentrum of the University of Basel, Switzerland, which he chaired from 1985 - 1987. His scientific achievements were honored by many prestigious international prizes, elections to scientific academies (including the US National Academy of Sciences and the Royal Swedish Academy) as well as by two honorary doctorates. He served as Secretary General of the European Molecular Biology Organization (EMBO), Councilor of The Protein Society, Member of the Swiss National Research Council, and as President of the Swiss Science and Technology Council. As a student he also worked as a violinist at opera houses in Graz and Vienna. He and his Danish wife have three children.

Jörg B. Schulz

Professor of Neurology and Chair, Department of Neurology, University Hospital, RWTH Aachen, Pauwelsstrasse 30, D-52074 Aachen, Germany, Phone: +49-241-808-9600, Fax: +49-241-808-2582, Email: jschulz@ukaachen.de, www.neurologie.ukaachen.de. Date of Birth: 01/04/64, Nationality: German, Degree: MD

CURRENT POSITION:

- Full Professor, Director of the Department of Neurology, University Hospital, RWTH Aachen, Aachen.
- Speaker of the German Network of Hereditary Movement Disorders (GeNeMove)
- Deputy Speaker of the German Competence Net for Degenerative Dementias (KNDD)

LICENSURE AND CERTIFICATION:

- 1991 Certification (Physician) by the State of Nordrhein-Westfalen, Germany
- 1992 Chamber of Physicians License Registration No. 7450 (Cologne)
- 1999 Board certification in Neurology
- Educational Qualifications and Research Experience
- University of Cologne - MD - 1984-1991
- Department of Neurology, University of Tübingen, 1991-1993, Clinical neurodegeneration and residency program
- Department of Neurology, Mass. General Hospital, 1993-1995, Post-doc in neurobiology, animal models of neurodegenerative diseases,
- Department of Neurology, University of Tübingen, 1995-1999, Residency, clinical neurodegeneration and molecular and cellular neurobiology
- Department of Psychiatry, University of Tübingen, 1998-1999, Residency

POSITIONS AND EMPLOYMENT, RESEARCH EXPERIENCE

- 1991-1993 and 1995-1999: Medical Residency, Neurology, University of Tübingen, Germany
- 1993-1995: postdoctoral training, Neurochemistry Laboratory, Mass. Gen. Hospital and Harvard Medical School, Boston
- 1999-2004: Assistant Professor of Neurology, Attendent, Center of Neurology, Tübingen
- 1998-2004: Head of Neurodegeneration Laboratory, Hertie Institute of Clinical Brain Research, University of Tübingen
- 9/2004-6/2009: Full Professor of Neurology and Restorative Neurobiology, Center of Neurological Medicine, University of Göttingen
- 9/2004- 6/2009: Director of Neurodegeneration and Restorative Research, Center of Molecular Physiology of the Brain (German Research Foundation Center of Excellence)
- Since 1/2009: Full Professor, Director of the Department of Neurology, University Hospital, RWTH Aachen

FIELDS OF SPECIALIZATION

Scientific: pathogenesis of neurodegenerative diseases (Parkinson’s, Huntington’s, Alzheimer’s disease and ataxias), neuronal cell death mechanisms, gene therapy and other experimental therapeutics, molecular mechanisms of motor learning, clinical studies in Alzheimer’s disease, Parkinson’s disease and hereditary movement disorders
Clinical: neurodegenerative diseases (Movement Disorders, Dementias) and stroke

FELLOWSHIPS AND HONORS

- 1985-1991: Scholarship: Studienstiftung des Deutschen Volkes,Germany
- 1991 M.D. summa cum laude, University of Cologne,Cologne
- 1993-1995 Fellowship: German Research Foundation, Germany
- 1/98 Gerhard Hess-Award of the German Research Foundation (DFG)

- 3/99 Schering-Award of the German Parkinson's Disease Foundation
- 8/99 International Society for Neurochemistry Young Scientist Lectureship Award
- 6/00 Elected as a corresponding member to the American Neurological Association
- 9/01 Pette-Award of the German Neurological Society

CURRENT MEMBERSHIPS:

since 1993 American Society for Neuroscience
since 1994 American Academy of Neurology
since 1995 International Society for Neurochemistry
since 1998 German Neuroscience Society
since 1998 German Parkinson's Disease Foundation
since 1999 German Neurological Association
since 2000 American Neurological Association

AD HOC REVIEWER:

Nature Genetics; Cell Stem Cell, PNAS, Annals of Neurology; Brain; Neurology; Circulation, Journal of Neurology, Journal of Neurology, Neurosurgery, and Psychiatry; Journal of Neuroscience; Journal of Neurochemistry; Brain Research; Neuroscience; European Journal of Pharmacology; Pharmacology Biochemistry and Behavior; European Journal of Neuroscience; Cell and Tissue Research; Neurotoxicity Research, European Journal of Clinical Investigation, Journal of Neuroscience Methods, Journal of Neuroscience Research, British Journal of Pharmacology, European Journal of Clinical Investigation, Trends in Molecular Medicine, Trends in Neuroscience, Movement Disorders, Experimental Brain Research
Wellcome Trust, German Research Foundation, European Science Foundation, Telethon Italy; Israel Science Foundation, MRC, INSERM, Österreichischer Nationalfond

EDITORIAL BOARD:

Journal of Neurochemistry since 2001
Journal of Neuroscience since 2008

Daniel G. Schultz

MD, F.A.C.S. Senior Vice President, Medical Devices and Combination Products Greenleaf Health LLC. Dr. Daniel Schultz joined Greenleaf Health following a distinguished 35-year career devoted to supporting and advancing Americans' public health as a physician, teacher, Food and Drug Administration (FDA) official and member of the U.S. Public Health Service (USPHS). He has been recognized many times for his contributions and dedication to public health. Dan continues his commitment to public health at Greenleaf, where as Senior Vice President for Medical Devices and Combination Products he provides strategic consulting services and works with Greenleaf clients to bring innovative devices to patients. As Director of the Center for Devices and Radiological Health (CDRH) at FDA from 2004 to 2009, Dan was responsible for seven FDA offices and more than 1,000 agency employees. He led the development, implementation and evaluation of regulatory policies concerning medical devices and radiation-emitting products. He also established national goals and policies to ensure that FDA and U.S. Department of Health and Human Services (HHS) objectives were met.
Dan began his 15-year FDA career in 1994 as a Medical Officer in the General Surgery Devices branch of the CDRH's Office of Device Evaluation, advancing in 1995 to the position of Chief Medical Officer in the Office of Device Evaluation in the division of Reproductive, Abdominal, ENT, and Radiological Devices. He served as Division Director from 1998 to 2001. Dan became Deputy Director for Clinical and Review Policy in the Office of Device Evaluation in 2001 and Director of the Office of Device Evaluation the following year. Named Director of CDRH in 2004, he remained in that role until stepping down in August of 2009. During his FDA stint, Dan also used his medical knowledge and experience as Assistant Professor of Surgery at the Uniformed Services University of the Health Sciences and as a member of the Surgical Staff at the National Naval Medical Center, Bethesda, MD. Prior to joining FDA, Dan served as a member of the U.S. Public Health Service (USPHS). During postings at Indian Health Service hospitals in Arizona and New Mexico, he provided medical care for people living in the Navajo Nation and Indian Pueblos. Dan received multiple awards for his service, including the Public Health Service Outstanding Medal. A New York City native, Dan is a graduate of the City College of New York. He received his medical degree from the University of Pittsburgh and is Board-certified in Surgery and Family Practice.

Simó Schwartz

Dr Simó Schwartz Jr (1967th, Barcelona) obtained his Medical degree in 1991 at the Faculty of Medicine of the Autonomous University of Barcelona, where he also got his PhD in 1996 working on oncogenic signalling pathways in prostate cancer.
He was a research fellow in the New York Univ. Medical Center in 1993, working on molecular mechanisms related to Ras/Raf activation in transgenic mice. In 1996 he moved to California as postdoctoral research at the Burnham Institute for Biomedical Research, where he work intensively under the guidance of Dr Manuel Perucho in the molecular pathways involved in the development of colorectal cancer. In 2000 he was appointed as head of the Molecular Oncology and Aging laboratory at the Molecular Biology and Biochemistry Research Center (CIBBIM) of the Vall d'Hebron University Hospital in Barcelona. He is nowadays a Board member of the CIBBIM and also member of the Science Committee of the Vall d'Hebron Research Institute.
In 2004, Dr Schwartz start few collaborations with biotech companies in the field of diagnostic and prognostic biomarkers and new therapeutic targets in colorectal cancer. The success of these collaborations lead to 6 patents transfered in the last four years to leading companies of the biotech and pharma sectors. Also, the development of several research projects among which are worth to mention projects of the National R+D Grants, CENIT (industrial consortiums) and few european and international innitiatives. Since 2006 he is a member of the Science Advisory Board of Oryzon Genomics, a Spanish leading biotech company. Recently (2007), he has been appointed as Coordinator of Nanomedicine at the CIBBIM (now CIBBIM-Nanomedicine) and coordinator of its strategic action plan for R+D, which is focused on the research of new biomedical nanotechnology-based applications. In particular, new drug delivery systems, image based diagnostic systems and preclinical validation of therapeutic conjugates and bio-nanosensors, mainly in the areas of oncology and rare diseases.
He also leads the "drug delivery and targeting group" at the CIBBIM-Nanomedicine. In this context, Dr Schwartz Jr is coordinator and collaborator of several research projects directly related with the obtention of therapeutic drug delivery systems. Among them are the CENIT "Oncnosis" project, two ERANET projects (IMMAPROT on Industrial biotechnology - ERA-IB; NANOSTEM - EuroNanoMed, coordinated by him), and an international project of the Iberian Nanotechnology Institute (OncoNanoTarget). All of them projects involving SME's in which animal models are being used for preclinical validation of new therapies directed against tumor cells. Dr Schwartz Jr is also member of the Nanomedicine Spanish Platform (NanomedSpain) and of the "European Platform for Nanomedicine" where he co-authors the 2006 Research Strategic Agenda intended to the European Commission. His research group is also a group member of the "CIBER de Bioingeniería, Biomateriales y Nanomedicina" (CIBER-BBN) of the Spanish Health Institute CarlosIII (ISCIII) which gathers a total of 50 research groups of national excellence in the field of nanotechnology and nanomedicine. Since 2007, Dr Schwartz Jr acts as the Nanomedicine Coordinador of CIBER-BBN at the national level where he also leads two intramural projects and collaborates in other seven coordinated projects. All of them are centered in biomedical applications of nanotechnology and nanomedicine for human health.

Giacinto Scoles

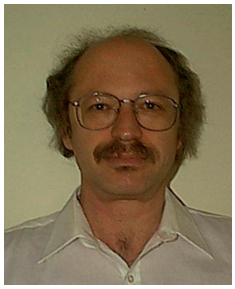
Emeritus Donner Professor of Science at Princeton University, is currently Professor of Biophysics at the International School for Advanced Studies, is a long term collaborator at the ELETTRA synchrotron and, finally, coordinator of Nanotechnology at the Consortium for Biomolecular Medicine, all in Trieste , Italy.
Scoles graduated in Chemistry in 1959 from the University of Genova, Italy and obtained a honorary doctorate in Physics from the same university in1996. In 2000 he obtained a honorary degree in Science from the University of Waterloo in Canada where he taught from 1971 to 1986. He is a Fellow of the Royal Society (UK) and is a foreign member of the Royal Academy of Arts and Sciences of The Netherlands. Other honors include the 2006 Franklin Medal for Physics, the E.K. Plyler Prize for Molecular Spectroscopy of the APS and the ACS's Peter Debye Award in Physical Chemistry.. Scoles, who has published in excess of 250 papers in refereed international journals and has edited four books, has spent his career at the boundary between Chemistry and Physics and currently active in the area of Science that extends from these two disciplines to Biology and Medicine.

Gert Storm

Professor, 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 (Drug Targeting chair) at Utrecht University. He is author/co-author of more than 300 original articles, reviews and book chapters, in the field of advanced drug delivery/drug targeting (in particular with liposomal systems), 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 course director of the GUIDE/UIPS/LACDR Course on Advanced Drug Delivery & Drug Targeting, co-sponsored and accredited by EUFEPS and the GALENOS Network held in The Netherlands. He is involved in organizing conferences in the field of advanced drug delivery. He is member of the editorial (advisory) board of a variety of scientific journals. He acts as a consultant to a number of pharmaceutical companies. 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 (TNPT). contact: g.storm@uu.nl

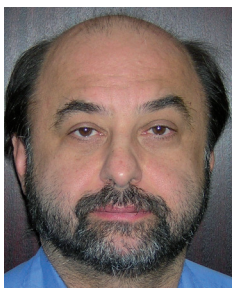
Rudolf W. Strohmeier

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

M.D., Ph.D., D.Sc., Med. Habil., immunologist, research director at the Nanomedicine Research and Education Center at the Bay Zoltán Foundation for Applied Research/Semmelweis Medical University in Budapest, Hungary.
He obtained M.D. diploma in 1978 at Semmelweis University, and then held various scientific positions in Hungary and abroad, including the Institute of Hematology in Budapest, Christchurch University in New Zealand, ETH in Zurich, University of Arizona in Tucson, Harvard University in Boston, National Cancer Institute at NIH and the Walter Reed Army Institute of Research, in Bethesda, Maryland, USA. In 2006 he returned to Hungary to establish the Nanomedicine Department with a liposomal drug pilot plant at the Bay Zoltán Institute of Nanotechnology in Miskolc, Hungary. His research over 25 years centered around two main themes: liposomes and the complement system, in which fields he is author in some 80 papers and book chapters. He is also editor of a manual on the complement system. Along with numerous social, editorial and university teaching commitments in Hungary and abroad, he is a founder and scientific director of an immune toxicity CRO in Hungary (SeroScience Ltd). He is best known for promoting the idea that complement activation underlies numerous drug-induced hypersensitivity reactions.



Laszlo Takacs

M.D., Ph.D. DSc., Foreign Member of the Hungarian Academy of Sciences

EXPERIENCE/EMPLOYMENT/EDUCATION:

- Doctor of Sciences. 1996 (advanced science degree, no US equivalent), Medical Biology, Molecular Sciences Hungarian Academy of Sciences (Document# 3,543)
- Ph. D. 1990, Ph.D. as evaluated by a US agency for immigration purposes) (Candidate of Sciences) Immunology, Hungarian Academy of Sciences (Document# 13,275)
- Medical Doctor: 1979, Semmelweis Medical University, Budapest (Document# 475/1979)

SIGNIFICANT ACCOMPLISHMENTS

- Building of Biosystems International, a startup company for biomarker discovery and validation.
- Discovery of a monoclonal antibody proteomics platform technology

- Successful translation of genome and bioinformatics research to biomarker and small molecule drug discovery, Discovery of many drug target and biomarker candidate genes.

EDUCATION

- Doctor of Sciences. 1996 (advanced science degree, no US equivalent), Medical Biology, Molecular Sciences Hungarian Academy of Sciences (Document# 3,543)
- Ph. D. 1990, Ph.D. as evaluated by a US agency for immigration purposes) (Candidate of Sciences) Immunology, Hungarian Academy of Sciences (Document# 13,275)
- Medical Doctor: 1979, Semmelweis Medical University, Budapest (Document# 475/1979)

EMPLOYMENT HISTORY

- 2004 - Chief Operating Officer and Chief Scientific Officer of Biosystems International SAS France. President Biosystems International Inc. USA, President Biosystems International Kft. Hungary
- 2004- Scientific Advisor, Full professor level at the University of Debrecen.

Publications: 70 peer reviewed publications. Meeting abstracts: approx. 90. Invited speaker: approx. 25. Patents and applications: >10

Marcus Textor

Professor, ETH Zürich, Switzerland. He is Professor at the Laboratory for Surface Science and Technology, Department of Materials, and head of a research group dedicated to the area of surfaces and interfaces in bio-related fields of material science. He studied chemistry at the University of Zurich. His thesis (1972) covered the synthesis and spectroscopic characterization of metal-organic complexes as well as interpretation of UV/VIS spectra in the fame work of molecular orbital calculations and ligand-field theory.

Receiving a fellowship of the Royal Society he spent the following two years at the School of Molecular Sciences, University of Sussex, Brighton, Great Britain. Research covered the preparation of metal single crystals, their characterization using LEED, XPS and UPS and the monitoring of catalytic model reactions at these surfaces. In 1978 he joined Alusuisse Central R&D Laboratories (now Alcan), Neuhausen am Rheinfall, Switzerland and started as a team leader in the area of surface analytical research and services (XPS, SIMS). He later became responsible for the development of new surface technologies (anodic oxidation, electrodeposition, CVD/PVD), before being appointed head of the Materials Department with worldwide responsibilities for materials aspects related to the fabrication and application of aluminium and composites for the industrial (automotive) and packaging sector. In 1994 he joined ETH Zurich, Department of Materials and started a research group and teaching activities in the area of surfaces and interfaces of light metals (aluminium, titanium) and biointerfaces. His current main interests cover both fundamental aspects in the behaviour of materials in contact with biological milieus and the design and making of surfaces that elicit biospecific responses. In terms of applications, his research activities aim at useful developments for the field of biosensors, biomaterials/medical implants, carriers for targeted drug delivery and functional nanoparticles for medical imaging and biosensing applications.

Donald A. Tomalia

Ph.D., President/Founder, NanoSynthons LLC, National Dendrimer & Nanotechnology Center, Midland, MI 48640 USA

Dr. Tomalia 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. 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 (2003) for discovery of the fourth major macromolecular architectural class, dendritic polymers.

In 1990, he joined the Michigan Molecular Institute (MMI) as Professor and Director of Nanoscale Chemistry & Architecture (1990-99). Dendritech, Inc., the first commercial producer of dendrimers, was co-founded by Dr. Tomalia in 1992 after which he was named founding President and Chief Scientist (1992-2000). He became V.P. of Technology for MMI (1998-2000) while simultaneously serving as Scientific Director for the Biologic Nanotechnology Center, University Michigan Medical School (1998-2000). Dr. Tomalia founded Dendritic Nanotechnologies, Inc. (DNT), Mt. Pleasant, Michigan, in a joint venture with Starpharma Pooled Development (Melbourne, Australia) (2002) and served as President/Chief Scientific Officer and Company Director (2002-2007).

Currently, he is the Founder and President of NanoSynthons LLC. Other positions currently held by Dr. Tomalia include Director of The National Dendrimer & Nanotechnology Center and Distinguished Professor/Research Scientist at Central Michigan Campus (2007-2010); Distinguished Visiting Professor, Columbia University; External Faculty, University of Wisconsin-Madison (School of Pharmacy); Chairman - Peer Review Panel for Environmental Protection Agency (EPA) "Nanotechnology White Paper" (2006), Washington, D.C.; Board of Directors, American Society of Nanomedicine; Advisory Board, European Foundation for Nanomedicine and Faculty Member, Faculty 1000 Biology.

He is listed as the inventor of over 110 U.S. patents and is author/coauthor of more than 240 peer reviewed publications. Over 170 papers are focused in the dendrimer/dendritic polymer field, including a monograph entitled "Dendrimers and Other Dendritic Polymers" (J. Wiley) co-edited with J.M.J. Fréchet (2001). Dr. Tomalia serves as Associate Editor for Nanomedicine (Elsevier) (2006-), editorial advisory board of Bioconjugate Chemistry (1999-) and is a founding member of the editorial advisory board for NanoLetters (2000-2004).

Gerald Urban

G. A. Urban received the Diploma (Dipl. Ing.) for technical physics at Technical University Vienna, afterwards he was a research assistant at the neurosurgical department, University Hospital Vienna. In 1985 he received the PhD in electrical engineering at the TU Vienna. He was co-founder of the company OSC in Cleveland and Vienna. In 1994 he received the Venia Legendi for Sensor Technology. In 1997 he becomes full professor of sensors at the Institute for Microsystem Technology at the Albert Ludwig University Freiburg/Germany. From 1998 to 2001 he was Dean of the faculty of applied science.

His main interest focuses on research and development of micro sensor applications including microthermistors, flow sensors and chemo- and biosensors including oxygen, pH, glucose, glutamate, glutamine and lactate probes for in-vivo applications and for clinical analyzers. Recently nano- and microsystem technology is the main field of interest including the development of microarrays for proteomics and cell based microassays. The integration of sensor and actuator systems with microfluidics for detection of µRNA is one of the main research topic. Additionally he is interested in coating of biosensors and implants with magnetron-enhanced deposited nanofilms.

In Nanoscience he is also involved the development of new quantum dot systems and using metallic and quartz nanoparticles for nanocatalysis and sensor modifications.

He has published more than 100 papers, 15 book chapters and 57

patents and got four awards.

EMPLOYMENT/EXPERIENCE

- 1980-1982: Scientific coworker, Neurosurgical Department AKH Vienna
- 1982-1986: Assistant professor, Electrical Engineering, TU Vienna
- 1986,1987: Guest scientist at neurophysiological department University Münster
- 1986-1990: Cofounder and employee of company OSC, Cleveland, USA
- 1990-2002: Head Ludwig Boltzmann Institut f. Biomedical Mikroengineering, Vienna
- 1997-present: Full Professor in Sensors, University Freiburg, Germany
- 1998: Co-Founder of „Zentrum für Applied Biosciences" (ZAB)
- 1999-2001: Dean of Faculty „Applied science"
- 2002- present: Director of Freiburger Materials Research Centre (FMF), Freiburg
- 2009-present: Member of the Freiburg Institute of Advanced STudies (FRIAS)

SCIENTIFIC HONORS:

he awarded: 1990 the Stefan Schuy price. 1993 AVL-List price. 1993 Best poster award Eurosensors. 1994 Hoechst Price. 1994 Applicant for a chair of Biomedical Engineering (succession: Prof. Anliker) ETH Zurich.

Pedro M. Valencia

EDUCATION

09/2007-Present Massachusetts Institute of Technology Cambridge, MA, PhD Candidate in Chemical Engineering, Thesis Advisors: Robert Langer, Omid Farokhzad, Rohit Karnik, Research: Development of targeted polymeric nanoparticles for cancer chemotherapy

- 09/2007-12/2008 Massachusetts Institute of Technology Cambridge, MA, M.S. in Chemical Engineering Practice GPA: 4.7/5.0
- 08/2004-08/2007 University of Wisconsin-Madison Madison, WI, B.S in Chemical Engineering GPA: 3.93/4.0 Class Rank: 1/47
- 06/2002-05/2004 Miami Dade College–Kendall Campus Miami, FL, AA in Engineering GPA: 4.0/4.0 Class Rank: 1

RESEARCH AND PROFESSIONAL EXPERIENCE

- 01/2011-Present PrivoTechnologies (Start-up Company from Langer Lab) Cambridge, MA, Senior Scientist, Developing a nanoparticle platform for buccal delivery of drugs and proteins in medicated chewing gum
- 09/2009-12/2009 Massachusetts Institute of Technology Cambridge, MA, Worked in 4 4-week intense consulting projects with General Mills (Minneapolis, MN), GlaxoSmithKline (Singapore) and Bioprocess Technology Institute (Singapore).
- Summer 2007 Vienna University of Technology Vienna, Austria, Summer school participant, Successful completion of different experiments in chemical engineering unit operations.
- 01/2006-05/2007 University of Wisconsin-Madison Madison, WI, Chemical Eng Undergraduate Researcher (Prof. Michael Graham's group), Study the behavior of polymeric microparticles in viscoelastic fluids and the use of polymers for drag reduction in blood flow.
- Summer 2006 Toulouse Center for Materials Studies (CEMES) Toulouse, France, NSF Undergraduate Research Fellow, Synthesis and characterization of a mixed-valence system for single-molecule computer applications.
- Summer 2005 Massachusetts Institute of Technology Cambridge, MA, Chemical Engineering Research Intern (Prof. Paula Hammond's group), Study the properties of a specific kind of polymeric micelles for drug delivery applications.

AWARDS

- National Science Foundation Graduate Fellowship (2008)
- Full Scholarship (tuition, room, and board) from U. Wisconsin-Madison (2004-2007)
- Carole Foster Scholarship at UW-Madison for research abroad experience
- Certificate of Academic Excellence from Miami Dade College
- Honors and Commends from the Center of Community Involvement and Civic Literacy

PUBLICATIONS

- P. M. Valencia, P. A. Basto, L. Zhang, M. Rhee, R. Langer, O. C. Farokhzad, R. Karnik, ACS Nano, 4, 1671.
- J. M. Chan, P. M. Valencia, L. Zhang, R. Langer, O. C. Farokhzad, Methods Mol Biol, 624, 163, 2010
- N. Kolishetti, S. Dhar, P.M. Valencia, R. Karnik, R. Langer, S. Lippard, O. Farokhzad , PNAS, 2010, October Early Edition
- C. Salvador Morales, P.M. Valencia, A.B. Thakkar, E. Swanson, R.Langer, Frontiers in Bioscience, Jan 2011
- M. Rhee*, P.M. Valencia*, R. Langer, O. Farokhzad, R. Karnik 2010, Advanced Materials online Feb 2011 *Equal Contribution
- P.M. Valencia, M. Hanewich-Hollatz, W. Gao, F. Karim, R. Langer, R. Karnik, O.C. Farokhzad, Submitted.

CONFERENCE PRESENTATIONS

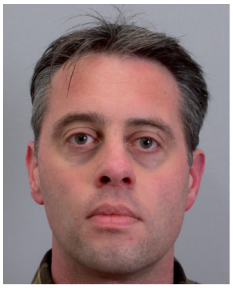
- MIcroTAS 2008, San Diego CA, USA
- NanoTech 2009, Houston TX, USA
- MicroTAS 2009, Jeju Island, South Korea
- US-Japan Symposium, Maui HI, USA
- Colombian Symposium in Health and Biological Sciences, Boston MA, USA
- MicroTAS 2010, Groningen, Netherlands

SEMINAR PRESENTATIONS

- Micro-Nano Seminar, Dept Mechanical Engineering MIT (Spring 2009)
- Centers of Cancer Nanotechnology Excellence Meeting, MIT (Fall 2009)
- MIT Koch Institute Center Focus Seminar (Fall 2009)
- Centers of Cancer Nanotechnology Excellence Meeting, MIT (Fall 2010)
- MIT Chemical Engineering PhD Student Seminar (Fall 2010)
- MIT Koch Institute Center Retreat (Fall 2010)
- Centers of Cancer Nanotechnology Excellence Meeting, MIT (Spring 2011)

TEACHING EXPERIENCE

Teaching Assistant in Heat and Mass Transport Processes. MIT Chemical Engineering Department (Fall 2010)



Cornelus F. van Nostrum

1995: Ph.D. degree in Supramolecular Chemistry, University of Nijmegen. 1995-1997: Postdoctoral fellowship, Philips Research Laboratories, Eindhoven. 1997-1999: Assistant professor, department of Polymer Chemistry and Coating Technology, Eindhoven University of Technology. 1999-present: Assistant professor, associate professor. from October 2004, department of Pharmaceutics, Utrecht University.

RESEARCH ACTIVITIES: design and synthesis of hydrogels, micelles and nanoparticles and application as drug and gene delivery devices.

AWARDS: two personal research grants, 1.5 million euro's in total, from the Dutch Organisation of Scientific Research and from Utrecht University.

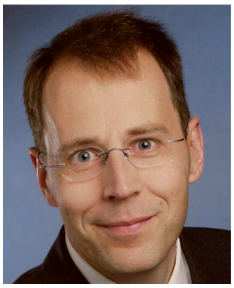
PUBLICATIONS RECORD >100 papers, which received more than 3000 citations, 7 book chapters and 8 patent applications.

Viola Vogel

Viola Vogel is Professor in the Department of Materials and Head of the Laboratory for Biologically Oriented Materials at the ETH Zürich since 2004. After conducting her PhD research at the Max-Planck Institute for Biophysical Chemistry in Göttingen, and spending her Postdoctoral years in the Department of Physics in Berkeley, she started her first faculty position in 1990 in the Department of Bioengineering at the University of Washington in Seattle, where she was later the Founding Director of the Center for Nanotechnology (1997 - 2003). She received various awards, including the Otto Hahn Medal of the Max-Planck Society in 1988, the 'First Award' from the National Institute of Health General Medicine in 1993, the Philip Morris Award in 2005, the Julius Springer Prize 2006 for Applied Physics, and the Advanced Investigator Award from the European Research Council in 2008.

Yuri Volkow

Prof. Yuri Volkov received his MD from the Moscow Medical Academy and subsequently a PhD in biomedical sciences at the Institute of Immunology, Moscow. He works 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 is coordinating 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.



Ulrich Walker

Ulrich Walker is a faculty member at the Basle University Dept. of Rheumatology. After being trained in internal medicine, immunology, rheumatology and infectious diseases, he heads the Rheumatology Outpatient Service at Basel University and its interdisciplinary Scleroderma Clinic. In his research laboratory Ulrich Walker is interested in mitochondrial lesions that are acquired in, and contribute to human pathology.

Heinrich Walt

(HW) is a tumor biologist who got his degrees (master diploma and Ph.D.) at the Universities of Zurich and Fribourg, Switzerland. After a postdoc phase performing cancer related research projects at the Institutes of Anatomy and Pathology of the University of Zurich, he built up a new Research Division of Gynecology at the Department of Gynecology, University Hospital Zurich from scratch. In 1993 he received his title as a professor from the medical faculty of the University of Zurich. Today he is group leader of oral oncology research at the University Hospital of Zurich, Department of Cranio-Maxillo-Facial Surgery (Director: Prof. Klaus W. Grätz). HW has strong scientific connections to the Jackson Laboratory, Bar Harbor, Maine, USA (TJL, Prof. Rick Woychik, Director). In 2006 he was appointed Adjunct Professor to TJL. From 2003-2008 he served as president of the Swiss Society for Oncology.
In 2008 he became vice-president of a new organisation, called European Platform for Photodynamic Medicine. This organisation is involved in the promotion of photodiagnosis and photodynamic therapy and includes nanomedicine as well. It fosters the formation of consortia for EU-projects. In parallel, HW serves as an associate editor of PD and PDT, a new scientific journal which recently became listed at MedLine.
In 2010 he became a board member of the Foundation for Research on Information Technologies in Society in order to develop new projects to fight against cancer.

Klaus-Michael Weltring

I am a molecular biologist by training with a PhD and a Habilitation degree from the University of Münster. Since 2001 I am the managing director of bioanalytik-muenster responsible for the development of the Münster region into a leading nanobioanalytic location at the European level. I set-up a local network of researchers from different disciplines and SMEs and organize the communication within the network and the outside. Between 2003 and 2008 I was the deputy-coordinator of the Nano2Life Network of Excellence and leader of the "ELSA" Board in this network. Furthermore, I co-managed the Nanomedicine Round Table and the EuroNanoBio projects (FP7 CSA projects) which analyzed the ELSA and economic environment and the infrastructure requirements for efficient development of Nanomedicine in Europe. For the last four years I have been a member of the Executive Board of the ETP Nanomedicine leading the ELSA advisory group of this platform.

Wolfgang Wenzel

Wolfgang Wenzel studied physics at the University Bochum starting in 1983. As a Fulbright fellow he moved to Ohio State University (Columbus, Ohio, USA) in 1985 where he graduated 1989 with a Ph.D. in physics. He stayed as a postdoctoral fellow in the laboratory of Nobel Laureate Ken Wilson until his return to Germany in 1992, where he joined the department of physics of Dortmund University. In 2001 he became a group leader for computational nanophysics at the newly founded Institute for Nanotechnology at the Research Center Karlsruhe, one of Germany's national laboratories which in 2009 merged with Karlsruhe University to form the Karlsruhe Institute of Technology.
Together with his group he works on the development of predictive

simulation methods to accurately describe slow processes in various scientific fields: these include the POEM (protein optimization with energy methods) for biomolecular structure simulation, including protein folding, docking and structure prediction; the FlexScreen high-throughput in-silico screening approach for drug development and efficient simulation techniques for the description of nano-materials (http://www.fzk.de/biostruct).

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Kenneth Kak Yuen Wong

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ACADEMIC QUALIFICATIONS: MB. ChB. (Edinburgh) July 1992, FRCSEd Feb 1997, Ph.D (London) April 2002, FCSHK April 2002, FAAN Sept 2006 FHKAM (Surgery) Dec 2006, FRCSEd (Paeds) Dec 2006

LEARNED SOCIETIES: Member of British Society for Immunology. Member of British Association of Paediatric Surgery. Member of International Pediatric Endosurgery Group. Member of Pacific Association of Pediatric Surgeons. Member of American Society of Nanomedicine. Founding Member of International Society of Nanomedicine. Executive board member of Asian Association of Pediatric Surgeons. Member of Hong Kong Society of Developmental Biology. Council member of Hong Kong Paediatric Society of Gastroenterology, Hepatology & Nutrition

AWARDS & HONOURS: International Guest Scholarship 2008, American College of Surgeons. Visiting Professor 2009-2010, Changchun Children Hospital, Jilin, China . Visiting Professor 2009-2010, Shenzhen Children Hospital, Shenzhen, China. Visiting Professor 2009-2010, Harbin Children Hospital, Heilongjiang, China. Overseas Medical Expert Award February 2011, Changchun, Jilin, China

RESEARCH SUPERVISION

Ph.D students x4; M.Phil students x4; Post-doc fellows x2

ACADEMIC ACTIVITIES / JOURNAL REVIEWS: Regular reviewer for: American Journal of Physiology; Archives of Diseases of Childhood; Asian Journal of Surgery; Current Pediatric Review; Journal of Investigative Dermatology; Journal of Magnetic Resonance Imaging; Journal of Nanobiotechnology; Journal of Pediatrics and Child Health; Journal of Pediatrics; Journal of Pediatric Gastroenterology and Nutrition; Journal of Pediatric Surgery; Nanomedicine: Nanotechnology, Biology and Medicine; Neonatology; Pediatric Surgery International; World Journal of Pediatrics; World Journal ofGastroenterology

MEMBER OF EDITORIAL BOARD OF: Nanomedicine: Nanotechnology, Biology and Medicine. World Journal of Gastrointestinal Endoscopy. Hong Kong Medical Journal. Reviewer for McMaster Online Rating of Evidence (MORE)

PUBLICATIONS/PRESENTATIONS: Book Chapters x6

RESEARCH EXPERIENCE & INTERESTS: My current research interests are: 1. Molecular biology of childhood tumours. 2. Immuno-modulation in transplantation tolerance. 3. Nanomedicine for wound regeneration and tumor therapy.

INNOVATION AND TECHNOLOGY FUND: Guangdong-Hong Kong Technology Cooperation Funding Scheme (2007-2010) Development of a new frontier in nanomedicine.

SEED FUNDING (2008-2010)

- Small Project Funding (2009-2011) The use of encapsulated gold-porphyrin nanoparticles as a novel treatment for neuroblastoma
- Small Project Funding (2009-2011) Inkjet printing of tissue-engineered skin construct for vascular regeneration in wound healing
- Small Project Funding (2009-2011) Small molecule inhibitors for use in the treatments for thyroid diseases

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PERSONAL DETAILS

Born February 26, 1974 in Interlaken, Switzerland. Swiss Citizen. Married to Marjel Zumbühl-Wegelin, 1 daughter (2008), 1 son (2011)

ACADEMIC APPOINTMENTS

12/2010– NCCR CHemBio, Member of the National Center of Competence in Chemical Biology, Geneva, Switzerland. 01/2008–current Maître Assistant (lecturer), Department of Organic Chemistry, University of Geneva, Geneva, Switzerland.

EDUCATION

- 09/2006– Postdoctoral Associate, Biozentrum Basel, University of Basel, Switzerland.12/2007 Biophysical Chemistry Advisor: Prof. Dr. Joachim Seelig
- 01/2005– Visiting Scientist, The Whitehead Institute for Biomedical Research, Cambridge, USA. 08/2006 Molecular Biology Advisor: Prof. Dr. Gerald R. Fink
- 08/2004– Postdoctoral Fellow/Associate, Massachusetts Institute of Technology, Cambridge, 08/2006 USA. Biomedical Engineering Advisor: Prof. Dr. Robert S. Langer
- 12/1999– Dr. Sc. nat. ETH, Swiss Federal Institute of Technology, Zürich, Switzerland. 06/2004 Organic Chemistry Advisor: Prof. Dr. Erick M. Carreira Thesis title: Novel Amphotericin B Conjugates: Synthesis and Biological Relevance
- 10/1995– MSc. (Dipl. Chem. ETH), Swiss Federal Institute of Technology, Zürich, Switzer
- 11/1999 land. Organic Chemistry Advisor: Prof. Dr. Andrew D. Miller (Imperial College London) and Prof. Dr. Donald Hilvert (ETH) Thesis title: Fluorescent Labeling of Cationic Liposomes
- 1990–1994 Matura in Mathematics and Science, Gymnasium Interlaken.

RESEARCH FOCUS

Research at the interface of chemistry, biophysics, and medicine. Using chemical synthesis to create phospholipid-based molecules and materials with potential applications in medicine and biology. Equilibrium between basic and applied research.

CLINAM European Foundation for Clinical Nanomedicine

4th European Conference for Clinical Nanomedicine

The Great Strides towards the Medicine of the Future

May 23-25, 2011 - Congress Center Basel, Basel, Switzerland

Conference Proceedings

PART II

Abstracts of the Speeches

IMAGING CONTROLLED TUMOR THERAPY WITH MAGNETIC NANOPARTICLES

Christoph Alexiou[1], Stefan Lyer[1], Rainer Tietze[1], Eveline Schreiber[1], Jenny Mann[1], Marc Schwarz[2], Tobias Struffert[2]

[1]Department of Oto-Rhino-Laryngology, Head and Neck Surgery, Section for Experimental Oncology and Nanomedicine, Else Kröner-Fresenius-Stiftung-Professorship, University Hospital Erlangen, Germany. [2]Division of Neuroradiology, University Hospital Erlangen, Germany

INTRODUCTION

In order to increase the dose of antineoplastic agents in the tumor area, the concept of Magnetic Drug Targeting (MDT) has been developed[1]. Magnetic nanoparticles consisting of iron oxide and a biocompatible cover layer suspended in an aqueous solution (ferrofluid) serve as carriers for chemotherapeutics being enriched by an external magnetic field after intra-arterial application in desired body compartments (i.e. tumor). Enrichment can be monitored using different methods. It could be shown that with this system a high and specific enrichment of the bound chemotherapeutic agent in a desired body compartment (i.e. the tumor) is possible. HPLC-analysis of the chemotherapeutic agent after MDT revealed a 75 times higher concentration of the administered dose in the tumor region compared to the regular systemic administration. Complete tumor remissions without negative side effects can be observed within a pilot study [2]. MRI technique enables imaging control of magnetic nanoparticle enrichment in the tumor area after administration.

METHODS

Experimental VX-2 squamous cell carcinomas were implanted at the left hind limb of rabbits. After four to six weeks MDT was performed with one cycle of treatment. Mitoxantrone was bound to superparamagnetic Fe3O4-nanoparticles (hydrodynamic diameter: ~100 nm) in an aqueous solution (= ferrofluid). The range of the body weights of the rabbits was between 3.1 kg and 3.9 kg. The applied doses were approximately less than 10 % compared to the regular systemic dose (10mg/m2). The drug loaded nanoparticles were given through the femoral artery close to the tumor. The magnetic nanoparticles were attracted to the tumors by a focused external magnetic field during the application. The magnetic field was generated by a water cooled electromagnet (Siemens Healthcare, Erlangen, Germany) with a field gradient of up to 45 T/m directly under the tip of the poleshoe. Imaging was performed using a standard 3 Tesla MRI device (Siemens Healthcare, Germany).

RESULTS

Imaging of the tumor region with a standard clinical 3T MRI was performed one day before and 20 to 30 hours after MDT. The imaging data show an impressive signal deletion (T1- weighting) in the tumor region, caused by a high enrichment of the applied magnetic nanoparticles. Due to this interfusing enrichment the majority part of the tumor can be reached by the nanoparticles and the respective therapeutic agent.

CONCLUSION

It could be shown, that nanoparticle-enrichment in a tumor area is possible with the chosen settings of magnetic field arrangement. This promising data shows, that there is a high potential of adjusting particle enrichment in certain body regions by specific placement of the external magnetic field. On top of that it opens possibilities for MDT to reach tumors in deeper body regions. To affirm these results a larger number of experiments have to be performed. In addition to that investigations with other technical methods, like magnetorelaxometry, µX-CT and histochemistry have to be done to confirm these data qualitatively and quantitatively.

ACKNOWLEDGMENT

These studies were supported by the Else Kröner-Fresenius-Stiftung, Bad Homburg v.d.H., the German Research Foundation (DFG, AL 552/3-1) and the BMBF (Exzellenzzentrum für Medizintechnik, FZ:01EX1012B)

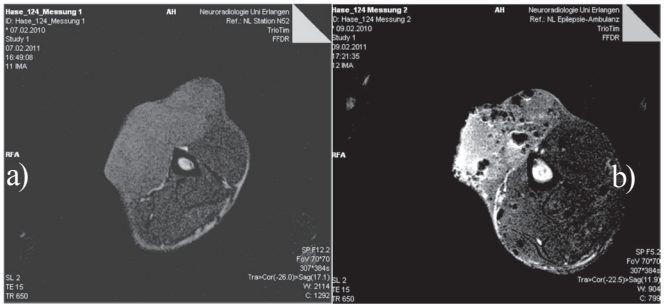

Fig.: a MRI (T1-weighting) of the VX2 Tumor (white circle) at the rabbits hind limb before MDT. b.: Signal deletion after MDT.

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NEW DEVELOPMENT OF DOXORUBICIN TRANSDRUG IN HEPATOCELLULAR CARCINOMA

Pierre Attali[1], Emilienne Soma[2], Severine Rochaud[2], Philippe Merle[3].

[1]Liver Unit, Hopital Paul Brousse, Villejuif, France and BioAlliance Pharma, Paris, France [2] BioAlliance Pharma, Paris France [3] Liver Unit, Hopital de la Croix Rousse, Lyon, France

Transdrug is a novel formulation of doxorubicin that associates a carrier, polyisohexylcyanoacrylate (PIHCA) nanoparticles, with doxorubicin molecules bound to the nanoparticles by absorption. Doxorubicin is compartmentalized within the PIHCA nanoparticles and, thus, presents new pharmacological properties Doxorubicin-Transdrug is far more cytotoxic on resistant cells and is more effective than free doxorubicin on both sensitive and resistant tumour models. Its activity is associated with a decrease in drug efflux and an increase in nuclear concentration. Doxorubicin Transdrug forms an ion pair that hides doxorubicin to the physiologic efflux pumps and consequently overcomes Pgp or MRP-induced multi-drug resistance (MDR).

The rationale for testing Doxorubicin Transdrug® in HepatoCellular Carcinoma (HCC) was based on:

- The ability of the drug to bypass multidrug resistance in vitro and to increase the efficacy in vivo by increasing tumour necrosis and tumour-cell apoptosis.
- The preferential hepatic distribution and proven efficacy in susceptible or resistant tumour models.

A phase 2-3 clinical trial was carried out in patients with advanced HCC according to a randomized, multicenter, open, 2 arms design. The main objective was to compare the efficacy and safety of 3 courses of hepatic intra-arterial injection of Doxorubicin Transdrug® every 4 weeks with best of care treatment according to each centre's usual practice, usually Trans-Arterial ChemoEmbolization (TACE). The primary efficacy endpoint was to assess the number of patients free of local progression at 3 months after randomization. Overall survival and safety were other secondary endpoints. Twenty-eight patients were enrolled, 17 in the Doxorubicin Transdrug® group and 11 in the Control group. Thirteen men and 4 women from 51 to 78 year-old received a single dose of 30 mg/m² Doxorubicin Transdrug® by hepatic intra-arterial injection for up to three courses. Ten men and one woman from 52 to 80 year-old were randomized in the Control group.

The study has been placed on hold following the occurrence of frequent and severe pulmonary adverse events in the Doxorubicin Transdrug® group. Recommendations from the independent Steering Committee were to monitor carefully the patients randomised in the study, to evaluate efficacy and particularly survival and review the clinical benefit taking into account serious adverse events. Clinical data were updated in March 2011 and will be presented: the number of patients free of local progression was not different between the 2 groups. In contrast, survival rate was increased in the Doxorubicin Transdrug® group as compared to the Control group (usually TACE). Based on these encouraging data, new approaches validated in animal models, have been designed for a safer use of Doxorubicin Transdrug® that could reduce the likelihood of occurrence of pulmonary adverse events, and

further improve the benefit/risk ratio of Doxorubicin Transdrug® in patients suffering from advanced hepatocellular carcinoma.

TOWARDS NANOMECHANICAL THERAPY: ENHANCED OPTICAL BREAKDOWN OF NANOCOMPOSITE LABELED CELLS

Lajos P. Balogh, Christine Tse, Marwa J. Zohdy, Jing Yong Ye, Matthew O'Donnell, and Wojciech Lesniak

Dendrimers are symmetrically branching macromolecules that offer the possibility to create a flexible and tunable platform for functional nanodevices for imaging and therapy.

Dendrimer nanocomposites (DNC) are nanosized organic-inorganic hybrid particles made from dendrimer templates by synthesizing small clusters of inorganic nanomaterials within the network of dendrimer macromolecules. The resulting hybrid nanoparticles combine the chemical and physical properties of both the inorganic components and their template.

DNCs can be synthesized with either cationic, anionic, neutral, lipophilic, lipophobic or mixed surfaces. As physical interactions of any individual nanoparticle with its molecular environment are dominated by the contact surface of the template molecules, the encapsulated inorganic nanoparticles can be manipulated as if they were organic macromolecules.

To effectively target any nanodevice to cells and tissues, we have to understand and optimize a number of biologic and pharmacokinetic responses (biodistribution, immunogenecity, and respective toxicity profiles etc.). For example, anticancer dendrimer composite nanodevices are made of dendrimers of defined size and net surface charge; they carry small peptides as targeting moieties, and biologic labels.

Reactive encapsulation of inorganic components permits the rapid immobilization of active materials in the pre-made multifunctional template, which then can be efficiently delivered to specifically label or kill tumor cells with minimal collateral damage.

In this work, enhanced optical breakdown of KB cells (a human oral epidermoid cancer cell known to overexpress folate receptors) targeted with silver/dendrimer composite nanodevices (CNDs) is presented.

CNDs $\{(Ag0)_{25}$-PAMAM_E5.$(NH2)_{42}(NGly)_{74}(NFA)_{2.7}\}$ were fabricated by reactive encapsulation, using a biocompatible template of dendrimer-folic acid (FA) conjugates. Preferential uptake of the folate-targeted CNDs (of various treatment concentrations and surface functionality) by KB cells was visualized with confocal microscopy and transmission electron microscopy (TEM). When irradiated with a near-infrared (NIR), femtosecond laser, the CND-targeted KB cells acted as well-confined activators of laser energy, enhancing nonlinear energy absorption, exhibiting a significant reduction in breakdown threshold, and thus selectively promoting intracellular LIOB. Intracellular laser-induced optical breakdown (LIOB) threshold and dynamics were detected and characterized by high-frequency ultrasonic monitoring of resulting transient bubble events.

NANOTECHNOLOGY BASED NOVEL JOINT BIO-LUBRICANT AND WEAR REDUCER: SUMMARY OF PRE-CLINICAL STUDIES

Yechezkel (Chezy) Barenholz

Lab of Membrane and Liposome Research, IMRIC, Hebrew University-Hadassah Medical School, Jerusalem, 91121, Israel

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 (Yaniv et al in preparation).

An efficient GMP production process was developed. "First in man" study of this DMPC/DPPC-MLV as a medical device is now awaiting final approval.

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THE ETP NANOMEDICINE - GUIDING THE STRATEGY FOR NANOMEDICINE IN EUROPE. THE NEED FOR CONSOLIDATED ACTION

Patrick Boisseau

Chairman working group Nano-diagnostics ETP Nanomedicine, CEA-Leti, Minatec Campus, Grenoble (FR)

The European Technology Platform Nanomedicine was established in 2005 as a joint venture of the European Commission and CEOs of large industrial companies such as Philips, Siemens and UCB, SMEs and academic research institutions to investigate and advance joint activities in the area of nanotechnology in medicine. Since 2005 the ETPN published a number of strategic documents outlining the strategic needs and roadmaps for nanomedicine research in Europe. Influenced by these strategic documents, the NMP unit of the Directorate General for Research funded projects worth 265 Mio. Euros so far in FP 7 including projects such as the NanoMed Round Table and the EuroNanoBio project which provided a first impression of the needs and conditions for a suitable social and economic environment and the structural requirements for an efficient

translation of R&D results into innovative Nanomedicines. Based on this large body of information and the partnership covering the whole value chain the ETPN is actively committed to the setup and implementation of pilot actions in the area of nanomedicine. This contribution is important, as Nanotechnology applied to medical applications – usually called Nanomedicine – will be one of the key enabling technologies for earlier diagnosis (the sooner, the better the treatment), better targeted therapies (less side effects) and better therapy monitoring (faster recovery). Beyond that, Nanomedicine is thought to be instrumental with regard to improved and cost effective healthcare, one of the key issues for the ageing population. Furthermore, the field of Nanomedicine will be an important contributor to European economic growth and employment if developed properly.

The ETPN implementation plan consists of five fundamental steps, namely:

- Analysing the future needs of stakeholders including patients, clinicians and industries and the contribution that Nanomedicine can bring,
- Improving translation in Nanomedicine by advancing project setup process,
- Establishing sustainable infrastructures for translation of R&D towards industry,
- Improving coordination with member states and regions,
- Fostering European harmonisation for increasing European global competitiveness and industrial attractiveness.

The ETPN believes that amongst others, Nanomedicine will be a, if not the, key enabling technology to achieve the envisioned benefits for the ageing population in Europe. Only by heavily investing in future medical innovations will Europe be able to keep up the race against the inflating costs and increasing demands by patients. Europe has the strength and capability to develop and master this important economic sector rather than being pushed into the role of being a nanomedicine "consumer".

The ETPN is ready to take a lead in this endeavour, since it already has established contacts to many major relevant stakeholders such as clinicians, industry, SMEs, academia and social and ethical scientists which are necessary to successfully implement Nanomedicines into the future healthcare of the ageing population. However, only a consolidated and synchronised action of European stakeholders could be effective in shaping the development of nanomedicine.

TARGETED PHOTO DYNAMIC THERAPY USING LIPID NANOCARRIERS

Patrick Boisseau

Coordinator of EuroNanoMed TARGET-PDT project, CEA-Leti, Grenoble (F)

Photo Dynamic Therapy -PDT- is an emerging modality for the treatment of various cancers. PDT consists of the systemic or local administration of a photoactive drug known as a photosensitizer, its preferential uptake and retention in malignant tissues, and its subsequent activation by a visible laser light. In the presence of oxygen, this activated photosensitizer can generate reactive oxygen species that are cytotoxic for the tumor tissue, leading to its destruction. The TARGET-PDT project presented here studies the delivery and targeting of photosensitizers encapsulated into lipid nano-particles. These lipid nanocarriers offer a high payload that will include antibodies targeting specific tumor biomarkers.

PDT offers strictly focused application, biocompatibility with other forms of treatment, the option for repeated use, excellent cosmetic or functional outcomes and fast recovery. But, the use of PDT has been restrained by limited effectiveness of the photosensitizers on reaching the tumor and the potential damage to healthy cells near the tumor. Improved targeting of the photosensitizer and nano-particles is necessary to prevent damage to the surrounding healthy tissue.

Various components and teh first results of the project will be presented : nano-carrier size and payload, photosensitizers, targeting method and types of laser irradiation.

WHAT'S THE GOOD OF NANOMEDICINE? AND SHOULD IT LEAD TO HUMAN ENHANCEMENT?

Donald Bruce

Edinethics Ltd., Edinborough, Scotland (UK)

In scientific and medical circles nanomedicine is expected to deliver many good things for addressing medical problems, some of whose

implications could be very far reaching. Some in the social sciences have been more sceptical, and worry about trends of technologisation in medicine. So what good will nanomedicine really do for society; and what problems may it also pose?

In particular, should those benefits be restricted to medical conditions? If a technique can also be used to try to enhance the \'performance\' or capabilities of someone who is well and able bodied, should we allow this also? Or is this a misuse of research that was justified because it was targeted at therapies and treatments? At a practical level, how would we assess the risk/benefit of a technique if its purpose is no longer directed to to saving life or reducing suffering? At an ethical level, should we also draw some kind of line at this distinction?

Where would we need to draw ethical lines, and how would we regulate them? If, on the other hand, we make no distinctions, do we reduce any moral implications of human enhancement to matters of private choice, in a free market for enhanced people? Some argue that enhancement be deeply unjust, primarily enabling the rich and privilged to become richer and their privilege more deeply embedded. An EC expert group argued that wider society should engaged to decide on such technologies, but what should be the guiding values? Should nanomedicine be the gateway to a new transhumanist humanity, or is that a misguided technocratic illusion? What, indeed, are human beings for?

ALGINATE/CHITOSAN NANOPARTICLES FOR ORAL DELIVERY OF BIOPHARMACEUTICAL DRUGS

Mateja Cegnar[1], Ana Miklavžin[2] and Janez Kerč[1,2]

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INTRODUCTION

Proteins are highly specific drugs and their therapeutic potential is broadly recognized, however, their bioavailability after peroral administration is very low, therefore, they can be delivered only by more invasive (injectable) route. A lot of effort has been done to deliver protein non-invasively, among which nanoparticles (NPs) represent particularly interesting approach to improve the absorption of protein from gastrointestinal tract (GIT). NPs are advantageous because they physically entrap the protein into carrier material, which may partly give protection from degradation in the GIT. Due to nanometer size and specific polymer used, NPs also have the ability to intimately interact with the intestinal epithelia thus increasing the residence time and concentration gradient of protein at the site of absorption.

In this study biodegradable NPs – polyelectrolyte complexes (PEC) from two polymers, alginate and chitosan, were designed for peroral delivery of the model protein albumin. A mild method

of polyelectrolyte complexation in aqueous medium was used. The influence of pH and ionic strength of the medium on the protein association with polymers and its release from PEC was studied. The capability of PEC in protecting albumin from pepsin degradation was also examined since the enzymatic degradation represents one of the major barriers in the oral protein delivery. The surface characteristics of PEC were also investigated due to their significant influence on the interaction with biological membranes (1, 2).

METHODS

Polyelectrolyte complexes consisting of alginate core entrapping the protein albumin and the outer coating of chitosan were prepared by dropwise addition of one polymer solution into another polymer solution. The influence of solution pH and ionic strength on the polyelectrolyte association was studied using colloid titration in combination with dynamic light scattering (DLS, Zetasizer Nano ZS, Malvern Instruments, UK). The same was investigated on the PEC dissociation and protein release by incubation of PEC in different release media (water, phosphate buffered saline (PBS, 0.15M, pH 7.4), and acidic saline solution (0.1M HCl, 0.9% NaCl, adjusted to pH 3.0) raised to pH 6.8 after 2 h incubation).

Protective capability of PEC against pepsin degradation was assessed by proteolysis assay incubating albumin, either free or associated in PEC, with specific amount of pepsin for different time periods. Degree of proteolysis was determined by visual analysis of SDS-PAGE gel after staining with Coomassie Brilliant Blue. Surface characteristics of PEC were evaluated using bis-ANS fluorescence spectroscopy.

RESULTS

The formation of nanocomplexes by polyelectrolyte complexation is primary driven by the intrinsic physico-chemical properties of protein and polymers and occurs spontaneously by a colloid formation of two or more components. Therefore, a step-wise approach was followed to tailor the conditions of polyelectrolyte association with regard to the specific properties of the particular protein/system. Results showed that the process of polyelectrolyte association between polyionic polymers (alginate and chitosan) and protein (albumin) was dependent on pH and ionic strength of the medium (3). Association/complexation of albumin with negatively charged alginate occurred only at pH bellow the isoelectric point of albumin (pI 4.8), where the protein's net charge was positive (observed as an increase in the scattering intensity in dispersions). At pH 5.0 albumin is negatively charged thus showing no interaction with negatively charged alginate (scattering intensity was similar to polymer solution). The presence of strong electrolytes (high ionic strength) in the polymer solution prevented the association of albumin and alginate in complexes although to pH 4.0, which indicated a charge shielding effect of strong electrolytes that weakened the electrostatic attraction between alginate and albumin. Non-electrolytes (mannitol) negligibly influenced alginate-albumin complexation.

Negatively charged complexes of alginate and albumin were further coated with chitosan, due to chitosan's ability to enhance the absorption, as reported (4). Optimal composition of PEC was determined for alginate:albumin:chitosan in their final concentration 0.5:0.5:0.05 mg/ml, yielding 280 nm sized particles with zeta potential -40 mV.

Ionic strength and pH of the release medium also markedly influenced the release of albumin from PEC. Immediate release up to 90 and 60% was detected in phosphate buffer pH 7.4 (PBS) and water, respectively. Initial burst release is common with the nanometer size particles, since the rate of protein diffusion is proportional to the particle's surface area. However, much higher release was observed in solution with higher ionic strength (PBS) confirming the previous observation of strong electrolytes weakening the interaction between polyelectrolytes thus enabling albumin release.

Under acidic saline solution, pH 3.0, the release of albumin was retained in spite of high ionic strength of the medium. After increasing the pH to 6.8, albumin immediately released from PEC. At low pH it is possible that there were stronger interactions between alginate and albumin that became dominant. Albumin became more positively charged showing strong interaction with negative alginate. It is also possible that at this pH some alginate molecules partly precipitated forming a network that prevented albumin release. When the pH was increased, the positive charge on the protein was reduced, which mitigated interaction with alginate. At this pH alginate was also converted into ionic and more soluble state, which contributed to higher albumin release. These differences in the release profiles could be beneficial in the protein protection against aggressive environment of the stomach (gastric pH) when administered orally.

The protective properties of PEC against pepsin degradation are presented in Fig. 1.

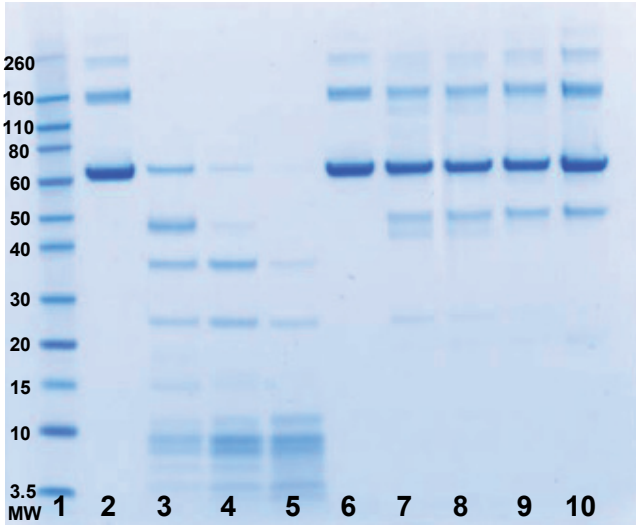

Figure 1. SDS-PAGE results obtained after proteolytic degradation of albumin (66.4 kDa, 0.1 mg/ml), either free or in PEC, with pepsin (34 kDa) at pH 4.0 (albumin/pepsin mass ratio, 20/1). Lane 1, MW standards. Lane 2, untreated free albumin. Lane 3, 4 and 5, free albumin treated with pepsin for 5, 15 and 30 min, respectively. Lane 6, untreated albumin-loaded PEC. Lane 7, 8, 9 and 10, albumin-loaded PEC treated with pepsin for 5, 15, 30 and 60 min, respectively.

Results showed that PEC were able to protect albumin against pepsin degradation up to 1 h of incubation. Strong staining could be observed for the band of albumin-associated within PEC. On the contrary, free albumin progressively degraded already after 15 min of incubation with pepsin, and after 30 min no staining could be observed for the band of albumin, indicating complete degradation of free albumin. Also, degradation products with lower MW could be observed for the free albumin.

Surface characteristic of PEC were investigated with bis-ANS dye. This dye shows no fluorescence in water but becomes highly fluorescent upon contact with hydrophobic surfaces (5). Fluorescence emission spectra were recorded in the presence of bis-ANS on each component and PEC (Fig. 2).

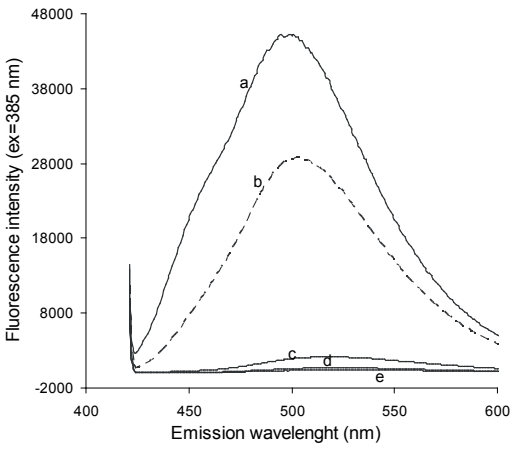

Figure 2. Increase in bis-ANS fluorescence intensity as a measure of surface hydrophobicity: PEC (a); albumin (b); chitosan (c); alginate (d); medium (e). All samples were recorded in the same dispersion media (pH 4.0).

Higher bis-ANS fluorescence intensity representing higher hydrophobicity was obtained for albumin after its association in PEC. This increase in surface hydrophobicity in comparison to free albumin might be an important parameter promoting the interaction with biological membranes (1, 2). In addition, it is also necessary to consider the mucus permeability (6) as well as colloidal stability of nanoparticles (7, 8) that can be provided by certain hydrophilic polymers, thus suggesting especially important impact of hydrophilic/hydrophobic balance of the properties of nanoparticles.

CONCLUSIONS

Polyelectrolyte complexation represents a promising method to formulate protein in nanoparticles under mild conditions. Association/complexation of albumin with alginate and chitosan in NPs as well as their dissociation and protein release were mostly dependent on the pH and ionic strength of the medium. In addition, particle size, surface characteristic, partial resistance to pepsin degradation and release profiles of protein from NPs indicate that such system possesses the characteristics suitable for oral drug delivery; nanometer-sized particles and increased surface hydrophobicity may assist in the particle transport and interaction with epithelial cells. Protection against enzymatic degradation and retained release in acidic pH may protect the protein from harsh conditions in the stomach.

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PREDICTIVE DIAGNOSTICS AND TARGETED PREVENTIVE MEASURES AS THE COST-EFFECTIVE PLATFORM FOR PERSONALISED MEDICINE

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European Association for Predictive, Preventive and Personalised Medicine, The EPMA-Journal, www.epmanet.eu

Optimistic versus Pessimistic Prognosis depends much on diagnostic, preventive and treatment approaches which healthcare will preferably adopt in the near future. Without innovation in healthcare, e.g. in years around 2030 the prevalence of Diabetes mellitus will reach the dimension of a half of billion of affected people worldwide additionally burdened with a spectrum of secondary complications (cancer, cardiovascular and neurodegenerative diseases) and concomitant enormous economical burden linked to the treatment. In the same period of time, neurodegenerative pathologies (Alzheimer's and Parkinson's diseases, glaucoma and macular degeneration, etc.) can reach more than 30% of global disease burden. In contrast, effective utilisation of advanced early/predictive diagnostics, preventive and personalised medical approaches could enable a significant portion of population to reach the 100-year age limit remaining vibrant in excellent physical and mental health as actively contributing members of society. Global research and implementation programmes in biomedicine, communication among scientific societies, healthcare-providers, policy-makers, educators and organised patient groups and, finely, a consolidation of professional groups in the branch of personalised medicine will play a decisive role in driving the situation in favour of one of two scenarios (Optimistic versus Pessimistic Prognosis) over the next 5-10 years.

The overall concept in the field is conducted by the "European Association for Predictive, Preventive and Personalised Medicine" (EPMA).

NANOTECHNOLOGIES FOR OVERCOMING CANCER RESISTANCE

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Even if new molecules are discovered to treat cancer diseases, the clinical use and efficacy of conventional chemotherapeutics is hampered by the following limitations : i) drug resistance at the tumor level due to physiological barriers (non cellular based mechanisms) ii) drug resistance at the cellular level (cellular mechanisms), and iii) non specific distribution, biotransformation and rapid clearance of anticancer drugs in the body. It is therefore of importance to develop nanodevices able to overcome resistance of cancer cells or tissues to chemotherapeutic treatments.

This is illustrated by the camouflage of doxorubicin into polyalkylcyanoacrylate nanoparticles, allowing to overflow the PgP detoxification capacity, thus inducing reversion of the multidrug resistance (MDR). The higher cytotoxicity of doxorubicin when loaded onto poly(isohexylcyanoacrylate) nanoparticles has been shown on the X/myc transgenic mouse model of hepatocellular carcinoma which mimics several steps of human hepatocarcinogenesis[1]. Based on these data, a phase II multicentric clinical trial is currently performed on patients with resistant hepatocarcinoma or liver metastasis.

Another illustration of this approach is squalenoylation[2], a technology that takes advantage of squalene's dynamically folded conformation to link this natural compound to anticancer and antiviral nucleoside analogues in order to achieve the spontaneous formation of nanoassemblies (100–300 nm) in water without the aid of surfactants[3]. When applied to the anti-cancer compound gemcitabine[4], this original concept was demonstrated to be able to overcome different mechanisms of resistance of gemcitabine[5], ie. deamination of gemcitabine by the blood deaminases, down regulation of nucleoside transporters[6] and/or insufficient phosphorylation by the deoxycytidinekinases (dCK). Indeed, the squalenoylated gemcitabine nanoparticles were found (i) to be resistant to deaminases, (ii) to diffuse intracellularly independently of the presence of nucleoside transporters and (iii) to improve the phosphorylation of gemcitabin by dCK.

Finally, the use of nanohybrids constructed with metal organic frameworks (nanoMOFs) will be reviewed for their ability to encapsulate unprecedent high quantities of the anticancer compound busulfan[7].

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EXPLORING NANO-BIOLOGICAL SYSTEMS ENCOUNTERING A NANOMEDICINE

Kenneth Dawson

Director of the Centre for BioNano Interactions (CBNI)

The importance of understanding the interactions between nanoscale materials / nanomedicines and living matter has now been appreciated by an extraordinary range of stakeholders. As the potential to manipulate materials at the nanometer scale grows, this leads to

opportunities to stipulate and study specific interactions with cells, tissue, organs and whole organisms, opening new directions in nanomedicine and nanodiagnostics.

Nanoparticles in a biologically relevant environment (cell media, plasma etc.) draw to themselves a number of proteins and lipids that form a sort of dynamical 'corona' in slow exchange with the environment. The exchange times can be so slow that many early biological responses are defined by the associated corona biomolecules. Even functionalized particles often have some residual long-lived protein corona, and an in-depth understanding of the nano-bio interface (corona) presented to cells and barriers is likely to be the key to understanding targeting. Some new approaches and tools to achieve this will be discussed.

Another key difference between nanoparticles and more conventional drugs is that, as a consequence of their size and protein corona (amongst other factors), nanoparticles are taken up into cells by active processes and trafficked to specific sub-cellular locations utilising well-defined pathways whereas drugs partition based on quasi-equilibrium principles. Thus, clearance can occur only where an established biological pathway. For example, at cell level, many nanoparticles enter the lysosomal pathway, where they accumulate (without clearance) within several hours. In vivo bioaccumulation and clearance are also likely determined by the nature of the protein corona,

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TARGETING THE IMMUNE SYSTEM WITH BIODEGRADABLE NANO-ENGINEERED CAPSULES

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Vaccines that can elicit strong T-cell responses against viral infections or cancer are undoubtedly one of the major challenges for medicine today. [1] For this purpose, dendritic cells (DCs) have to internalize antigen, process them into peptide fragments and present them to T-cells. As DCs have evolved to recognize micro-organisms rather than soluble antigens, particulate antigen delivery systems tend to strongly increase the potency of the induced response. Generally spoken at least three major advantages of microparticulate antigen delivery can be defined. First, compared to soluble antigens, antigens encapsulated in particles with sizes varying from 1-10 µm are far more efficiently taken up, processed and presented. Second, uptake of antigens in a particulate form appears to strongly augment antigen presentation via MHCI, a feature hardly achievable when using soluble antigens. MHCI mediated presentation is crucial for the induction of CD8 cytotoxic T cell responses. Third, microparticulate formulations allow co-encapsulation of immune-modulating compounds and recent studies have highlighted that the potency of an immune response is strongly increased when both antigen and immune-modulator are co-delivered to the same intracellular compartment of the same antigen presenting cell.

Here, we present a highly versatile strategy to formulate vaccine antigens into microparticles that target and modulate dendritic cells in vivo. For this purpose vaccine antigens are encapsulated into biodegradable capsules using all aqueous non-denaturating conditions. Modulation of the Th1/Th2/CTL balance is possible through fictionalization of the capsules with specific ligands and protective immunity in mice models for cancer and viral infection were demonstrated.

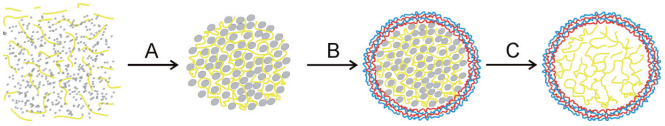

Figure 1. Polyelectrolyte microcapsule synthesis. (A) Antigen (yellow) is mixed with CaCl2 and Na2CO3, resulting in the generation of macromolecule-filled CaCO3 microparticles (gray), which are (B) subsequently coated with alternating layers of dextran sulfate and poly-L-arginine (red, blue). (C) Dissolution of the CaCO3 core by EDTA results in the generation of a hollow microcapsule composed of macromolecules surrounded by the polyelectrolyte shell.

Figure 1 schematically shows the encapsulation procedure of antigen into hollow polyelectrolyte capsules. [2,3] In a first step, antigen loaded CaCO3 microparticles (3 µm diameter) are fabricated by co-precipitation of CaCl2 and Na2CO3 in the presence ovalbumin (OVA) as model antigen. Subsequently these CaCO3 microparticles are coated (2 bilayers) by sequential deposition of dextran sulfate and poly-L-arginine using electrostatic interaction as driving force. Finally hollow polyelectrolyte capsules are obtained after dissolution of the CaCO3 core templates in aqueous EDTA medium.

As demonstrated by transmission electron microscopy (Figure 2A) and confocal microscopy (figure 2B), polyelectrolyte capsules are efficiently taken up by DCs and both the capsule membrane as well as the encapsulated antigen becomes readily processed. [4,5] Antigen presentation to T-cells was assessed by incubating DCs with OVA loaded capsules followed by co-culturing with respectively OT-I and OT-II cells (Figure 2C). OT-I and OT-II cells are transgenic CD8, respectively CD4 T cells that specifically recognizes the OVA CD8 peptide, respectively CD4 peptide. Compared to soluble antigen a dramatic increase in T-cell presentation is observed. Especially cross-presentation to CD8 T-cells, which are crucial to induce cellular immune responses, is strongly promoted. [4]

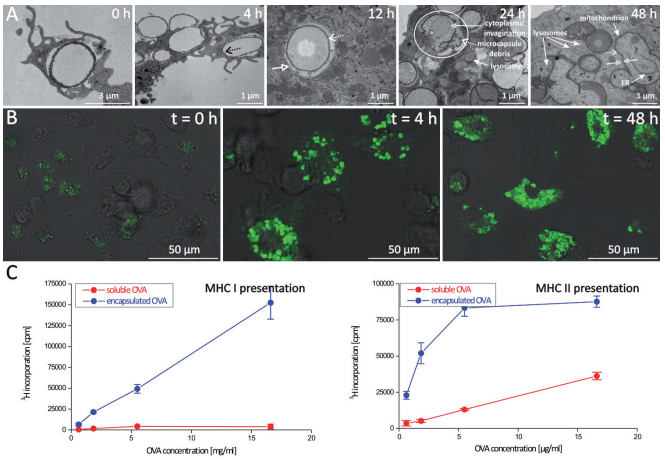

Figure 2. (A) TEM images of BM-DCs that have internalized dextran sulfate/poly-L-arginine microcapsules at the indicated time intervals. Microcapsule shell: dotted arrows; membranes surrounding the microcapsules: open arrows. In the encircled area, microcapsule rupture and cytoplasmic invagination are clearly distinguishable. Lysosomes, endoplasmatic reticulum (ER), and a mitochondrion are indicated by the solid arrows. (B) Processing of dextran sulfate/

poly-larginine microcapsule encapsulated OVA was analyzed using DQ-OVA. Confocal microscopy images of BM-DCs incubated with OVA-DQ microcapsules for 0, 4 and 48 h (overlay of green fluorescence and DIC). (DQ-OVA is ovalbumin oversaturated with BODIPY dyes. Upon proteolytic cleavage, quenching is relieved and green fluorescence appears. (C) Antigen presentation by BM-DCs after uptake of soluble and encapsulated OVA. Proliferation of OT-I cells was used as a measure for MHC-I-mediated cross-presentation of OVA (left graph), proliferation of OT-II cells as a measure for MHC-II mediated presentation (right).

In vivo studies show mild tissue reactions [6,7] upon subcutaneous injection [5 while potent humoral and cellular immune responses [8], including cytotoxic T-cells are induced which show protective immunity against viral infection as well as cancer. Moreover in recent studies we have also demonstrated an easy strategy – involving main stream pharmaceutical technology – to scale the production of polyelectrolyte microcapsules using a one-step procedure which encapsulates antigen with extremely high yields while barely hampering its biological activity. [9]

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ABRAXANE® IN PANCREATIC CANCER TREATMENT

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Pancreatic cancer continues to be a major unsolved health problem, with only 1–4% of patients with adenocarcinoma of the pancreas alive 5 years after diagnosis. Chemotherapy is still the only option in metastatic pancreatic cancer treatment, although in the majority of patients, it is purely palliative with minimal impact on survival. Neither gemcitabine alone nor gemcitabine-based combinational chemotherapy has achieved a favorable outcome in advanced disease. A hallmark in pancreatic cancer is the presence of 'desmoplasia,' which is defined as proliferation of fibrotic tissue with an altered extracellular matrix (ECM) conducive to tumor growth and metastasis. One of the challenges to drug development for pancreatic cancer is poor tumor perfusion due to the unique stromal organization of pancreatic tumors, which may contribute to inefficient drug delivery and chemoresistance in pancreatic cancer.

Therefore, novel strategies to treat this disease are urgently needed. ABRAXANE (nab-paclitaxel), a solvent free 130 nm albumin-bound nanoparticle form of paclitaxel, represents the first in a new class of protein-bound drug nanoparticles that take advantage of the natural transport pathways of albumin. These include caveolae-mediated albumin transcytosis across tumor blood vessel endothelium and potential association in the tumor with albumin-binding proteins such as SPARC, a protein present in majority of pancreatic tumors, to achieve enhanced drug penetration. ABRAXANE is currently approved for the treatment of metastatic breast cancer on the basis of a pivotal Phase III trial, which demonstrated increased response rate, longer time to tumor progression and improved survival in patients previously treated for metastatic disease when compared to Taxol®, the conventional cremophor-based paclitaxel.

Recently, the efficacy of ABRAXANE and gemcitabine combination therapy against pancreatic cancer has been demonstrated in both preclinical and clinical settings. In pancreatic cancer xenografts and the genetic engineered KPC pancreatic model, combination of ABRAXANE with gemcitabine significantly improved antitumor efficacy compared with either agent alone. Importantly, ABRAXANE also enhanced the delivery of gemcitabine into the tumors, resulting in significantly higher intratumoral gemcitabine concentration for the combination versus gemcitabine alone. In a phase I/II study in patients with metastatic pancreatic cancer, the combination of nab-paclitaxel 125 mg/m2 and gemcitabine 1000 mg/m2 on days 1, 8, and 15 of an every 28-day cycle (n = 44) resulted in impressive clinical activity with an overall response rate of 48%, median progression-free survival of 7.9 months, and median overall survival of 12.2 months. Impressive and rapid responses in these pancreatic cancer patients were observed via PET scans. An assessment of SPARC in patient tumors showed a correlation of high SPARC level with improved overall survival, lending support to the proposed mechanism. The efficacy of this combination is being confirmed in an ongoing phase III trial in patients with advanced pancreatic cancer. Further studies are ongoing to investigate the effect of ABARXANE on stromal organization and vasculature in pancreatic cancers. ABRAXANE and albumin-based nanomedicines may be a new avenue for improving the delivery and efficacy of therapeutics in patients with pancreatic cancer.

NANOMEDICINE – CAN EUROPE ADAPT ITS NANO-RESEARCH TO HELP PATIENTS OR WILL IT BE A CASE OF REVERSE INNOVATION ?

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Nanomedicine is at a several cross-roads. Should Nanomedicine be researched and funded separately to mainstream drug research or should it be integrated? The same conundrum applies to nanotechnology in all industrial applications. There remains additionally the problem of definition, quantizing what is in effect a continuous function - size. Non-translation of applied nano pharmaceutical research is still a major problem for funders, industry and patients. Failure to adapt in Europe to open innovation will lead in to nanodrugs being researched and manufactured in the Far East, where there is less separation between academia and industry – this is Reverse Innovation1.

No one organisation is responsible for improving or advising on translation - there is a hope of establishing a translation facilitator, but the best advice for now is for each research unit to come up with its own commercialisation strategy to bring nano to the patients. The problem areas inter alia remain :

- Choice of translatable research areas
- Project selection
- Peer reviewers with no translation knowledge

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PRESENTATION TITLE: NANOTECHNOLOGY AND MEDICINES: THE INNOVATION TASK FORCE INITIATIVE AND THE PRE-COMPETITIVE QUALIFICATION PATHWAY

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The European Medicines Agency follows the latest developments in nanotechnology that are relevant to the development of medicines. Recommendations from the Agency's Committee for Medicinal Products for Human Use (CHMP) have already led to the approval of a number of medicines based on nanotechnology. These include medicines containing liposomes (nanosized fatty structures containing an active substance), such as Caelyx (doxorubicin), Mepact (mifamurtide) and Myocet (doxorubicin) or nano-scale particles of the active substance, such as Abraxane (paclitaxel), Emend (aprepitant) and Rapamune (sirolimus).

The development of medicines using innovative nanotechnology techniques may raise new challenges for regulators in the future. The identification of characteristics of 'nanomedicines' and of their interactions with the biological systems at an early stage of development is key in the preparedness for their evaluation.

Specific properties and characteristics of nanomedicines in their final product formulation for clinical applications should be definable and relate to the specific expected advantages in the intended clinical use (e.g. preferential organ/tissue/cellular distribution, uptake and persistence of effect) as well as potential risks of the nano-engineering, taking into account route and frequency of administration and dose.

In this context and in order to promote R&D and the development of nanomedicines in the interest of public health, the European Medicines Agency offers a pre-competitive qualification pathway, a voluntary, scientific pathway leading to either a CHMP opinion or a Scientific Advice on innovative methods or drug development tools.

This qualification process addresses innovative drug development methods and tools. It focuses on the use of novel methodologies developed by consortia, networks, public/private partnerships, learned societies and pharmaceutical industry for a specific intended use in pharmaceuticals R&D.

To complement and support the qualification pathway, the European Medicines Agency offers a forum for early dialogue with applicants via Briefing meetings under the umbrella of the Innovation Task Force (ITF), a multidisciplinary group that includes expertise from the EMA scientific network, regulatory and legal competences.

The scope of the briefing meetings covers regulatory, technical and scientific issues arising from innovative medicines development, new technologies and borderline products.

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

Rutledge Ellis-Behnke

Director of the Nanomedicine Translational Think Tank Department of Ophthalmology, Medical Faculty Mannheim, Ruprecht-Karls-University, Heidelberg (D)

Glaucoma is currently the leading cause of irreversible blindness, and one major risk factors for developing glaucoma is elevated intraocular pressure (IOP). In this study, an elevated IOP of around 120mmHg was introduced into the right eye balls of Sprague-Dawley rats for around 1 hour in vivo, while their left eye balls were 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 suffered from elevated IOP was significantly higher at 4.89 to 5.58 MPa. Scanning electron microscopy 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, and this may cause strain-stiffening effects in them and therefore result in the observed increase in elastic modulus of the cornea.

MORE THAN HUMAN: THE TRANSHUMANIST AGENDA OF TRANSFORMING HUMANS INTO POSTHUMANS

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In The Metaphysics of Virtual Reality Michael Heim has observed, 'Behind the development of every major technology lies a vision.1 It will be no different with the technologies used by postmoderns to transform themselves into posthumans. Critically examining these mythical and religious themes helps to explain why postmodernity is not displacing modernity as a historical era, but is subsuming and transforming it within a thoroughgoing historicist vision. Postmodernity is simultaneously the affirmation and negation of modernity. Following David F. Noble, despite the Enlightenment's apparent victory of displacing theology with science as the dominant force of cultural formation, religious motivations were never eliminated but only muted.2 Rapid technological development was often praised in profane, progressive and scientific terms, but it was 'driven also by distant dreams, spiritual yearnings for supernatural redemption3. Indeed, 'modern technology and religion have evolved together, and as a result the technological enterprise has been and remains suffused with religious belief4. Consequently, it is not surprising that religious themes continue to inform the development of postmodern technologies, for postmodernity is modernity's prodigal child. Yet since postmodernity is also the negation of modernity, its religious themes are radically reinterpreted and redirected. Modern millennial expectations for an Edenic and Adamic recovery, for instance, were reinforced by advances in modern science and technology. Humankind, it was believed, was entering a golden age when it would faithfully exercise its divinely mandated dominion over creation, and, more importantly, would obtain the state of perfection humans enjoyed prior to the fall. 5The postmodern turn is to insist that such a restorationist program is too confined. Complete mastery over nature, and derivatively human nature, cannot be achieved until humans perfect themselves by becoming a superior species. If the modern project is to make humans better, then the postmodern goal is to make creatures that are better than human.

FOOTNOTES

- 1 Michael Heim, *The Metaphysics of Virtual Reality* (Oxford: Oxford University Press, 1993) p. 118.
- 2 David F. Noble, *The Religion of Technology; The Divinity of Man and the Spirit of Invention* (New York: Knopf, 1997) pp. 3-6
- 3 Ibid., p. 3.
- 4 Ibid., p. 5.
- 5 Ibid., pp. 21-100; John Passmore, *The Perfectibility of Man* (Indianapolis, IN: Liberty Fund Inc., 1970, 2000) pp. 116-327.

NANOTECHNOLOGY AND DRUG TOXICITY: BETWEEN SCYLLA AND CHARYBDIS

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To paraphrase Paracelsus: all drugs are poison; only the dose permits something not to be poisonous. Nanotechnologies offer exciting opportunities for targeted drug delivery which is anticipated to increase the efficacy of the drug and reduce potential side-effects, through the reduction of the dose of the drug in bystander tissues and an increase of the drug at the desired target site. Nevertheless, understanding whether the nanoplatform itself may exert adverse effects is also of great importance. The small size may enable nanoparticles to negotiate various biological barriers in the body which, in turn, could give rise to unexpected toxicities. On the other hand, the potential of nanoparticles to cross the blood-brain barrier may open up new approaches for drug delivery into the brain. Determining the ultimate fate of nanoparticles following their therapeutic or diagnostic application is critical: are nanoparticles excreted from the body, or biodegraded by cells of the immune system, or do the bioaccumulate, thereby leading to potentially harmful long-term effects? The surface of nanoparticles can be modified using targeting ligands, etc but as these particles enter into a biological system, it is likely that the particles are covered with biomolecules that modify the properties of the nanoparticles and the way in which they interact with cells. Moreover, the binding of proteins to nanoparticles may also induce modifications of the proteins. Understanding such nano-bio-interactions is critical for the safe application of nanoparticles in medicine.

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NANOROBOTICS – MEDICAL REALITY TOMORROW?

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Nanotechnology offers interesting perspectives for medical treatment of various diseases, including e.g. cancer. Particles and objects of this size show properties which can be exploited to improve imaging with existing and new technologies and even to deliver therapeutic agents. One of the main challenges for therapy is the targeting efficiency. Chemotherapeutic agents loaded on suitable nanoparticles still affect broad regions in the human body. Recently approaches have been developed to further optimize the delivery accuracy and reduce side effects by exploiting and using robotic approaches for targeting. Core issues with the robotic control of nanoscale particles and agents are the sensory feedback, the actuation mechanism and the particle design and functionalization. Existing nanorobotic approaches and promising technologies for visual feedback are discussed in the talk. A widely-used actuation principle is the actuation by external magnetic fields. Apart from specialized setups using tailored coils, existing magnet resonance imaging systems, MRI, can be used for this purpose. Both crucial features, actuation using the gradient coils and visual feedback using MRI imaging, are already integrated into a system which is commonly available at hospitals. The talk gives a view on the most promising medically feasible perspectives of MRI-based nanorobotic technology.

DESIGN OF NANOVECTORS FOR THE TARGETED DELIVERY OF ANTIMALARIALS

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Malaria is the most devastating parasitic disease of humans in developing countries and one of the main medical concerns worldwide. There is a pressing need for new therapeutic strategies because the disease has to be fought from different fronts and the currently available treatments will not guarantee its eradication. This includes the constant search of new drugs [1] and of improved ways for their efficient and specific targeting. At present, administration methods of antimalarial drugs release the free compound in the blood stream, where it can be taken up by all cells, and not only by Plasmodium-infected red blood cells (pRBCs). 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.

Liposomes have been assayed in the past for the encapsulation of compounds against murine malaria, but there is a lack of cellular studies on the performance of targeted liposomes in specific cell recognition and on the efficacy of cargo delivery, and very little data on liposomedriven antimalarial drug targeting to human-infecting parasites. We have used fluorescence microscopy to assess in vitro the efficiency of liposomal nanocarriers for the delivery of their contents exclusively to pRBCs [2]. 200-nm liposomes loaded with quantum dots were covalently functionalized with oriented, specific half-antibodies against P. falciparum late form-infected pRBCs. In less than 90 min, liposomes dock to pRBC plasma membranes and release their cargo to the cell. 100.0% of late form-containing pRBCs and 0.0% of non-infected RBCs in P. falciparum cultures are recognized and permeated by the content of targeted immunoliposomes (Figure 1). Liposomes not functionalized with antibodies are also specifically directed to pRBCs, although with less affinity than immunoliposomes. In preliminary assays, the antimalarial drug chloroquine at a concentration of 2 nM, $\geq$ 10 times below its IC50 in solution, cleared 26.7 $\pm$ 1.8% of pRBCs when delivered inside targeted immunoliposomes.

Next in our agenda is to advance towards a nanovector-based antimalarial delivery strategy suitable to enter preclinical trials. Liposomal nanovectors are adequate for parenteral delivery, indicated in cases of complicated malaria, those at risk of developing severe disease, or if the patient is vomiting and unable to take oral antimalarials. Parenteral treatment can also be required in the last mile of a malaria eradication protocol for the single-dose, individualized administration of drugs specifically targeted to pRBCs with good accuracy. Notwithstanding, formulations adequate for the oral intake of targeted nanovectors would be a valuable contribution to treating malaria now in endemic areas with poor health care systems. However, liposomes and whole antibodies are difficult to formulate for oral intake, which will likely benefit from smaller (but equally 100% specific) targeting agents and drug-containing structures in the form of polymeric nanoparticles. Our first objective along this path is the development of new highly specific pRBC targeting agents of varied chemical nature adequate for the functionalization of both liposomes and polymeric nanoparticles, with a special emphasis on non-immu-

nogenic molecules sufficiently small to be adequate for the design of orally administered nanovectors.

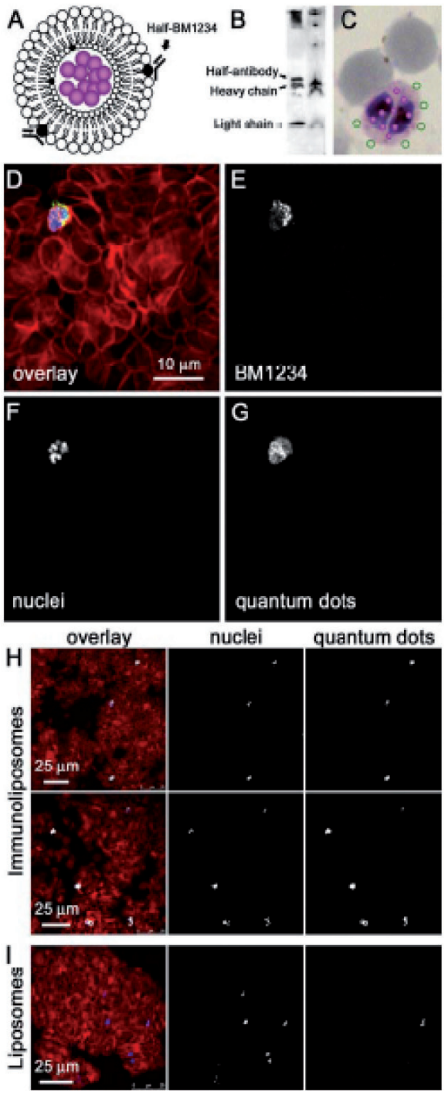

Figure 1. Confocal fluorescence microscopy analysis of the delivery of immunoliposome cargo to pRBCs. (A) Cartoon showing a quantum dot-containing liposome functionalized with half-antibodies. (B) Western blot analysis of the result of treating the monoclonal antibody BM1234 with 2-mercaptoethylamine (MEA), (left lane) after MEA treatment, and (right lane) after addition to MPB-PE-containing liposomes. Immunoliposomes were purified byultracentrifugation prior to electrophoresis. (C) Graphical scheme of the expected performance of nanovectors when added to a P. falciparum living culture containing both infected and non-infected cells. (D) Confocal fluorescence microscopy section of a suspension of living RBCs containing ca. 5% pRBCs that had been treated, for 90 min and prior to fixation, with a preparation of immunoliposomes assembled as depicted in panel (A). The selected field contains a single pRBC among tens of non-infected cells, showing the fluorescence of RBC plasma membranes (red), antibody detection (green), quantum dots (white), and nuclei (blue). For an easier visualization of the colocalization of quantum dots and antibodies only in pRBCs, the fluorescence signals for (E) antibody, (F) nuclei, and (G) quantum dots are shown separately in white color. (H,I) Examples of images used for the quantitative confocal fluorescence microscopy analysis of suspensions of RBCs containing ca. 5% pRBCs, treated (H) with quantum dot-containing immunoliposomes or (I) with quantum dotloaded liposomes not functionalized with antibodies. The fluorescence signals for nuclei and quantum dots are shown separately in white color, where only the originally weak quantum dot signal in panel (I) (see overlay) has been deliberately increased with the imaging software. From Urbán et al. (2011) [2].

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IN-VIVO MOLECULAR IMAGING OF RHEUMATOID ARTHRITIS USING OPTOACOUSTIC TECHNIQUES

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INTRODUCTION

Rheumatoid arthrtis is a systemic inflammatory disorder that predominantly attacks synovial joints. The clinical diagnosis of arthritis can be supported by medical imaging technologies such as Doppler-enhanced ultrasound, X-ray or nuclear magnetic imaging. Current imaging methods either detect late anatomical changes like erosions of cartilage and bone or unspecific signs of inflammation like hypervascularisation and edema. Imaging-based detection of molecular markers that are directly linked to the disease process could allow a better identification of patients at risk for developing severe disease and thus allow a better selection of treatment options. Optoacoustic imaging (OAI) represents a promising alternative to existing techniques [1] combining the benefits of optical methods with those of ultrasound imaging. It is based on the generation of broadband acoustic transients as a result of localized optical absorption of laser pulses. It provides high contrast imaging, high resolution and low scattering. This modality can be used for imaging of the distribution of intrinsic tissue chromophores (i.e. hemoglobin) [2]. If specific antibodies are coupled to suitable photoacoustic contrast agents, it can be used for imaging with molecular specificity. Different types of gold nanoparticles have shown to be particularly suitable as optoacoustic contrast agent since their strong surface plasmon resonance results in remarkable absorption cross sections [3,4,5]. Among them, gold nanorods are best suited since their absorption maximum can easily be tuned by adjusting the synthesis parameters (concentration of educts, temperature). Further, their known surface chemistry allows an uncomplicated conjugation with biological targeting agents. In the context of rheumatoid arthritis, the inflammation is driven by an enhanced release of tumor necrosis factor α (TNF- α). For imaging and identification of inflamation sites, we used gold nanorods coupled to the anti-TNF- α antibodies Infliximab. The suitability of the molecular contrast agents was investigated in a mouse model of arthritis.

MATERIALS AND METHODS

The gold nanorods used as contrast agents were produced by a seed growth method [6, 7]. The synthesis is a two step process including the generation of gold colloids based on the reduction of HAuCl4 and the subsequent anisotropic growth of the colloids by further reduction of ionic gold. The synthesis parameters (concentration of reagents, temperature) were adjusted in order to produce nanorods having an absorption maximum in the range of 1064 nm, so that a Nd:YAG laser could be used for signal generation. For coupling with the antibody, the nanorods were first washed in order to remove synthesis residues. Heterobifunctional PEG molecules were then used for the binding of the antibody Infliximab (Remicade®, Essex-MSD). While control particles were coated with mPEG-SH, the molecular nanoprobes were modified with HS-PEG-NH2 and NHS-PEG-MAL. In another reaction, the antibodies were prepared for coupling to the particles by thiolation with Traut´s reagent (Sigma). The actual coupling of the thiolated Infliximab to the PEGylated nanorods was achieved by incubation at 25°C and 600 rpm over 12h. The optical properties of the gold nanoparticles before and after coupling to the antibodies were analyzed by measuring the absorption spectrum. For generation of optoacoustic signals, light from a nanosecond-pulsed Nd:YAG laser was coupled into a fibre ring allowing confocal illumination and acoustical detection. The signals

were acquired with a homemade focusing transducer with 30 MHz centre frequency and a focusing distance of 5 mm. For amplifying and digitization, a single channel hardware platform (AMI-US/OA, kibero GmbH) with 200 MHz sampling rate was used. A schematic of the experimental set-up is shown in figure 1.

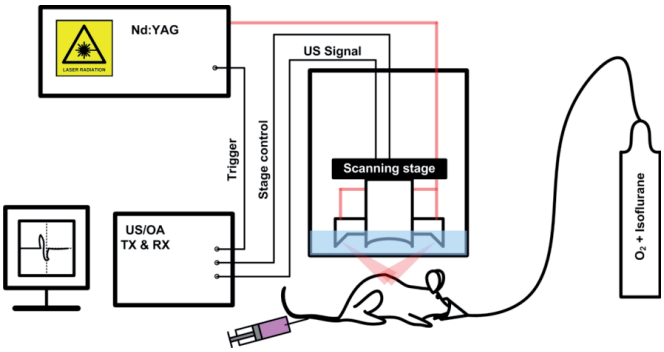

Figure 1: Experimental set-up used for in-vivo optoacoustic molecular imaging including Nd:YAG laser, high frequency transducer and digitizing electronics.

Optoacoustic imaging was performed on 5 mice. The group consisted of 2 healthy control animals and 3 animals affected with collagen induced arthritis (CIA). For measurements, animals were anesthetized with isoflurane and an infrared lamp was used to prevent cooling. The region of interest (ROI) to be investigated was defined as the tissue fraction between the patella and the tibia, since an overexpression of TNF-α could be expected in this area as the result of the CIA. A volume of 200 µl of the nanoprobes (or of the control particles) at a concentration of about 70 nM was administered to the animals by injection in the tail vein and the ROI was imaged prior to the injection, and 1h and 15h after.

RESULTS

Optoacoustic and acoustic C-scan images were obtained by maximum amplitude projection (MAP) of the threedimensional data sets of the ROI. The comparison of the measurements made prior to injection and 1h after the injection in the animals showed an enhancement of the optoacoustic signals after 1h irrespective of the disease status of the animals and independent of the presence of coupled antibodies. The amplitude increase of the signals suggested that nanoparticles are within the blood vessel system. For the demonstration of the specific targeting ability of our nanorod-infliximab nanoprobes, optoacoustic 3d data sets were acquired 15h after the injection. Control experiments were performed by the injection of infliximab- or certolizumab-coupled nanoprobes into healthy animals (M1) and by the administration of pegylated nanorods without antibody coupling to a healthy animal and an animal affected with arthritis (M2, M3). The results of the control experiments (animals M1-M3) revealed a maximum dynamic range of 27-30 dB, corresponding to the signal amplitudes prior to injection with a dynamic range of 25-28 dB. On the other hand, the MAP-images of the ROI of the arthritic animals M4 and M5 injected with Infliximab-coupled nanoprobes showed a maximum dynamic range of 38 dB (Fig. 2). The signals obtained 15h after injection of infliximab-coupled nanorods were enhanced by 10 dB with respect to the pre-injection background measurements.

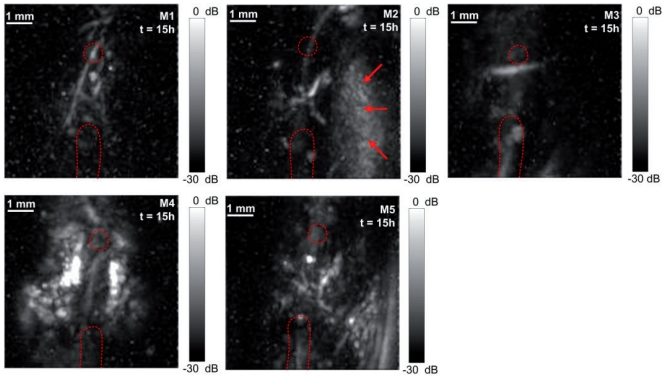

Figure 2: MAP-images of the optoacoustic 3d data obtained after 15h (M1-M3 = control experiments, red arrows show skin artefacts in M2-data)

CONCLUSION

This study shows that the increased expression of TNF-α in arthritic knee joints of mice can be detected with optoacoustic imaging techniques and antibody-coupled gold nanorods as contrast enhancer. The use of heterobifunctional PEG molecules as cross-linkers allows an easy transfer of the approach to other disease models by replacing the used antibody by alternative targeting molecules. In summary, the suitability of a novel technique based on optoacoustic imaging of tissue areas showing a high expression level of TNF-α could be demonstrated in an animal model of arthritis.

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FROM VISION TO REALITY: NANOMEDICINE IN THE ONCOLOGY PRACTICE

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Most of the currently used anti-tumor agents have problematic toxicities compromising efficacy, and often resulting in life-threatening events. Nanomedical devices such as liposomes and other nanoparticles can provide effective control of the release rate and of the tissue distribution of many of these agents. These pharmacokinetic changes often have a major pharmacodynamic impact with attenuation of toxic effects and protection of sensitive tissues from dangerous and unwanted drug exposure. Polyethylene-glycol (PEG) 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 which has been described in a broad variety of experimental tumor types, and appears also to be a relevant phenomenon in human cancer. Nanomedicine can offer a delivery platform for future pharmacologic agents such as oligonucleotides, siRNA, and aptamers protecting them from degradation and nonspecific interactions and facilitating their interaction at the nano-scale level with target cells. However, the main focus of nanomedicine currently relevant to oncology practice remains the controlled delivery of chemotherapeutic agents to improve their therapeutic index. An example of nanaomdicine with demonstrated clinical added value in cancer therapy is pegylated liposomal doxorubicin[1], which has demonstrated a favorable safety profile and

proven efficacy against various common malignancies and can be considered as the first anti-cancer nanomedicine approved for clinical use. Other forms of nanomedicine in clinical use include a cremophor-free albumin-based nanoparticle formulation of paclitaxel[2], and a mutivesicular lipid-based formulation of cytosine arabinoside for intrathecal administration[3]. Based on preclinical and early clincial studies, other formulations such as liposomal vincristine, and pegylated liposomal irinotecan hold promise to offer an important clinical edge in cancer chemotherapy.

Major changes in the pharmacokinetics and biodistribution of cancer chemotherapeutic agents can be obtained using properly engineered nanocarriers as has been shown for liposomes. The ensuing pharmacodynamic changes may result in a substantial improvement of the toxicity profile and in a significant enhancement of the therapeutic index of the delivered drugs. Nanomedicine offers a unique platform for a variety of manipulations that can further enhance the value of the delivered drugs, depending on the drug and the formulation selected. These include enhanced sensitization to ionizing radiation, hyperthermia and ultrasound-induced drug release, enhanced tumoricidal effect of radiofrequency ablation, co-encapsulation of synergistic drugs, and real-time imaging of drug biodistribution using the nanocarrier as a theragnostic platform.

CHALLENGE: THE EXISTING REGULATION ENVIRONMENT IN RELATION TO NANOMEDICINE

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The Health Canada statement will review the current regulatory approach and initiatives with respect to nanotechnology in Canada

TODAY'S PERFORMANCE OF NANODRUGS

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The first nanomedicines "nanopharmaceuticals" already came onto the market more than 20 years ago and first used in clinical trials almost 3 decades ago. Since a steady stream of products have followed. These first generation products were able to meet the evolving general regulatory standards (1).

However, with increasing complexity in nanomedicine structure, use of new materials, new characterization methods and manufacturing processes, and not least, the convergence of science that is bringing together nanopharmaceuticals and nanodevices, there will be pressure to define a new regulatory environment able to bridge the gap in biomedical nanotechnology between medicines and medical devices regulation (1,2).

Appropriate risk assessment and risk/benefit analysis are major issues for Regulatory Agencies in respect of all new medicinal products (3). The development of better nanopharmaceuticals will necessarily benefit from public and private contributions with appropriate funding and looking at strategically important scientific issues and search for new analytical and production tools; but still the most important job will be performed everyday by scientists looking at a better understanding and integration of complex events like whole-body pharmacokinetics and intracellular trafficking or appropriate physico-chemical characterisation of macro and supramolecular architectures within heterogeneous systems.

Some of the most important issues currently discussed in nanomedicine include the question of whether the currently required toxicological studies will be adequate for new nanomaterials, and whether the major differences in biofate and increased complexity of clinical use (integrating different technology subsets from therapeutics to imaging as well as integrated non-invasive diagnosis) are adequately covered. Increased use of nanotechnology in biomedicine brings highly complex problems and critical questions arise looking for the opportunities of using a globally integrated regulatory structure that would promote better (fast and safe) access to these new technologies (4).

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SELF-ASSEMBLED PEPTIDE NANOSTRUCTURES: A NEW FRONTIER IN ORGANIC NANOTECHNOLOGY

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The formation of ordered amyloid fibrils is the hallmark of several diseases of unrelated origin. In spite of its grave clinical consequence, the mechanism of amyloid formation is not fully understood. We have suggested, based on experimental and bioinformatic analysis, that aromatic interactions may provide energetic contribution as well as order and directionality in the molecular-recognition and self-association processes that lead to the formation of these assemblies. This is in line with the well-known central role of aromatic-stacking interactions in self-assembly processes.

Our works on the mechanism of aromatic peptide self-assembly, lead to the discovery that the diphenylalanine recognition motif of the Alzheimer's beta-amyloid polypeptide self-assembles into ordered peptide nanotubes with a remarkable persistence length. Other aromatic homodipeptides could self-assemble in nano-spheres, nano-plates, nano-fibrils and hydrogels with nano-scale order. We demonstrated that the peptide nanostructures have unique chemical, physical and mechanical properties including ultra-rigidity as aromatic polyamides. We also demonstrated the ability to use these peptide nanostructures as casting mold for the fabrication of metallic nanowires and coaxial nano-cables. The application of the nanostructures was demonstrated in various fields including electrochemical biosensors, tissue engineering, and molecular imaging. Finally, we had developed ways for depositing of the peptide nanostructures and their organization. We had use inkjet technology as well as vapour deposition methods to coat surface and from the peptide "nano-forests".

We are currently using this notion, as well as a novel b-breakage strategy that was developed in our laboratory, for the development of novel inhibitors of the process of amyloid formation by utilizing hetero-aromatic interactions. Our lead compound is a novel chemical entity that inhibits the formation of b-amyloid oligomers in vitro and protects cultured cell and isolated cortical neurons from cytotoxic effect of b-amyloid aggregates. Chronic administration of the compound was shown safe and significantly effective in preventing memory impairment in this animal model as assayed by Morris Water Maze experiments.

Taken together, our hypothesis provides a new approach to understand the self-assembly mechanism that governs amyloid formation and indicates possible ways to control this process.

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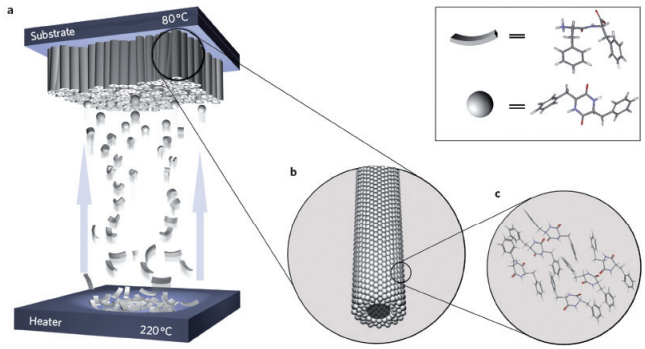

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FOOTNOTE

1 Known commercially as Doxil or Caelyx. 2 Known commercially as Abraxane. 3 Known commercially as Depocyt

THE DISCOVERY OF PLASMODIUM BY ALPHONSE LAVERAN (1880) AND TODAY'S GLOBAL FIGHT AGAINST MALARIA

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What is common between Laveran's discovery of the Plasmodium in 1880 and nanomedicine? Both are dealing with malaria diagnosis eventually. Great hope for better better diagnosis and cure surrounded Laveran's discovery, and this stays true for nanomedicine potential to improve the fight against malaria.

Laveran found the causative agent of malaria while he was serving as a doctor in the Army Medical Service in Algeria (1878–83). He made his discovery in 1880 while doing autopsies of patients that had died of malaria. He noted that these patients had numerous pigmented bodies in their blood. Most of these bodies were in the red blood cells but others were free, at the edge of which he observed moveable filaments or flagella. The extremely fast and diverse movements of these flagella indicated to Laveran that they must be parasites. He found such parasites in 148 out of 192 cases and thus assumed them to be the cause of malaria.

From then, the world has experienced a dramatic reduction of malaria during the first decades of the 20th century thanks to the application of many control measures, namely environmental measures to reduce vector density and man-vector contact (destruction of breeding and nesting sites, better housing, windows, doors, screening and DDT spraying) and mass drug administration. Unfortunately, the hope for malaria eradication vanished in the 60s when mosquitoes became resistant to the insecticides used and Plasmodium parasites to the drugs administered. Then, a change in paradigm occurred and the malaria world decided to switch from the eradication concept to a more realistic one which was the control of malaria and its devastating consequences on the health of the unprivileged populations. New interventions were investigated for their potential to reduce malaria transmission and mortality. At the end of the 90s, insecticides-impregnated nets proved to be a very effective tool and large-scale national programmes of distribution were introduced targeting the most vulnerable groups, namely under five year children and pregnant women. The new century saw another dramatic improvement, which was the progressive change from the old and no more effective drug chloroquine to the new artemesinine-based combinations that came from China and allowed to successfully curing all species of malaria. At the same time, funding for malaria control increased dramatically from 200 millions in 2004 to almost 2 billions in 2009, thanks to the commitment of governments of the industrialized countries through the Global Fund for AIDS Tuberculosis and Malaria, many private-public partnerships and philanthropic organizations such as the Bill and Melinda Gates Foundation. This huge investment and strong commitment from high level politicians and local health authorities allowed scaling up effective interventions such as universal coverage of ITNs, indoor residual spraying, accurate diagnosis and prompt treatment with artemisinine combinations to achieve reduction of the burden of malaria. A huge impact of these measures has been observed with malaria having been halved in more than 15 countries in Africa in the last 10 years. This rapid decline worldwide has led to a change of paradigm from malaria control to potential for malaria elimination, end even ultimately eradication. This will take time and sustainability of considerable investment, even when malaria will no more be a public health problem. In the meantime, new tools are developed such as new diagnostic tests, new drugs and vaccines to circumvent the problem of resistance and access to health goods. The nanomedicine (the medical application of nanotechnology) shows great potential to assist in this endeavor, and even radically revolutionize new and current diagnostic procedures and treatments for infectious diseases of poverty.

INTELLIGENT OPERATION OF THE 3D DENDRITIC SUPRAMOLECULAR ARCHITECTURE CONNECTED TO MOLECULAR MACHINES: IN-VITRO HUMAN CELL STUDY

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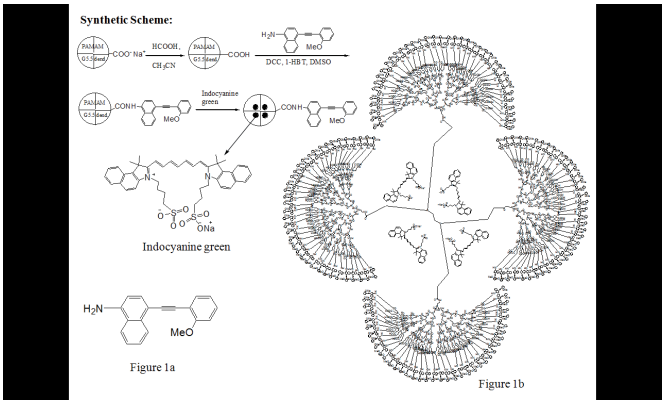
The name nano-brain has been proposed[1] for a well-organized standalone, massively parallel molecular super computer,[2] -conceptualized to perform remotely works by itself without human intervention. The proposed core architecture of a nano-brain consists of a potentially redox-active molecular switch[3] based central control unit (CCU)2 connected to all execution parts i.e. molecular machines/ switches[4] to execute one-to-many communication.[5] Due to radial connection, by tweaking the central molecule one can logically control all radially connected units at a time, that is a simultaneity in operation is added to the system. The complete jaunt multi molecular system is called nano-brain (NB). It is able to send a series of logical instructions to the execution units during its operation, executing series of operations one after another by itself. Spherical shape is preferred to provide feed-forward information processing through 360° orientation, better accessibility of the machines to the external world and sustainability to environmental noise. To create such standalone processor, we use a composition of conjugated and non-conjugated dendritic box (some organic molecular redox switches doped inside the dendrimer cavities) and the derived system is radially connected to molecular machines. It's dynamics generates background noise to the molecular machines during operation. Therefore, we have chosen commercially available non-conjugated PAMAM dendrimers, of different generation from minimum 4 (64 end terminals) to maximum 8 (1024 end terminals), as the basic sphere like architecture with its tree shaped outward branching, mimicking the neural network. The terminal groups have been successfully connected to the functional units i.e. the newly developed molecular machine (see Figure 1a). To construct CCU, redox active multilevel switching molecules like indocyanine green, eosin B, copper-phthalocyanine trimer etc. have been trapped inside the dendritic core cavities. The final machine looks like Figure 1b. The architecture and its different characterisation have been performed using NMR, MALDI-TOF, absorbance/ fluorescence spectroscopy, AFM etc. Currently, we are studying the dynamic feed-forward information processing of NB both in-vivo and in-vitro in different human brain cell lines and computational studies is being performed to find out the operability of nano-brain against the cancer and cell aging.

Figure Caption 1 (a) The molecular machine (b) Two dimensional view of nano-brain developed from molecular machine connected PAMAM dendrimer 5.5 generation, four eosin B molecules encapsulated inside dendritic cavities, three fundamental functional sections are shown with arrows.



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CANCER STEM CELLS AND INFLAMMATION – FRIEND OR FOE?

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The cancer stem cell concept has important implications for understanding carcinogenesis as well as for the development of cancer therapeutics. According to this concept, tumors are initiated and maintained by a cellular subcomponent that displays stem cell properties. These properties include self-renewal which drives tumorigenesis and differentiation, albeit aberrant, which contributes to tumor cellular heterogeneity. In addition to driving tumorigenesis, cancer stem cells may contribute to tumor metastasis as well as to tumor recurrence following treatment. These observations suggest that the development of more effective cancer therapies may require effective targeting of the cancer stem cell population.

We have developed a strategy to target these "breast cancer stem cells" through blockade of the IL-8 receptor CXCR1. We demonstrate that CXCR1 blocking antibody or repertaxin, a small molecule CXCR1 inhibitor, selectively targets the cancer stem cell population. Furthermore, this is followed by the induction of massive apoptosis in the bulk tumor population via FAS-Ligand/FAS signalling. CXCR1 effects on cancer stem cell viability as well as FAS-ligand production are mediated by the FAK/AKT/FOXO3A pathway. Furthermore repartaxin is able to specifically target the cancer stem cell population in breast cancer xenografts retarding tumor growth and reducing metastasis. CXCR1 blockade may provide a novel means of eliminating breast cancer stem cells. Because these cells may drive tumor progression and metastasis such strategies may lead to improving outcomes for women with advanced breast cancer.

Constant refinement of the cancer cell hierarchy underlines the importance to develop new targeted therapies that will specifically eradicate the cancer stem cell population. Nanomedicine will have to play a major role in the development of Targeted-NBS (Nanotechnology Based Systems) that will allow the selective deliver of effective non-classical therapies against CSC, blocking specific molecular pathways involved in the maintenance of cancer cell "stemness".

MODULATION OF THE INNATE IMMUNITY BY NANOPARTICLES FOR RESTENOSIS THERAPY

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Intimal hyperplasia is a universal response of the arterial wall to mechanical injury and it is a major cause of restenosis following angioplasty. Experimental and clinical data indicate that the innate immunity and inflammation are of major importance in the pathophysiology of restenosis. We validated the hypothesis that systemic and transient depletion of circulating monocytes inhibits the inflammatory cascade. Monocytes/macrophage depletion was achieved with a systemic injection of nanoparticulated dosage forms (PLGA-based NP and liposomes) containing bisphosphonates (BP), which were formulated for effective phagocytosis. Following phagocytosis the vesicles discharge their encapsulated drug like a Trojan Horses, inactivating the cell with no effect on non-phagocytic cells. We investigated the effect of different BP, NP type (polymeric or liposomal), and size on the formulation properties, biodistribution, and monocytes sub-populations. Bioactivity and mechanism was examined in tissue cultures, and in animal models of restenosis, MI and endometriosis. Partial and transient depletion of blood monocytes following NP systemic injection correlated with the therapeutic effect. Phase I and phase II clinical studies, in stented patients (one IV injection at the time of angioplasty), confirmed the safety and efficacy of the liposomal delivery system in preventing restenosis.

UNSOLVED PROBLEMS IN IMPLANTATION

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Orthopaedic related conditions are a major cause of pain and disability with significant cost on health and social services in developed countries [1]. This scenario is further stressed in view of the rapid aging of population. In Europe, population over 60 will surpass that under 20 in this decade. In USA, population over 65 will double by 2030 [2]. Joint disorders affect half of the population above 65, and functional impairment is present in 25%. These disorders, basically degenerative and inflammatory joint diseases, are efficiently treated today by joint arthroplasties based on prosthetic implantation. Data from hip and knee arthroplasty national and European registries confirm the success of these techniques. Over 85% of these implants may survive over 15 years. However, the maintained failure rate related to infection encourages the search for more infection-resistant materials in the implant surface. The increasing aseptic loosening related to osteolysis and mechanical problems in the long-term also fosters research to improve fixation over time, particularly in osteoporotic bone. Besides, the generation of bone defects surrounding the implants promotes the increasing exploration of orthobiologics to reconstruct the supporting bone surrounding prosthetic implants.

Therefore, despite significant advances that permit the development and social benefit of today's implant surgery in Orthopaedics, persistent failure rate is a niche for innovation and improvement that will benefit more demanding patients with functional requirements at younger and older ages.

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DIAGNOSTICS OF INFECTION BY NANOMECHANICAL ARRAY-TECHNOLOGY

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Nanomechanical sensing platforms for label-free qualitative and quantitative bio-analytical measurements provide high-sensitivity, fast and specific bio-assay results. Silicon based cantilever array sensor platforms functionalized with native biomolecules present an excellent tool for genomic, proteomic and micro-organism applications.

The latest findings applying membrane protein functionalized sensors for future viral diagnostic applications will be presented. We show an approach for fast microbial antibiotics resistance testing and discuss its potential for up-scaling and routine use.

CLEANING BLOOD: APPLICATIONS OF ULTRASTRONG METAL NANOMAGNETS IN NANOMEDICINE

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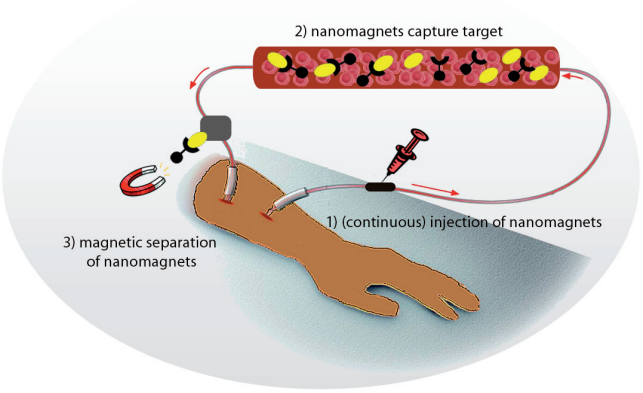
Nanomagnets with metal core have recently been shown to be promising candidates for magnetic drug delivery and hyperthermia due to superior magnetic properties compared to commonly used metal oxide beads. This presentation will discuss the direct removal of harmful substances from human whole blood by the use of functionalized magnetic nanoparticles. As successful application strongly relies on a safe implementation, a particular focus is put on possible interactions of nanomagnets with the vascular compartment. The presentation will also discuss the implementation of the technology into an extracorporeal blood purification device (ex vivo) and further steps in the direction of a clinical application of the concept.

METHODS

Carbon coated metallic nanoparticles were equipped with various functional groups, including heavy metal scavangers, fab-fragments and antibodies. Carbon coated metal nanomagnets were added to fresh human whole blood where they scanned the liquid volume and captured the target compounds (Figure 1). After removal of the toxin-loaded nanomagnets by magnetic separation, the blood was analyzed for remaining toxin or inflammatory mediators, iron metabolism and blood integrity.



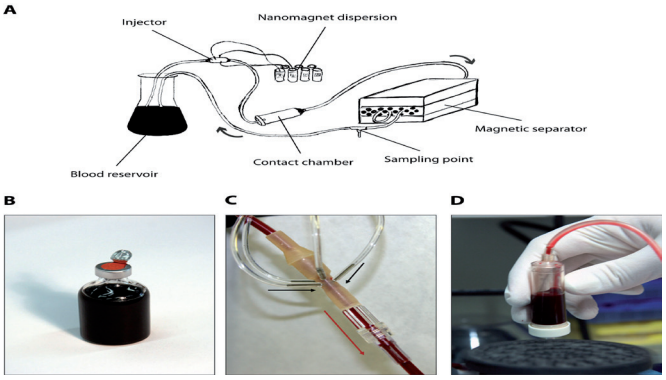


Figure 1: Extracorporeal magnetic-separation based blood purification device (top) and experimental setting (bottom). Pre-dispersed nanomagnets are injected into the blood stream. After passing through the line, the toxin-loaded nanomagnets are then recollected by magnetic separation before the purified blood is recirculated.

RESULTS

The applicability of the concept is demonstrated utilizing three examples: The removal of a heavy metal (lead), a steroid drug (digoxin) and a whole protein (Interleukin-6) was achieved by spiking human whole blood with the contaminant and applying appropriately functionalized magnetic beads for the detoxification. The contaminant concentration in intoxicated whole blood could be significantly decreased in a dose-dependent manner using a magnetic separation-based blood purification technology (Figure 2). The integrity of the blood was not affected by the process as depicted by monitoring a series of clinically important parameters.

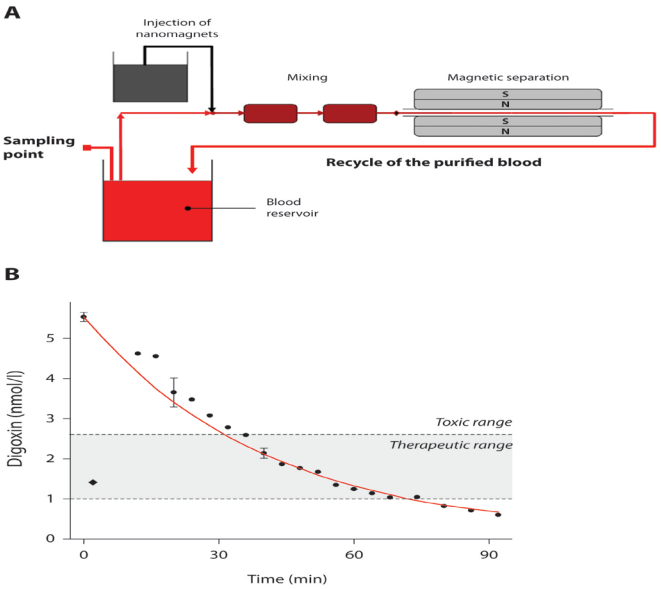


Figure 2: Digoxin levels in a 500mL blood volume can be decreased from an initially toxic down to therapeutically relevant concentrations by magnetic separation-based blood purification.

CONCLUSIONS

Noxious compounds differing in chemical nature (ions, small molecule drugs, and proteins) can be efficiently and selectively removed from whole blood without being limited by filter cut-offs or slow pore diffusion. Combined with existing therapies, these results may have major implications for the treatment of severe intoxications (digoxin, barbiturates), sepsis (specific filtering of cytokines or toxins), metabolic disorders or auto-immune diseases.

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CHALLENGES IN MANUFACTURING AND APPLICATION OF NANOPARTICLES AS DIAGNOSTIC TOOL

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The interaction of nanoparticles with cells is still a challenging research field. Recent results show, that beside size, surface charge, coating, adsorbed proteins the behavior of particles can also be influenced by specially derivatized peptides and proteins. Finally a nanoparticle in a biological application is a very complex system and still not all interactions are known. To investigate such complex systems in vitro and in-vivo is the subject of lot of research projects carried out during the last year. To control the behavior of nanoparticles in complex biological systems, the particles needs sophisticated coatings and very often additional functionalisation with biomolecules like peptides, antibodies, plasmids etc. To carry out such coating and functionalisation in a reproducible way, the synthesis and coating processes need also a further development.

This paper will give a short overview regarding the activity in the field of superparamagnetic iron oxide nanoparticles (SPION) used as contrast agent, for hyperthermia, molecular imaging and protein and cell separation. The most important results and conclusions will be presented, but even more important the authors will try to formulate the most important open questions which need to be answered regarding synthesis, characterization and in-vitro and in-vivo behavior in view of a clinical application of such particles.

WITH NANOMEDICINE TOWARDS PERSONALISED MEDICINE – NOW AND FUTURE

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Prof. Dr. med., Deputy Head of the Intensive Care Clinic of the University of Basel and President, of the European Society for Nanomedicine, Basel (CH)

The current practice of medicine is characterized by a ‘one-size-fits-all’ approach, the search for “the single best” drug for a class of diseases: “the best antihypertensive drug”, “the best heart failure drug combination”, “the best antithrombotic drug for acute coronary syndromes” and so on. This approach has been dictated by a number of reasons: First, the development of so-called “evidence-based medicine”, often understood as a medicine where

The one-size-fits-all approach to medicine has important downsides: to produce ‘megatrial’ evidence, only frequent diseases and multicenter studies with broad inclusion criteria are suited – the resulting trial heterogeneity leads to statistical dilution of treatment effects which may reduce the chance to achieve overall statistical significance for the all-important primary endpoint even when subgroups might profit. Treatment duration is usually standardized in trials and therefore finds its way in clinical treatment guidelines; as treatment benefit in drug therapy often is largest early on in the treatment, but incidence side effects and cost are typically linear with time, leading to overtreatment and increased costs in certain subgroups who would not need long treatment periods.

Nanomedicine brings two key aspects to this topic: Tools for individualized analyses and thus better understanding of the all-important interaction of a specific disease with a specific individual, and therapeutic approaches which allow, in principle, tailoring therapies in a more personalized manner than the “one-size-fits-all” approach. Important questions, however, are open to debate: How to link “individualized diagnosis” with “individualized therapy” in practice ?

How to develop, assess, regulate, and monitor "personalized" nanodrugs based on "personalized" diagnosis ? Which trial designs are suited for clinical evaluation; what industries are needed to put such approaches into clinical practice ? What does this imply for medical education ?

The "personalized medicine" approach will require new thinking from all partners: care givers, industry, regulators, and will also require significant efforts to inform patients and families alike.

ARTERIOSCLEROSIS – A LIFE-THREATENING INFLAMMATORY DISEASE

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Atherosclerosis, once considered a degenerative disease of the elderly, has in the past two decades increasingly recognized as a disease where genetic, environmental and nutritional factors interact with the immune system in various ways. The immune system is not only a key player in the silent progression of vascular plaques, it is also, using different biological pathways, responsible for sudden complications of atherosclerosis like rupture of 'vulnerable' plaques; furthermore, inflammatory reactions play an eminent role in the complications of current atherosclerosis therapies like stent restenosis. Enhanced understanding of the role of the immune system and progression of atherosclerosis is key for the development of new diagnostic and therapeutic approaches to this most lethal disease of the western world.

BOTTOM-UP APPROACH TO INTELLIGENT NANOSYSTEMS

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A key feature of body defenses including the immune system, the coagulation system, and many other biologic systems is the observation that they are intelligent, locally and systemically regulated, reactive systems, rendering them capable of protecting from injury and repairing organs in a targeted manner. This concept is very different from the current approach to drug therapy, usually consisting of simple small molecules, systemically applied, not regulated, and not particularly more active in a diseased organ than in any other location in the body. This presentation looks at bottom-up approaches to therapy using nanoscale building blocks that act in an intelligent manner. The increasing complexity of bottom-up systems and the decreasing size of top-down nanosystems will lead to their unification in the future.

SMART NANOSTRUCTURED ACTIVE IMPLANTS FOR TISSUE ENGINEERING APPLICATIONS

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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 layer-by-layer build-up of multilayered films (PEM films) from oppositely charged polyelectrolytes1 offers new opportunities for the preparation of functionalized biomaterial coatings. This technique allows the preparation of supramolecular nanoarchitectures 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 polyelectrolytes or Cyclodextrins (CDs), adsorbed or embedded in PEM films, have been shown to retain their biological activities2-12. Recently, we have demonstrated for the first time the sequential induction of nuclear and /or cytoplasmic expression products, mediated by β-cyclodextrin embedded in a multilayered films7.

We present here that embedded BMP-2 and TGFβ1 in a multilayered nanostrutured film can drive stem cells to the cartilage or bone 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 multilayered films on a planar surface or as a nanostructured capsules for bone induction in vivo 8,12 .

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NANOTHERM® THERAPY

Andreas Jordan

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NanoTherm® therapy (thermotherapy using magnetic nanoparticles) is a new approach for the local treatment of solid tumors and one of the first clinical applications of nanotechnology in cancer therapy. Principle of the method is the direct injection of a dispersion of magnetic nanoparticles (NanoTherm®) into a tumor and their subsequent activation by a 100 kHz alternating magnetic field (NanoActivator™) 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.

In a clinical trial, 59 patients with recurrences of glioblastoma multiforme, the most common and most aggressive type of primary brain tumors in humans, received local thermotherapy combined with fractionated stereotactic radiotherapy with a median dose of 30 Gy in a median fractionation of 5x2 Gy/week.

Median overall survival after diagnosis of first tumor recurrence was 13.4 months (95% CI: 10.6–16.2 months) and 23.2 months (95% CI 17.2 – 29.2 months) after primary tumor diagnosis compared to 6.2 and 13.4 resp. of a historical control. Due to this positive outcome, MagForce received European regulatory approval for its medical products NanoTherm and NanoActivator for the treatment of brain tumors.

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, hyperthermia can be combined with chemotherapy. Additionally, in order to develop multifunctional nanocarriers with multiple therapeutic applications, MagForce is also exploring the use of targeting ligands with tumor localizing properties along with stealth coatings for systemic administration.

NANO-CARDIOLOGY FOR ATHEROSCLEROSIS TREATMENT

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Nanocardiology entails nanoscale tools and materials for the effective treatment of cardiovascular disease. This is necessitated by the fact that the pathogenesis of atherosclerosis happens at the molecular and even atomic level. It involves a complex series of events, with the first step of the endothelium dysfunction that ends to the formation of plaques and thrombotic events that may cause the arterial obstruction. Until now, the vascular stents are the landmarks of reopening of stenotic arteries in clinical practice. The most commonly used drug eluting stents (DES) have as drawbacks the late stent thrombosis and the in- stent restenosis caused mainly by the delayed endothelialisation owing to the polymers or the drug release from DES. Thus, there is a need for new class of non-polymeric biomaterials that are thrombo-protective and promote endothelialisation.

MATERIALS & METHODS

Herein, novel nanostructured biomaterials made of carbon based (amorphous hydrogenated films- a:C-H films and carbon nanotubes-CNTs) and titanium boron nitride (TiBN) films were grown by vacuum deposition techniques and their surface properties were characterized and examined in terms of platelets response. Platelet adhesion studies were made via Atomic Force Microscopy (AFM), providing detailful imaging at nanoscale.

An enhanced endothelialisation of the a:C-H nanocoatings was achieved by the immobilization of peptides that trigger endothelial cell (EC) attachment and proliferation. The surface morphology and the real-time investigation of the binding of the EC selective peptides onto the carbon nanocoatings were investigated by non-destructive characterization techniques involving: i) AFM, ii) Real-time Spectroscopic Ellipsometry (SE) in the Vis-UV energy region, applied both in air and in liquid environment with ultra fast measurements, and iii) Fourier Transform IR Phase Modulated SE (FTIRSE) (900-3500 cm). SEM experiments were also carried out to visualize the Umbilical Vein Endothelial Cells isolated from normal human umbilical vein, attached onto the samples.

RESULTS

The AFM data indicate that nanotopographic modifications of surfaces and stoichiometry, as controlled by fabrication variables, can elicit desired interfacial platelet response. An effective biofunctionalization process was achieved as presented by the peptides tethering onto the carbon nanomaterials. SEM studies shown enhanced endothelialisation onto the bioactive a:C-H coatings in comparison with the bare ones. In general, the results were discussed in correlation with the nanotechnology strategies for advanced nanomaterials to be used as stent coatings.

WHAT CHARACTERIZES A NANODRUG?

Costas Kiparissides and Olga Kammona

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Most front-line drugs are untargeted, toxic compounds that act in a nonspecific fashion, often eliciting unwanted, dose-limiting and sometimes debilitating side effects. The ability to use nanotechnology to alter the characteristics of a drug, to increase solubility, decrease degradation during circulation, and concentrate the drug at the desired site of action promises to increase efficacy while decreasing unwanted side effects. The development of an effective, highly selective, nanocarrier-based therapeutic modality (i.e., nanodrug) has thus the potential to radically improve disease outcomes.

In general, nanodrugs comprise biologically active molecules (e.g., small molecules, macromolecular therapeutics), encapsulating material (e.g., polymers, dendrimers, lipids), surface coating and targeting sequences. The controlled release of drugs from a nanocarrier is achieved by the release of the encapsulated ingredient via surface or bulk erosion and diffusion, or it is triggered by the external environment, such as changes in pH, light and temperature. In order to increase circulation time, nanoparticles are usually surface functionalized with poly(ethylene glycol) (PEG) and polysaccharides. Aiming to reduce toxic effects associated with non-targeted delivery, targeting molecules, such as antibodies and peptides that recognize specific cell surface proteins and receptors are conjugated to the nanoparticle surface to target specific cell types. Aptamers, a class of DNA- or RNA-based ligands, can be also used as targeting moieties (e.g., aptamer-nanoparticle conjugations to target prostate cancer cells) helping to overcome some of the limitations associated with antibody- and peptide-based drug delivery like batch-to-batch variations and potential immunogenicity.

Nanodrugs exhibit various advantages like, for example, the large number of potential combinatorial variations that can be developed by selecting different nanoparticle materials, payload molecules and targeting ligands, the fact that they can travel through the blood stream without sedimentation or blockage of the microvasculature, circulate in the body and penetrate tissues such as tumors and be

taken up by the cells via endocytosis. Future generations of nanodrugs promise not only to deliver their payload to the desired sites within the body, but to do so in a temporally regulated manner. For example, nanoparticles have recently been generated that can be used to sequentially deliver drugs to cancer cells so that each drug is delivered at the proper time to induce cell death as well as to prevent angiogenesis.

However, the fact that only a few nanodrug formulations (e.g., Doxil, Abraxane) have reached the market indicates that the successful transfer of the lab research results to the clinic is very difficult. Apart from the small size, nanodrugs have on one hand to fulfil specific physicochemical criteria like biocompatibility, biodegradation, colloidal stability, protection of active therapeutic, controlled release kinetics, improved pharmacokinetics, toxicity issues, etc. in order to meet Food and Drug Administration (FDA) requirements and on the other hand to be manufactured in a simple scalable manner, with relatively low cost.

THE USE OF NANOTECHNOLOGY IN NEURODEGENERATIVE DISEASES: A CASE STUDY ON PARKINSON'S DISEASE

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Carbon nanotubes possess properties that make them attractive materials for biomedical applications. One of the key advantages that carbon nanotubes (CNT) offer for biomedical applications is their facile cellular internalisation that has allowed their proposition as novel delivery vehicles for molecules relevant to therapeutic and diagnostic applications. Moreover, exploitation of their unique electrical, thermal and spectroscopic properties in a biological context has been shown to offer further advances in detection, monitoring and therapy of disease. Both therapeutic and diagnostic applications using CNT are intensively explored by many laboratories – academic and industrial – around the world. A critical factor in the use of novel nanomaterials such as carbon nanotubes is the exemplification of efficacy advantages achieved using specific therapeutic models. In this Statement, the capacity of chemically functionalised CNT (f-CNT) as a prospective gene delivery vehicle for the neurodegenerated brain tissue will be shown based on our hypothesis that amine f-CNT will be able to complex siRNA and transport it effectively through the plasma membrane in neuronal tissue of the subthalamic nucleus (STN) to suppress pathological neuronal over-activity.

CLINICAL IMPACT OF ALBUMIN ON DRUG DELIVERY

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Over the past decade albumin has emerged has emerged as a versatile carrier for therapeutic and diagnostic agents, primarily for diagnosing and treating diabetes, cancer, rheumatoid arthritis and viral diseases. In this short overview, I will focus on the clinical developments of:

I. Fatty acid derivatives of polypeptides as albumin-binding adducts for treating diabetes
II. Albumin as a carrier for antibody fragments for treating cancer and rheumatoid arthritis
III. Maleimide derivatives and nanoparticles of anticancer drugs in oncology

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FUNCTIONALIZED GOLD NANOPARTICLES AND THE BLOOD BRAIN BARRIER

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One of the major obstacle for an effective treatment but also diagnosis of neurodegenerative diseases as well as cerebral tumors is the blood brain barrier. Recently it was that in dependence of the size gold nanoparticles stabilized by citrate entered the brain1,2. The mechanism as well as the distribution within the brain structure remains still unknown.

In the present work we will show with different imaging techniques as well as in vitro primary cell culture that polyelectrolyte multilayer coated nanogold (15 nm) functionalized with different fluorophores were able to cross the blood brain barrier and distribute in the brain with a specific pattern. We start with *in vivo* tracking by near infrared time domain (NIR-TD) imaging, then *ex vivo* x-ray tomography, confocal fluorescence microscopy and standard cell stains. The resolution goes from 0.5 mm in the whole body to 300-400 nm in confocal microscopy. Additionally we will study in *in vitro* primary cell culture the up-take mechanism.

We observed that 19 h after injection the nanoparticles accumulate mainly in the hippocampus, thalamus, hypothalamus, and cortex.[3] The signal intensity in fluorescence microscopy and its distribution indicates an endocytotic uptake of non-negligible amounts of the albumin- or apolipoE-coated poly-styrenesulfonate (PSS)/poly-allylamine (PAH). *In vitro* studies with prion proteins showed that this particle composition was most effective in prion protein aggregation inhibition.[4] Albumin was required to protect the endothelium of the brain from a potential toxicity of PAH[5] and to trigger the passage through the blood brain barrier. Basing on these results we will propose a possible uptake mechanism for nanoparticles.

With different microscopic techniques we showed that the nanoparticles accumulate in brain regions close to diseased areas in prion, Alzheimer's and Parkinson's disease.

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NANOSCIENCE AND NANOTECHNOLOGY IN THE 21ST CENTURY - THE ENGINEERING OF NANOSTRUCTURES WITH APPLICATIONS FROM CIVIL ENGINEERING TO MEDICAL SCIENCE

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As the 21st Century unfolds, chemistry-based, bottom-up approaches to the creation of materials with exactly specified atomic and molecular infrastructures have become more-and-more feasible and the top-down approach, which has served us so well, appears to be approaching some fundamental limits. The aim has been to access the promise that such new approaches hold for the formation of new materials with advanced function eg highly improved tensile strengths and/or novel electronic and magnetic properties, intelligent nanoscale machines for medical applications etc. As these new approaches to research in materials science have shifted more-and-more towards this more intrinsic chemical perspective, the field has acquired a new name, Nanoscience & Nanotechnology (N&N). It is of course not new as biology has done it this way since the first organisms appeared as all living systems are created atom-by-atom, molecule-by-molecule on the basis of a chemically coded recipe stored in DNA.

At the same time that these new approaches were developing, a totally unexpected new form of pure carbon, a family of cage molecules with fascinating properties was discovered - the Fullerenes (Buckyballs) together with their elongated cousins the Nanotube (or Buckytube). These nanoscale molecules and related structures have properties that should be able to fulfill some of the exciting promise of 21st Century Materials Science and Technology. The structures, which have also become the iconic images of N&N, are now the subject of intense study as they promise to play key roles in almost every possible area of future technology, from medicine and molecular electronics to civil engineering. In addition to carbon-based structures, the nanoscale behaviour of numerous other related materials is being explored with similarly exciting prospects. Ingenious strategies for the creation of molecules with complex, exactly-specified, structures and function are being developed – basically molecules that "do something" are now being made. As an example the drug industry has so far focused on the production of "relatively" simple molecules with exactly specified rigid structures but a plethora of complex molecules from electric motor enzymes and pulsing molecular machines abound within the body. These molecules are now highlighting incredibly exciting new directions for molecular biological research and challenging new perspectives for pharmaceutical research and development.

In fact, the cross-disciplinary field of N&N has resulted from a deep understanding and expert application of the fundamental chemical principles that underlie condensed matter physics, molecular biology and materials engineering. Indeed N&N may be considered "Frontier Chemistry of the 21st Century". However, the mechanisms whereby various types of nanostructures assemble are still poorly understood. Over the last decade or so, we have examined a wide range of methods for nanostructure formation and from these studies important new insights have been gained. We have focused particular attention on the factors governing the creation of materials with intrinsically 1- and 2-D nanoscale infrastructures. Although some improvement in materials behaviour has already been achieved, it is unlikely that the technological paradigm shifts that N&N promise will be forthcoming until key fundamental mechanistic issues can be resolved and we have significantly improved fine control over bottom-up (chemical) self-assembly.

REALIZING THE CLINICAL POTENTIAL OF TUMOR-TARGETED NANOMEDICINES THROUGH RATIONALLY DESIGNED COMBINATION REGIMENS

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Nanomedicines are submicron-sized carrier materials designed for improving the biodistribution of systemically administered (chemo-) therapeutic agents. Many different types of nanomedicines have been evaluated over the years, including e.g. liposomes, polymers and micelles. In animal models, by increasing drug accumulation at the pathological site and by decreasing its localization to healthy non-target tissues, nanomedicines generally improve both the efficacy and the toxicity of systemic chemotherapeutic interventions. In patients, however, they are often only able to attenuate the toxicity of the conjugated or entrapped chemotherapeutic drug, and they generally fail to improve the efficacy of the intervention. To overcome this shortcoming, and to broaden the clinical applicability of tumor-targeted nanomedicines, we have in the past 5 years developed several concepts for improving the efficacy of combined modality anticancer therapy. To this end, various different types of polymeric nanomedicines were synthesized, and they were shown to be highly useful for enhancing the efficacy of radiochemotherapy and of chemotherapy combinations. Based on these insights, and on the fact that the concepts proposed are considered to be broadly applicable, we conclude that long-circulating and passively tumor-targeted nanomedicines might hold significant potential for implementation in multimodal anticancer therapy.

NANOMEDICINES FOR THE THERAPY OF INFLAMMATORY BOWEL DISEASE

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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 reduction of drug carrier size to nanoparticles ~200 nm may help to minimize the diarrhoea effect and to increase nanocarrier accumulation in the inflamed tissue: Size-dependent accumulation of nanoparticles at inflamed areas of the colonic mucosa in rats was previously demonstrated by Lamprecht et al. [1]. Moreover, polylactide-co-glycolide (PLGA) nanoparticles loaded with the anti-inflammatory drug rolipram showed higher and prolonged anti-inflammatory activity and reduced central nervous adverse effects in a TNBS rat model [2]. The mechanisms of this enhanced permeability and retention ("EPR-effect") at the inflamed colonic mucosa is presently not yet understood. However, an increased permeability of the inflamed epithelium and uptake into invading intestinal macrophages are likely to play an important role.

Further investigating the size dependent accumulation, a novel 3D cell culture model of the inflamed intestinal mucosa based on the co-culture of the intestinal epithelial Caco-2 cells with blood derived macrophages and dendritic cells has been developed [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 compared in the novel in vitro model for their 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. A liposomal budesonide formulation further reduced the barrier function and increased IL-8 release, likely due to unspecific cytotoxic effects.

In parallel to the in vitro investigations, the possibility to target inflamed mucosal tissues by particulate carriers is being further validated in vivo in human IBD patients using confocal laser endoscopy to detect covalently fluorescence labelled PLGA nano- and microspheres [5, 6]. Here, an accumulation of microcarriers but not nanocarriers in the rectal mucosa of the patients could be observed which was correlated to the endoscopic activity score.

Budesonide loaded PLGA nanoparticles are now being tested in vivo in chronic and acute mouse models of colitis and adapted to GMP compliant preparation and scale-up within the context of the EuroNanoMed project 'BiBa'. In addition, the passive targeting of inflamed intestinal areas via the oral or rectal route is being investigated for biological such as IL-10 the next generation of IBD drugs.

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PATHOGEN-LIKE SYNTHETIC NANOMEDICINES AS THERAPEUTIC VACCINES: CHARACTERIZATION THEIR STRUCTURE, BIOLOGICAL ACTIVITY, IMMUNOGENICITY AND CLINICAL EFFECTS IN HIV-INFECTED PEOPLE

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There is great interest in developing nanomedicines through the complexation of DNA and RNA with synthetic polyelectrolytes to obtain effective gene expression and optimize their delivery. We have characterized the structure, stability and intracellular mode-of-action of the DermaVir nanomedicine that is under clinical development for the treatment of HIV/AIDS. Atomic-force-microscopy reveals that these polyplexes are pathogen-like nanoparticles (NPs) having a size and shape resembling spherical viruses that naturally evolved to deliver nucleic acids to the cells so this mode of gene delivery resembles viral infection. Simple optical-absorption measurements provide a useful determination of the NP structural stability, as well as biological activity relevant to their ability to escape from the endosome and release the DNA at the nucleus. Salt, pH and temperature influence the nanomedicine shelf-life and intracellular stability. This approach should facilitate the development of diverse polyplex nanomedicines where the delivered DNA-expressed antigens induce immune responses against chronic diseases.

The novel mechanism of action of DermaVir has been consistently demonstrated in mice, rabbits, primates and human subjects: NPs are naturally transported by epidermal Langerhans cells to the lymph nodes to express the pDNA-encoded HIV antigens and induce precursor/memory T cells with high proliferation capacity. These T cells transiently suppressed virus replication in chronically infected macaques leading to improvement of median survival time from 18 to 38 weeks compared to no treatment.

The primate experiments provided the rationale to investigate repeated DermaVir immunizations prior to initiation of ART in HIV-infected individuals. As DermaVir immunizations in combination with ART did not show any product- or administration-related adverse (AE) effects higher than grade 2, we developed a Phase II protocol to evaluate the safety and to test the immunogenicity and antiviral efficacy of repeated DermaVir immunizations. Thirty-six HIV-infected adults (CD4 > 400 mm3 and HIV RNA 5,000 to 150,000copies/mL) were randomized to receive one of three DermaVir doses (0.2, 0.4 or 0.8 mg pDNA) or placebo at Weeks 0, 6, 12, and 18. The primary endpoint of the trial was safety at Week 24 and secondary endpoints were HIV-RNA and immunogenicity. No subject stopped vaccinations due to an AE and only one subject initiated ART. Only one Grade 2 AE occurred in the 0.2 mg DermaVir group judged to be possibly related to treatment. Based on secondary analyses the 0.4 mg DermaVir dose was superior to the others. In this group, the HIV-specific memory/precursor T cells measured by PHPC increased from 5,055 to 9,978 cells/million PBMC (P=0.07) and the median log10 HIV-RNA decreased from 4.5 to 4.0, significantly different from the placebo (P=0.045). Viral load suppression by DermaVir vaccinations occurs slowly, as predicted by its mechanism of action, similarly to cancer vaccines. Consistent with the primate results, four DermaVir immunizations did not suppress viral load to an undetectable level and did not increase CD4+ T cell counts within 24 weeks. These results suggested that repeated DermaVir immunization boosted HIV-specific precursor/memory T cells and the improved immunity contributed to the preservation of the health of HIV-infected individuals.

These results demonstrate the pathogen-like mode-of-action of pDNA/PEIm nanomedicines and provide the human proof of concept for using these nanomedicines for the development of Langerhans cell-targeting therapeutic vaccines against chronic infectious diseases.

ETHICS OF LONG-TERM DEVELOPMENTS AND SUSTAINABILITY IN NANOMEDICINE

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When talking about Ethics in Nanomedicine the questions about the risks and benefits of many future medical applications, about the patient as a human being and about the view of researchers and about the society and last but not least about the purposes of Nanomedicine are widely discussed. Nanomedicine is seen as a novel technology and the reflexions regarding such technologies must be issue of careful analysis of ethical aspects in view of existing standards and regulations.

This speech addresses the field of the development of Nanomedicine to the benefit of mankind under an ethical behaviour and shows, why contrary to ethical reflexion about research in a humanistic way we have hardly room in a market economy that seeks for benefit on short term only. The development of Nanomedicine is a long term return on investment project alike practically all projects that you can find in the United Nations list of the biggest human challenges in the next 20 years.

We live in a globally economical system that is dominated by the need of ever growing pecuniary return on investment and the domination of the "shareholder value". Therefore novel projects and technologies undergo the thorough examination whether they bring back the investment with reasonable dispatch. The time is the decision factor that leaves space for further developing or narrows the possibilities for the long term project. This statement elucidates Nanomedicine as a project of long term return on investment and the question how the huge necessary investments can be generated and who will be willing to further the development without expecting an immediate return ob on investment?

In a highly competitive global market that understands the term innovation only as the motor of growth the only proof of investment is the scientific and application result. Once that Nanomedicine will be widely accepted there will be funding for it. Those who now engage for Nanomedicine are pioneers with a moral attitude and the hope to succeed. What is sustainable innovation and why is Nanomedicine more than Innovation thus rather Sustenation"? The statement includes the paradigm of CLINAM as a network to further Nanomedicine Development as a "long return on investment endeavour".

DEVELOPMENT OF LIPID NANOPARTICLE SIRNA-BASED DRUGS

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Using our modular lipid nanoparticle (LNP) siRNA delivery platform, Tekmira has confirmed RNAi mediated efficacy in several preclinical models of oncology, infectious and metabolic disease. Several LNP-based product candidates are now in development and by the end of 2011 we expect there to be five SNALP based products in clinical trials. Ongoing formulation development efforts, informed by this clinical experience, continue to produce SNALP formulations with substantially increased potency and reduced toxicity. While lipid nanoparticles are generally considered to be non-immunogenic, their *in vivo* efficacy and safety can be severely compromised due to the inherent immunostimulatory properties of their nucleic acid payloads. While the use of chemically modified siRNA with minimal immunostimulatory capacity has allowed for a more accurate delineation of the mechanism of action of siRNA based therapeutics, an essential precursor to the development of safe and effective siRNA-based drugs, recent clinical experience has suggested that even the most comprehensive battery of non-clinical testing may fail to reveal the true immune stimulatory potential of a given siRNA. Here we report new assay methodology that more completely reflects the clinical response to siRNA drugs, allowing for the elucidation of previously cryptic immune stimulatory activity and redesign of siRNA drug substances that are immune silent. Approaches towards the integration of these improvements into new and ongoing product development efforts will be discussed.

MEASURING INFLAMMATION IN ATHEROSCLEROTIC PLAQUES USING PARAMAGNETIC NANOPARTICLES AND MRI

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Despite the therapeutic advances over the last 20 years, cardiovascular disease is still the leading cause of death worldwide. This is mainly related to the increasing prevalence of atherosclerosis, owing to the ageing population, but above all, to the widespread underrecognition and undertreatment of individuals with risk factors for atherosclerosis.

The progression of atherosclerotic plaques involves the accumulation of cholesterol in the arterial wall, inflammation, leukocyte recruitment, and development of fibrotic lesions. Monocyte accumulation in the intima characterizes fatty streaks, the earliest visible lesion of human and experimental atherosclerosis. In the intima, monocytes differentiate into macrophages, ingest modified lipoproteins via scavenger receptors, and secrete inflammatory mediators that can stimulate smooth muscle cell migration and proliferation. Lipid-rich macrophages, i.e., foam cells, become key constituents of the plaque's lipid core. The dynamics of macrophage accumulation in atherosclerotic lesions is an important factor in the plaque progression and its evaluation in vivo can be of great value in the assessment of therapeutic efficacy for patients under pharmacological regimen.

Among the several imaging techniques which can provide information with diagnostic and prognostic value in atherosclerotic patients, Magnetic Resonance Imaging (MRI), has emerged as a versatile and non-invasive technique that can be used to assess the occurrence of track changes in arterial disease and to test the effects of therapies for atherosclerosis. In addition, MRI can be used to dynamically capture the accumulation of gadolinium ion (Gd3+)-based contrast agents that occurs in pathological tissues characterized by altered endothelial permeability providing an additional tool for smaller plaques localization and characterization.

Indeed, in the present work, we demonstrate that using mice with targeted deletion of the gene for apolipoprotein E (ApoE -/-) , after intravenous administration of paramagnetic lipidic nanoparticles, it become possible to discriminate among several states of the atherosclerotic plaque progression following the MRI signal of the arterial wall.

The intensity and the dynamic evolution of the MRI signal are related to both the permeability of the fibrous cap of the lesion and the selective uptake of the nanoparticles by the available foam cells.

These findings have the potential to be translated into a diagnostic procedure to assess the effects of a pharmacological treatment on patients under statins regimen.

INFLAMMATION: A PROCESS AT THE HEART OF DISEASE-DEVELOPMENT AND BODY DEFENCES

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Inflammation is an adaptive response that is triggered by pathogenic stimuli such as infection and tissue damage. During the past decade it became clear that inflammation is not only a central component of the immune system but is also a key feature of various pathologies and conditions such as type 2 diabetes, atherosclerosis and cancer. Recently, knowledge of the cellular and molecular mechanisms that control inflammatory responses has rapidly grown. However, crucial questions about how inflammatory responses originate and what is their role in the initiation and progression of non-infectious diseases have yet to be answered. The recent characterization of large cytoplasmic complexes called inflammasomes that link the sensing of microbial products and metabolic stress to the proteolytic activation of the pro-inflammatory cytokines IL-1beta and IL-18 has shed some light on new molecular pathways promoting inflammation. Inflammasomes have been associated with several inflammatory conditions including gout and type 2 diabetes. Here, we focus on the role of inflammasomes in the recognition of microbial and danger signals and discuss how these findings may underline the role of inflammatory pathways in health and diseases.

ANTICANCER AGENTS INCORPORATING POLYMER MICELLES UNDER CLINICAL EVALUATION. PHASE II: LESSONS LEARNED FROM EARLY CLINICAL TRIALS

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Tumor-targeted delivery of therapeutic agents is a longstanding pharmacological goal to improve the treatment selectivity and therapeutic index. Most scientists have sought to use 'active' receptor-mediated tumor-targeting systems. However, the 'passive' targeting afforded by the "Enhanced Permeability and Retention (EPR) effect" provides a versatile and non-saturable approach for tumor-selective delivery (1). Polymeric micelles are ideally suited to exploit the EPR effect, and have been used for the delivery of a range of anticancer drugs in preclinical and clinical studies (2,3).

NK105 is a micellar formulation for paclitaxel (PTX) whose recommended dose (RD) is 150 mg PTX equivalent/m2 administered every 3 weeks, as determined in a phase I trial. Following the phase 1 study, a phase 2 study of NK105 was conducted to evaluate the efficacy and safety of NK105 in patients with advanced gastric cancer after failure of first-line chemotherapy.

Eligible patients had measurable disease and one chemotherapeutic regimen except taxane. NK105 (150 mg PTX equivalent/m2) was administered by a 30-minute intravenous infusion every 3 weeks without anti-allergic premedication until disease progression, unacceptable toxicity or patient refusal. The primary efficacy endpoint was best overall response rate (ORR) post baseline. The secondary endpoints were progression-free survival (PFS), time to treatment failure (TTF) and overall survival (OS). All adverse events were reported using CTCAE v3.0. Between November 2007 and July 2009, 57 patients were enrolled and 56 were evaluable for efficacy. Two complete responses and 12 partial responses were observed for an ORR of 25%. The median PFS was 3.0 months, the median TTF was 2.8 months, and the median OS was 14.4 months. Causally related toxicity was mainly mild (grades 1-2) to severe (grades 3-4); other data: neutropenia (64.9%); leukopenia (17.5%); lymphopenia (8.8%); neuropathy-sensory (1.8%); fatigue (3.5%); and stomatitis (1.8%). There were no treatment-related deaths. This first phase 2 study of NK105 (150 mg PTX equivalent/m2) proves the concept for the high activity and tolerability of a new drug delivery system formulation for PTX. A phase III trial will be evaluated to clarify survival benefit .

In addition to the above clinical study, other anticancer agents incorporating micelles under preclinical and clinical evaluation will be discussed (4,5).

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THERMOSENSITIVE MAGNETOLIPOSOMES FOR MRI-GUIDED DRUG DELIVERY

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The development of new activatable drug nanocarriers, with multiple functionalities, presents a promising approach for cancer treatment.

Improved drug delivery and controlled drug release at the tumor site may have considerable benefit by increasing treatment efficacy while reducing side effects and toxicity. Further, the possibility to monitor both nanocarrier accumulation and drug release via current clinical imaging techniques may be particularly relevant for an optimal treatment.

Within the European project "Sonodrugs", we investigated the opportunity of triggering the drug release from new nanocarriers (temperature and pressure-sensitive) thanks to High Intensity Focused Ultrasounds (HIFU) and monitoring the release profile of the drug at the tumor site thanks to Magnetic Resonance Imaging (MRI) imaging.

A new versatile thermosensitive liposome has been designed and developed to efficiently encapsulate a drug (doxorubicin) and a contrast agent (superparamagnetic iron oxide nanoparticles).

Doxorubicin release profile from the thermosensitive liposome upon heat activation was characterized using the autoquenching properties of the molecule. Fluorescence intensity of the doxorubicin is quenched when the molecule is confined inside the liposomes, but is recovered when the doxorubicin is released from the liposomes in the external media. Efficient release of the doxorubicin was observed when the thermosensitive liposome solution was heated above its melting temperature (Tm) corresponding to the main gel-to-liquid crystalline phase transition of the liposome composition. Whereas, non significant doxorubicin release was observed upon aging the thermosensitive liposome solution at 37°C (below Tm).

Magnetic Resonance Imaging (MRI) experiments were performed on a 1.5 T clinical MRI Philips Achieva, using a head SENSE coil, to monitor the release of the drug at temperature above Tm. Thermosensitive liposomes encapsulating superparamagnetic iron oxide nanoparticles (TSL), non-thermosensitive liposomes encapsulating superparamagnetic iron oxide nanoparticles (NTSL), and free nanoparticles in solution (free NP) were tested. A marked positive contrast enhancement was observed on Magnetic Resonance images upon heat activation (above Tm) of the thermosensitive liposome (TSL). On contrast, no change in the MR images was observed for both the non-thermosensitive liposome (NTSL) and the free nanoparticles (free NP) (Figure 1). The signal changes were assessed by longitudinal and transverse relaxivity measurements, r1 and r2* respectively. A marked decrease in the ratio r2*/ r1 was observed for TSL only, upon heating the samples above Tm.

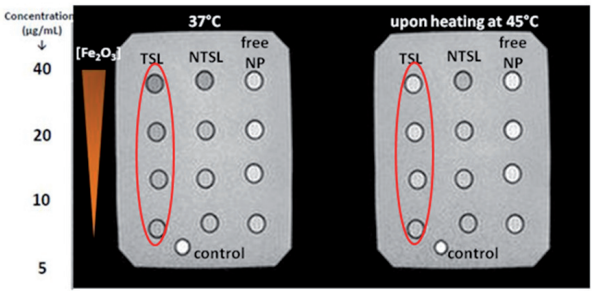

Figure 1 : Evolution upon heating of Magnetic Resonance (MR) signal at 1.5 Tesla of iron oxide encapsulated in thermosensitive (TSL) and non-thermosensitive liposomes (NTSL), and free nanoparticles (free NP).

Furthermore, heat activation (above Tm) using HIFU, and subsequent thermosensitive liposome membrane permeabilization was demonstrated through T2*-mapping, strongly suggesting the possibility of real-time imaging of drug release.

In vitro toxicity and efficacy assays, and in vivo biodistribution and antitumor efficacy are to be investigated to confirm the full potential of such new type of nanocarriers for cancer treatment.

ACKNOWLEDGEMENT

This project is granted by the EU Project SONODRUGS (NMP4-LA-2008-213706).

EX VIVO LASER MEDIATED NANOPHOTOTHERMOLYSIS OF HUMAN PANCREATIC CANCER WITH ALBUMIN FUNCTIONALIZED MULTIWALLED CARBON NANOTUBES.

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SUMMARY

The process of laser mediated ablation of cancer cells marked with biofunctionalized carbon nanotubes is frequently termed "nanophothermolysis". Here we present a method of selective nanophotothermolisys of pancreatic cancer (PC) using multiwall carbon nanotubes (MWCNTs) functionalized with human serum albumin (HSA). With the purpose of testing the therapeutic value of these nanobioconjugates, we developed an ex vivo experimental platform. Surgically resected specimens from patients with PC were preserved in cold medium and kept alive via intra-arterial perfusion.

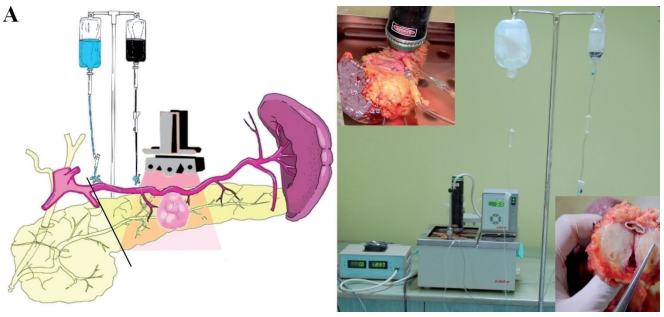

Figure 1: (A) Schematic illustration of ex vivo thermal ablation of human pancreatic adenocarcinoma on surgically resected specimen of body and tail of pancreas with spleen. (B) The proposed ex vivo laser mediated ablation of human pancreatic cancer system

Preliminary data from literature supports the involvement of albumin in tumor growth. In the present study, we tested a carrier-linked carbon nanotube system based on human albumin for selective delivery and laser mediated ex vivo ablation of living human PC. Fluorescently labelled HSA-MWCNTs were intra-arterially administered in greater pancreatic artery under ultrasound guidance. The ability of FITC-labeled bioconjugate of HSA-MWCNTs to internalize into the tumor cells after administration via vascular supply was evaluated by confocal fluorescence and electron microscopy imaging.

The area with the highest concentration of fluorescently labeled HSA-MWCNT`s was observed in central part of the tumor, where most of the malign cell were marked with the fluorescent dye. The malign tissue extracted from the periphery of the tumor also presented intracytoplasmic FITC-HSA-MWCNTs. However, as compared to the central region of the tumor, a lower density of fluorescent nanotubes was noted. No fluorescence outside the tumor in the healthy, surrounding parenchyma was observed.

Importantly, the external laser irradiation of the specimen after intra-arterial administration of HSA-MWCNTs produced extensive necrosis of the malign tissue with no harmful effects on the surrounding healthy parenchyma. There was no difference in tissue temperatures (using real time measurements based on intratumorally inserted termisthors) among the two measured tumoral areas at baseline: (1) central region of the tumoral mass; (2) peripheral region, 2-5 mm inside from the edge of hypoechogenic tumor mass; Statistical analysis of the maximal tissue temperature among the three areas revealed that area under curve (AUC) for heating curves corresponding to central and peripheral regions of tumor were significantly higher ($p>0.05$) compared to the heating curved obtained from pancreatic tissue temperature measurements. This indicates that the heating process following irradiation and MWCNTs-HSA treatment occurred mainly in tumor mass. The maximal tissue temperatures (4.2 oC in healty surrounding tissue; 25.6 oC at the periphery of tumor; 29.3 oC in central region of the tumor were achieved approximately after 20 minutes of irradiation combined with simultaneous administration of HSA-MWCNTs.

After irradiation combined with intra-arterial perfusion of HSA-MWCNTs, all sections were evaluated in detail for any changes in cellular shapes (cellular shrinkage, extraordinary cytoplasmic eosinophilia, clear cell change, cytoplasmic vacuolization), nuclear changes (nucleomegaly, nucleolomegaly, multi-nucleation, hyperchromasia, symplastic changes) and coagulative necrosis. At histopathologic examination, we consistently observed on all the examined malign slides in the entire tumor mass, mostly in its central area, foci with sizes ranging in between 500 μm and few millimeters with common signs of thermal cell necrosis.

To investigate the selectivity and efficacy of the proposed treatment, terminal transferase dUTP nick end labeling (TUNEL) assay was performed in order to stain the necrotic nuclei of apoptotic cells on the examined slides. As seen in Figure 6 A-C, based on this method we obtained strong imagistic evidence that more than 95 % of

the tumor cells were apoptotic after the irradiation. In contrast the apoptotic rate of healthy, surrounding cells was less than 2 % (Figure 6 G-I) on all the examined slides. ($p<0.0001$) These observations strongly suggest the specific nature of our treatment and confirmed its efficacy in inducing selective apoptosis of human pancreatic adenocarcinoma.

We obtained a selective photothermal ablation of the malign tissue based on selective internalization of MWCNTs with HSA cargo inside the pancreatic adenocarcinoma after ex vivo intra-arterial perfusion. In this experiment, all the existent situations met in surgical practice were replicated. We reasoned that, from the surgically point of view, ultrasound identification of the tumor and its vascular supply, the intra-arterial administration of the functionalized MWCNTs, as well as the external laser irradiation could all be safely achieved on patients using minimally invasive surgery with major benefits for the patient (e.g. laparoscopic approach or percutaneuos approach under ultrasound/CT guidance).

The above results impose human albumin as having a good potential for usage as selective delivery carrier for the development of carbon nanotube- based targeting agents for PC treatment. This original therapeutic strategy (intra-arterial administration of nanobiosystems in living human organs) presented in this paper will likely increase the knowledge of CNTs mediated photothermal therapy in cancer and will open new doors in oncological research.

Financial support by the Romanian Ministry of Education and Research (NANOPAN 41-009, NANOHEP 42-115 and NANOCITOX 42-112) is gratefully acknowledged.

COMPLEMENT ATTACK ON BLOCK COPOLYMER-COATED LONG CIRCULATING NANOPARTICLES

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Surface camouflaging of nanospheres with poly(ethylene glycol) (PEG), and block copolymers such as poloxamine 908 [a tetrafunctional polyethylene oxide (PEO)-polypropylene oxide (PPO) ethylenediamine block copolymer] is believed to combat body's defenses (notably against the macrophage recognition and clearance) and conferring longevity to nanospheres in the blood [1]. This communication will demonstrate that poloxamine 908-coated nanospheres incite complement activation (a part of innate immune system) in human serum and regardless of PEO chain configuration, but remarkably alteration of copolymer architecture on nanospheres from 'flat' to 'mushroom-brush' configuration switches activation from classical to lectin pathway, Figure 1 [2]. Also, different alterations in adsorbed polymer configuration triggers alternative pathway activation differently, where properdin-mediated activation is only restricted to particles displaying PEO chains in a transition mushroom-brush configuration [2]. Since poloxamine 908 have repetitive recognition patterns of polarity and hydrophobicity, the patterns can change as the density of attachment of the copolymers to the surface changes. Consequently, this creates new targets for complement recognition sites and shifts the activation pathway from one to another. Nanoparticle-mediated complement activation is of significant clinical concern [3,4] and these findings advance and provide a rational basis for precision surface engineering and design of immunologically safer stealth nanosystems with polymers for clinical medicine.

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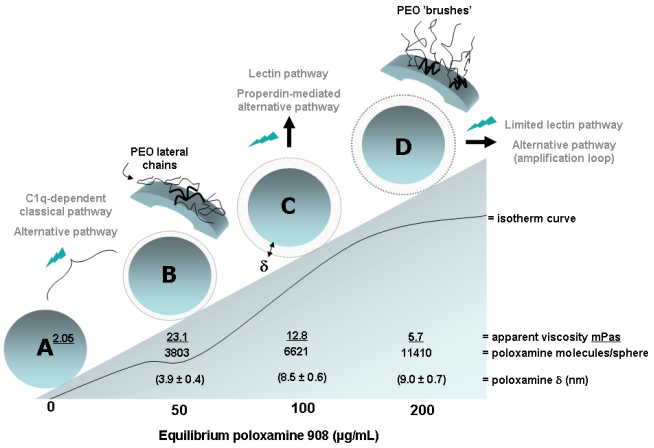

Figure 1. Physicochemical characteristics of poloxamine 908-coated nanoparticles and associated patterns of complement activation in human serum.

WHERE SIZE MATTERS? - THE PROBLEMS OF NANODRUG DEFINITIONS

Jan Mollenhauer

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The definition of the term nanodrug is in many regards of crucial importance. Both from the scientific and the legal perspective it is required to make a certain categorization. This may serve to derive, for example, guidelines for characterization, for testing of toxicity, and eventually for future prediction in order to achieve a rational design. In terms of communication to the public and relevant organs, a simple and clear definition would be required. On the other hand, it has to be avoided to make too strict definitions in order to not exclude reasonable approaches with medical potential right from the beginning. These different facets will be evaluated and examples will be provided. The question will be discussed, how drugs that per se would not match the present definition of a nanodrug, but contain nanostructured or nanoscaled components can be regarded. Finally, a pragmatic definition for a nanodrug will be offered.

PROBLEMS AND PERSPECTIVES IN PERSONALIZED CANCER NANOMEDICINE

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Personalized cancer nanomedicine is conceivable via two general strategies. Many efforts concentrate on the retrospective identification of molecular markers to identify responders to existing drugs with, however, so far limited success. An alternative is to design nanodrugs right from the beginning to match individual fingerprints of tumors. A possible strategy is outlined and problems on the regulatory side are discussed, which may limit the degree of personalization in cancer nanomedicine.

PRE-CLINICAL AND CLINICAL DEVELOPMENT OF TRANSBUCCAL INSULIN PHARMFILM®

Jan Mous

CEO PharMida AG / COO Midatech Ltd.

Insulin treatment is chronic and due to its physicochemical properties the hormone is not readily absorbed via the oral administration route, but routinely administered by subcutaneous injection. It has long been realized that this invasive method of long term dosing is not ideal for the patient, resulting in reduced patient compliance and even a risk of tissue damage. Such problems can result in poor disease management.

Oral administration of insulin remains a real challenge, because it is susceptible to hydrolytic degradation by acid and enzymes in the gastrointestinal tract. Moreover, the bioavailability of insulin is very low because it has low permeability through the mucous membranes. The use of buccal delivery systems allows for the delivery of drugs systemically, bypassing the portal circulatory system and avoiding first pass metabolism of the drugs by the liver. Since it is readily accessible, an expanse of smooth muscle, and relatively immobile, the buccal mucosa is a suitable option for the administration of retentive dosage forms.

The stability and bioavailability of insulin could be improved by attaching non-modified, monomeric insulin on very small (<5nm), glycan-coated gold nanoparticles (GNP) and administered through the buccal membrane using muco-adhesive, FDA-approved PharmFilm®.

The pharmacokinetics of insulin after application to the buccal membrane as well as its immediate effects on glucose clearance have been studied in various animal models, including streptozotocin-treated, diabetic mice, healthy minipigs and Type-2 diabetic Rhesus monkeys. These studies have shown that: i) that transbuccal insulin had a much faster onset and shorter duration of action than subcutaneously injected regular insulin; ii) transbuccal administration provides a cephalic insulin response; iii) bioavailability of transbuccal insulin reached 20-30% of a commercial, subcutaneously injected insulin analogue in minipigs and Rhesus monkeys.

Numerous safety assessments , including acute and 28-day repeated dosing toxicity studies, in rats, dogs, minipigs and Rhesus monkeys showed no signs of adverse reactions. Furthermore, there were no signs of mutagenicity or genotoxicity observed with high doses of GNP.

A single-center, inpatient, randomized, double-blind, placebo-controlled, dose-escalating study to evaluate the safety, tolerability and PK/PD profiles of single doses of a transbuccal formulation of natural human insulin with glycan-coated gold nanoparticles buccally administered to healthy subjects has been proposed and submitted for regulatory review.

BRIDGING THE GAP BETWEEN MEDICINE AND NANOMETER-SCALE PHYSICS

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Clinical imaging facilities currently reach sub-millimeter resolution and already lead to huge datasets. It is a dream of physicists, nanoscientists and clinicians to visualize the human body on the atomic scale to realize nature-analogue, biomimetic materials and implants. The talk deals with X-ray-based techniques to make visible the micro- and nanoanatomy of human tissues (tumors, teeth, urethra, cochlea, brain), the morphology of implant materials and the characterization of the interface between man-made material and biosystem. Related to these imaging data, clinically relevant problems including local drug release using nanocontainers, laser-based bone cutting and removal, and nanotechnology-based artificial muscles are discussed with the aim to demonstrate how patients can benefit from sophisticated physics-based approaches.

TOP-DOWN APPROACH TO INTELLIGENT NANOSYSTEMS

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Helical structures similar in geometry and scale to bacterial flagella can be fabricated using the self-scrolling technique. This method is based on the coiling of strained 2D thin films to form 3D structures after the films detach from the substrate by selective etching, a type of self-assembly. To fabricate rolled-up helical nanobelts, the 2-D films are patterned into ribbon-like mesas. Compared to nanohelices grown solely from "bottom-up" processes, these rolled-up helical nanobelts can be designed with a specific geometrical shape, i.e., their diameter, chirality, pitch, helicity angle and length can be precisely controlled. Nanorobotic manipulation shows that the as- fabricated helical nanobelts are highly flexible and retain a strong "memory" of their original shape. Inspired by the helical-shaped flagella of bacteria, we have developed artificial bacterial flagella (ABFs) as wireless swimming microrobots (Fig. 1). An ABF consists of a rolled-up helical nanobelt similar to a natural flagellum in both size and shape, and a thin soft-magnetic head for magnetic actuation. Experimental investigation shows that an ABF can be propelled and steered in 3D with micrometer precision by a low-strength, rotating magnetic field. Moreover, the swimming properties are tunable by changing the input magnetic field frequency and the shape of the ABF. Swarm-like behavior of multiple ABFs as a single entity is also demonstrated under the control of the magnetic field, and two approaches can be applied to decouple or immobilize an individual ABF from the swarm, i.e. using the step-out frequency in a high frequency range or the wobbling effect at low frequencies. These miniaturized devices made of helical nanobelts provide a mechanism for mimicking and investigating many natural micro-organisms, and can be used as magnetically driven wireless manipulators for medical and biological applications in fluid environments, such as cell manipulation and removal of tissue. Due to its large surface-to-volume ratio, surface functionalized ABFs have the potential to sense and transmit inter- or intracellular information, and to perform targeted drug delivery.

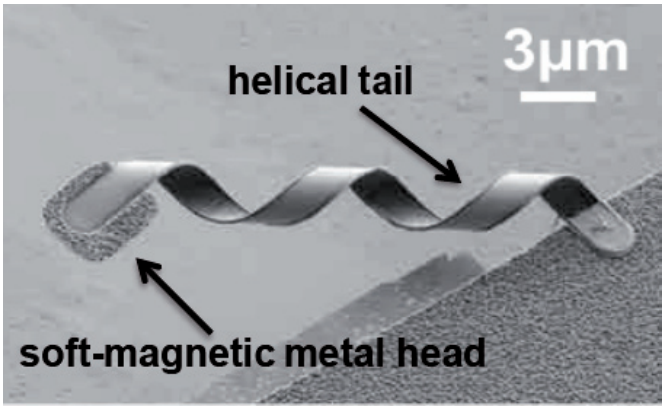

Figure 1. An artificial bacterial flagellum (ABF).

NANOROBOTIC SYSTEM

When engineering nanorobotic systems, efficiency is an important factor as power supply is difficult and limited. Applying an external magnetic field is a common actuation method as it provides a wireless power supply. Even though rotating a helical tail is not efficient from a fluidic propulsion point of view [3], the definition of efficiency can be misleading when taking into account the magnetic actuation of nanorobots [1]. Evaluating the nanorobotic system as a whole, considering both fluid mechanics and magnetic actuation, helical swimming is one of the most promising methods of propul-

sion for nanorobots.

The linear relationship between the forward and rotational velocity, u and ω , respectively, of a helical filament under the influence of an external force F and torque τ can be represented by a propulsion matrix.

The parameters a, b and c are functions of geometrical parameters and can be found by modeling or empirical methods. The rotation and translation of a helical filament are inherently coupled, i.e. b != 0, due to the lack of shape symmetry. This coupling is exploited by using a torque to rotate the filament, which in turn creates the desired translational motion.

$$\begin{pmatrix} F \\ \tau \end{pmatrix} = \begin{pmatrix} A & B \\ B & C \end{pmatrix} \begin{pmatrix} u \\ \omega \end{pmatrix}$$

Figure 2. Locomotion model of an artificial bacterial flagellum (ABF).

Using an external magnetic field to apply the required torque to the ABF removes the need to replicate the highly complex molecular rotary motor of real bacteria [2]. The challenge remains in fabricating the helical tail, which is a non-trivial 3D structure, at a micro-nano scale. The first method to successfully fabricate ABFs in a batch process is the self-scrolling technique [4]. The controlled release of internal stresses in the material allows arrays of ribbons to roll-up into helical shapes. The prototypes described here have a helical tail with an overall length of approximately 30 um, depending on the number turns, and a diameter of 2.8 um. The tail is attached to a

200nm thin and 4.5 um wide square plate made from a soft-magnetic material such as Ni.

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EU FUNDED RESEARCH PROJECTS IN NANOMEDICINE – FUTURE REGULATORY IMPLICATIONS

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The European Union, through the European Commission is funding nanotechnology research in many application fields such as energy, ICT, environment, transport and health, including nanomedicine. The nanomedicine projects are at the forefront of developing new pharma-ceutical products for targeted drug delivery and medical devices, processes/procedures for therapeutics and diagnostics at nanoscale. There is also research for developing biomaterials as support for cell therapies in regenerative medicine.

For approval of pharmaceutical products the regulatory framework is based on the three basic pillars; Quality, Safety and Efficacy of the product. The presentation will give a hint of what type of products we can expect in the future based on the research projects funded and what the scientific and regulatory challenges may be.

Further reading about Nanomedicine: :Nanomedicine; Nanotechnology for Health, European Technology Platform, Strategic Research Agenda for Nanomedicine, 2006. http://cordis.europa.eu/nanotechnology/nanomedicine.htm

SIRS – THE INFLAMMATORY KILLER DISEASE IN INTENSIVE CARE MEDICINE: CHALLENGES AHEAD

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SUMMARY

A high rate of death from septic shock persists despite general improvements in care. The relative failure of mechanistically-based therapies in various clinical trials should also trigger a reconsideration of such mechanistic approaches. Despite reversion of shock by hydrocortisone, the similar death rate compared to non-steroid treated patients suggests that factors other than shock itself might be responsible for death. This may be predetermined and relate to gene variants, the functionality of gene expression, age, an association with chronic diseases such as diabetes and cancer, or perhaps the treatments being given for these diseases. These aspects will be discussed in the light of arguments that support a hypothesis of outcome pre-determination. Not only constitutive factors but also acute and chronic environmental factors may be responsible. An important consequence would be the ability to perform an early prognostication in an individual patient using biomarkers. Such a view stimulates the devopment of nanotechnologies for rapid detection of biomarkers and sensors. On this basis, new therapies could be tested to reduce mortality rates with the response and toxicity of these therapies being predefined using pharmacogenetic testing.

UNSOLVED PROBLEMS IN DIABETES

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Diabetes continues to be one of the world's foremost healthcare problems. The frequency of diabetes is increasing at epidemic rates. There is almost no area of diabetes where the problems are solved and the opportunities for nanomedicine approaches are numerous.

In type 1 diabetes, major research themes include immune interventions in newly diagnosed or at-risk subjects to prevent or slow the autoimmune process that causes pancreatic beta-cell destruction and at least preserve long-term beta-cell function. An example is treatment with anti-CD3 monoclonal antibodies which disrupt T cell-mediated immunity (trailled in humans), but iron oxide-MHC linked nanoparticles which expand regulatory T cells have already been used to prevent diabetes in an animal model of diabetes. Improved insulin replacement in established diabetes is needed; transplantation of islet cells is still not routine and immune rejection might be prevented and longer survival ensured by, for example, nano-encapsulation technologies. We have encouraging preliminary results for mouse islets encapsulated with nanofilms and transplanted into diabetic animals.

The technology might also be useful for transplanted stem cell. Faster-acting insulins for meal time control of blood gluc ose in might be achieved by inhaled insulin nanoparticles, and longer-acting insulins for basal delivery by delayed-release subcutaneous nano-formulations of insulin.

In type 2 diabetes, prevention may be achieved by appetite-controlling therapies to prevent obesity – perhaps nanoformulations targeted at brain appetite centres . The realization that chronic inflammation plays a crucial role in the pathogenesis of type 2 diabetes and its complications argues that new anti-inflammatory agents may help in management. Some nanomedicines show promise as anti-inflammatory drugs. Early introduction of insulin therapy is often resisted by type 2 diabetic patients, but switching to insulin might be encouraged by non-injectable formulations such as nano-formulations of inhaled or oral insulin.

The extent of islet cell damage or inflammation in both type 1 and 2 diabetes could be assessed by non-invasive imaging. In both types of diabetes, improved glucose testing is necessary – more stable and accurate continuous glucose monitoring using implanted sensors are needed (e.g. 'smart tattoos' of skin-impregnated nanosensors) and, if possible, non-invasive glucose monitoring. Closing-the-loop by coupling glucose monitoring to insulin delivery is making progress using electromechanical devices but has not reached routine practice – the possibilities of developing an 'artifical nanopancreas' should be considered.

Better detection and treatment of diabetic tissue complications such as retinopathy are needed in both type 1 and 2 diabetes, and research is needed on non-invasive imaging and therapy using perhaps functionalized nanoparticles targeted to affected tissue.

EU PROJECT GAMBA: GENE ACTIVATED MATRICES FOR BONE AND CARTILAGE REGENERATION IN ARTHRITIS

Christian Plank

Institute for experimental oncology and therapy research of the Technical University of Munich, (D)

The GAMBA consortium develops a gene-activated matrix platform for bone and cartilage repair with a focus on osteoarthritis-related tissue damage. The S&T objectives of this project are complemented with an innovative program of public outreach, actively linking patients and society to the evolvement of this project. The GAMBA platform is going to implement a concept of spatiotemporal control of regenerative bioactivity on command and demand. A gene-activated matrix is a biomaterial with embedded gene vectors that will genetically modify cells embedded in the matrix. The platform comprises modules that self-adapt to the biological environment and that can be independently addressed with endogenous biological and exogenous physical or pharmacological stimuli, resulting in a temporally and spatially coordinated growth factor gene expression pattern. This reproduces, within the matrix, key elements of natural tissue formation. The modules are a biomimetic hyaluronan gel, a ceramic matrix, growth factor-encoding gene vector nanoparticles, magnetic nanoparticles and mesenchymal stem cells. Anatomical adaptivity is achieved with engineered thermal properties of the polymer matrix, which embeds other modules, selected according to functional requirements. Mechanical support is provided by Micro Macroporous Biphasic Calcium Phosphate (MBCP™), a resorbable material approved for clinical use. Spatiotemporal control of bioactivity and responsiveness to physiological conditions is represented, firstly, in the spatial distribution and release profiles of gene vectors within the composite matrix and, secondly, by letting local and external biological or physical stimuli activate the promoters driving the expression of vector-encoded transgenes. This innovative concept is implemented by a multidisciplinary team from leading European institutions combining scientific excellence with a focused plan of dissemination, public participation, gender equality and transition to market.

NANOSHELL ASSISTED LASER TISSUE FUSION: AN OPPORTUNITY FOR BYPASS SURGERY

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BACKGROUND & OBJECTIVES

Laser tissue soldering (LTS) is conventionally limited by the lack of reproducibility especially due to the changing content of the energy absorber Indocyanine Green (ICG). Nanotechnology enables to quantitatively bind the ICG to the soldering architecture. Aim of this study was to establish a highly reproducible and sufficient tissue fusion by using ICG packed nanoshells in combination with our common laser tissue soldering technique. We hypothesized, that by introducing our new technique of directly implemented chromophore into the scaffold would prevent energy absorber dilution during soldering procedure. We focused on the feasibility of this approach and compared the common and the new nanoshell soldering technique regarding heat development and tensile strength.

MATERIALS & METHODS

A previously described porous polycaprolactone (PCL) scaffold doped with albumin-indocyanine-green solder was compared to our new technique. We implemented the chromophore directly into the scaffold using ICG packed nanoshells of 250– 270 nm diameter. The nanoshell-scaffold was used in a flexible semidry formulation allowing formation of different shapes such as tubes and the possibility to optimally adapt the scaffold to the surgical requirements. Standardized tissue soldering procedures and measuring equipments were used.

RESULTS

Electron microscopy pictures of the nanoshell-scaffold revealed homogenous implementation of the nanoshells bound and incorporated into the PCL. Rabbit aortic arteries have been successfully soldered with our new technique. Tensile strength of the nanoshell soldered anastomoses were estimated to 734 ± 327 mN (median = 640 mN), compared to the common soldering technique 649 ± 389 mN (median=545 mN). Required heat deposition (>80°C) has been reached and thermal damage was restricted to the adventitia at the irradiated area. In addition, absorber dilution has been effectively reduced during the soldering procedure using these nanoshell-scaffolds and there was significantly less variance at temperature maximum ($p=0.03$) compared to the common soldering technique.

CONCLUSION

Using nanoshells, the quantified chromophore amounts could successfully be bound into the polymer scaffold. Diode laser soldering of vascular tissue using ICG-nanoshell-scaffolds leads to strong and reproducible tissue bonds with less variance at Tmax. With optimally chosen settings of irradiation time, nanoshell coating and scaffold properties, our improved LTS procedure has the potential of a clinical applicable anastomosis technique.

IMMUNOSTIMULATORY NANOPARTICLES FOR THE IN TREATMENT OF ALLERGIC ASTHMA SHOWN TO BE SAFE AND EFFICACIOUS IN PLACEBO-CONTROLLED PHASE II CLINICAL TRIAL

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BACKGROUND

Stimulation of the innate immune system via Toll-like receptor 9 aims at modifying the response of the immune system away from Th2-mediated allergic reactions. CYT003-QbG10 is a TLR 9 agonist consisting of the unmethylated CpG oligonucleotide G10 packaged into the protein nano-particle Qbeta.

Methods: The present study was a multi-center, parallel-group, double-blind, randomized and placebo-controlled phase II clinical trial. 63 patients with persistent allergic asthma that required long term treatment with inhaled corticosteroids (ICS) were randomized to 7 s.c. injections of placebo (PBO) or 0.9mg CYT003-QbG10 at weekly/bi-weekly intervals. After a stable add-on phase, the ICS dose was reduced after 4 weeks to 50% and completely withdrawn after 8 weeks, if possible. Clinical efficacy was assessed over the 12 week study period by the Juniper Asthma Control Questionnaire, by electronic diaries in which daytime and nighttime asthma symptoms and use of relief-medication were recorded, and by spirometry (FEV1).

RESULTS

All objective and patient reported outcome parameters were significantly improved vs. placebo from week 6 to the end of the study. The fraction of patients whose allergic asthma was “well controlled” (ACQ score ≤ 0.75) increased from 42% under ICS therapy to 67% under QbG10 treatment despite corticosteroid withdrawal, while under placebo the fraction of “well controlled” patients fell from 40% to 33% (p=0.008). Asthma symptoms decreased by 33% under QbG10 treatment despite corticosteroid withdrawal, while they increased by 29% under placebo treatment (p=0.01). Use of relief medication doubled in the placebo group, while it remained stable in the CYT003-QbG10 group (p=0.01). Symptomatic improvement was further supported by significant improvement in lung function (FEV1) vs. placebo (=233ml), p=0.009. Anti-inflammatory effects of treatment were demonstrated by a significant reduction in blood eosionophils under CYT003-QbG10 therapy versus placebo (p=0.043).

DISCUSSION

Achieving asthma control is an important therapeutic goal. The proposed disease modifier CYT003-QbG10 aims at eliminating the cause of allergic asthmatic inflammation and should provide patients with a therapeutic alternative to either replace or complement commonly used asthma medications, e.g. inhaled corticosteroids, while maintaining or improving asthma control. The current study demonstrated that CYT003-QbG10 improved asthma control, lowered symptoms and use of relief medication and improved lung function vs. placebo, in a highly significant and to a medically relevant extent

AN INDUSTRY VIEW ON TECHNOLOGY INTEGRATION GAPS AND BRIDGES TOWARDS NANOMEDICINE PRODUCTS

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Market sucess of nanomedicine products has proven which clinical benefit and value can be delivered by nanotechnology. Nanosuspensions, liposomes or nanosized drug conjugate formulations may enable the parenteral delivery of poorly soluble drugs and/or achieve a targeting effect. Nanoparticulate formulations may lead to a targeting effect that enables higher drug efficacy at a lower adverse effect rate compared to a simple parenteral solution formulation.

However, the development of such nanomedicine products involves a higher levels of complexity and risk. Thus decision for nanomedicine development competes with a conservative option of drug portfolio development: i.e. to invest into the next backup compound instead of a more complex nanoformulation for molecule endangered by undesired tox effects.

A common perception is, that despite an early hype for nanomedicine, not many products or technology platforms have created value on the market so far. Nevertheless there is growing evidence that nanotechnology should be applied more widely.

But how to select a portfolio of technologies for an industrial compound pipeline? Features of a prefered nanotechnology are: success as marketed products, clinical success differentiating it from proven technology, ease of manufacturing under cGMP, viable regulatory acceptance. The technology should be flexible for delivery of molecules with a wide range of physicochemical properties and doses. Excipients involved should be biodegradable and safe. Simplicity of composition and manufacturing beats complexity

The speed and extent of utilization of nanomedicine in industrial drug product development depends heavily on the shared understanding and collaboration of innovation stakeholders, industrial decision makers and regulatory bodies.

CRIPEC: TRANSLATION OF A NANOPARTICULATE DRUG DELIVERY PLATFORM INTO PHARMACEUTICAL PRODUCTS

Cristianne Rijcken

CEO Cristal Delivery B.V.

The pharmaceutical industry is exploring possibilities to improve the performance of their products, to prolong life-cycles or to obtain line extensions of current compounds. Cristal Delivery develops proprietary polymeric technologies (CriPec®) to design innovative pharmaceutical products on the base of new and existing compounds for various therapeutic areas.

Our custom-made polymers offer opportunities to tailor the presentation of an active ingredient to improve efficacy, safety and/or patient convenience, as well as to provide a differentiated and commercially protected drug product. The initial product development efforts are in the area of nanomedicine, i.e. to achieve enhanced disease treatment by using nanoparticles to (re)formulate drugs.

Cristal Delivery’s lead technology combines the best features of known nanosized drug delivery systems into 1 single particle: preservation of the particles’ integrity in the dynamic environment of the blood stream, sustained release upon a prolonged circulation and selective tissue targeting. The proprietary nanoparticles-drug conjugation method provides for truly controlled release of entrapped cargo, thus improving the therapeutic window. The technology platform allows for nanoparticle customisation through conjugation chemistry, particle composition and formulation, and depends on the type of drug, indication and route of administration.

Currently, preclinical development steps (in vivo safety and upscaling studies) of the first products are ongoing. Preliminary results demonstrate the long blood circulation of nanoparticles in mice with a sustained release of encapsulated drugs up to at least 72 hours. In case of paclitaxel entrapment, the maximum tolerated dose (MTD) was not reached even at 85 mg/kg, so at least 6-fold higher than the MTD of the current commercial formulation Taxol. No acute toxicity is observed upon a single intravenous administration of empty nanoparticles to mice at a dose up to 1700 mg/kg.

Cristal Delivery is actively pursuing strategic partnerships with pharmaceutical companies to conduct custom-made feasibility stud-

ies on compounds that are either in the pipeline, on the market or shelved, to create innovative products with a clear added value for the patient. Moreover, Cristal Delivery aims at expanding the applicability of CriPec® to different therapeutic agents, other routes of administration and a variety of diseases.

TOPICAL PHOTO-MIX: NANOMEDICINES FOR A FRIENDLY TREATMENT AGAINST CUTANEOUS LEISHMANIASIS

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Cutaneous leishmaniasis (CL), an endemic disease in South and Central America, Mediterranean region and Asia, has an overall prevalence of 12 million people for an overall-at risk population of 350 millions. Standard treatments consist of repeated, painful parenteral injections of pentavalents antimonials. The global project NANOSKIN (EULANEST: Germany, Portugal, Argentina) is aimed to design topical nano structures for prophylaxis and therapeutics against CL and skin cancers. Here we show our preliminary pre-clinical results on a self administered treatment for fast healing and radical cure of CL.

Photo-MIX is a 1:1 blend of ultradeformable archaeosomes (UDA made of total polar lipids extracted from Halorubrum tebenquichense, soy Pc and sodium cholate) loaded with sodium alendronate (ALE) at 1 mg/ml (ALE-UDA) and ultradeformable liposomes (UDL made of soy Pc, sodium cholate) loaded with tetrakis-2 adamatoxil Zn phtalocyanine (ZnPc, a sunlight photoactivable zinc phtalocyanine) at 1 mg ZnPc/g lipid matrix (ZnPC-UDL). Vesicles are unilamellar, 100 nm mean size. Keratynocytes and fibroblasts are refractory to Photo-MIX damage. However, after topical application on intact or ulcerated skin, Photo-MIX exerts a multiple and selective cytotoxicity against host macrophages and leishmanias up to a minimal depth of 50 m in mice skin. A synergy of factors contribute to the multiple and selective cytotoxicity of Photo-MIX: 1) UDA matrix is internalized with high avidity by macrophages 2) free ALE and ALE-UDA are toxic to macrophages (neutrophiles and skin monocytes) and to infective leishmanias 3) UDL matrix is toxic to leishmania promastigotes 4) ZnPc-UDL is toxic to leishmanias amastigotes 5) toxicity of ZnPc-UDL is accelerated by photoactivation after a short sunlight exposition. Remarkably, unacceptable high doses of parenteral bisphosphonates are needed before leishmanicidal effect could be exerted on the skin. However, topical application of Photo-MIX allows avoiding systemic circulation and subsequent sequestration of ALE to bone matrix upon Ca+2 chelation. Thus, ALE in Photo-MIX distributes in epidermal/dermal cylinders of surface area  4 mm 2 and several microns depth, at amounts 100-400 folds higher than if administered by parenteral route, at the same dose. A similar effect is achieved by topical application of ZnPc in Photo-MIX, since systemic absorption of ZnPc is minimized and because after internalized by skin infected macrophages and promastigotes a pronounced chemical and photochemical damage is caused.

We have determined the activity of Photo-MIX on an experimental model of american CL, caused by L. braziliensis on BALB/c mice. This model takes into account features of the natural course of transmission, such as the inoculation of a lower number of parasites into a dermal site. The resulting clinical outcome is similar to that observed in the human host, particularly in terms of lesion ulceration, parasite persistence, and immune response. Briefly, stationary-phase promastigotes (105 parasites in 10 l of saline) were inoculated into the right ear dermis of age-matched BALB/c mice using a 27.5-gauge needle. Lesion size was monitored weekly for 10 weeks using a digital caliper. After three weeks of infection the lesions achieved a maximal size. At this moment, groups of 10 mice received 4 drops (50 l each) of Photo-MIX on and around the close periphery of the lesions, once a day, for two weeks. Then the mice were daily submitted to 15 minutes sunlight irradiation for two weeks. After three weeks, Photo-MIX significantly accelerated the healing of lesions and the number of parasites in draining lymph nodes as well in lesions, was reduced as compared to control infection. Ongoing research is being carried out to assess if this regimen could offer a radical cure and to extend this treatment against different species causing CL.

INFLAMMATION IN THE GASTROINTESTINAL TRACT: BASICS AND POTENTIAL THERAPEUTIC INTERVENTIONS

Bernhard Sauter

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Inflammatory bowel disease (IBD) - Crohn's disease (CD) and ulcerative colitis (UC) - are autoimmune diseases in which chronic inflammation may result in bowel destruction associated with a significant morbidity and in certain cases mortality for the patients. These conditions are polygenic in nature and many genes have been linked with IBD using genome wide association scans.

The prevalence of inflammatory bowel disease (10-70/100'000 for CD; 20-130 /100'000 for UC) has been increasing over the last decades similar to other autoimmune diseases (e.g. multiple sclerosis, asthma, rheumatoid arthritis etc.). In addition, IBD is more prevalent in countries with advanced sanitary conditions providing evidence for the so-called "hygiene hypothesis" of IBD: Reduced exposure to environmental pathogens leads to the loss or break of tolerance to the commensal bacterial flora, which is the basis for the development of IBD.

The gut microbiome consists of 1017 microbes that equals about ten times the number of cells of the human body. Recently, three distinct (independent of nation or continent) host–microbial symbiotic states have been described based on species composition, termed enterotypes. The host–microbial interaction plays a central role in disease development: The maintenance of the epithelial barrier (e.g. IBD5), the evolutionary well preserved antimicrobicides (defensins), the antigen presentation of the innate immune system and correct function of pattern recognition receptors (e.g. TLR4, NOD2), the autophagy functions (e.g. ATG16L1, IRGM), and an appropriate cytokine response of the innate immunity to guide an adequate secondary (adaptive) immune response play a key role in the host–microbe homeostasis. The importance of the commensal flora in intestinal inflammation is underscored by the fact that mice raised in germ-free conditions are resistant to IBD. The inflammatory cycle in IBD can be started by an insult to the mucosal integrity through medications, infectious agents, smoking, environmental particles such as titanium dioxide, etc. Many times, however, the triggering agent cannot be identified.

In summary, IBD develops in a susceptible host (genetic predisposition) usually after a non-specific insult (smoking, medications, infectious gastroenteritis, etc.) resulting in a pathological host–microbe interaction (chronic inflammation) eventually leading to bowel destruction.

The complex nature of IBD presents multiple opportunities for therapeutic interventions. At the moment, the therapeutic efforts are guided towards the late steps of inflammation, i.e. the suppression of the increased inflammatory activity mainly driven by the adaptive immune system by "anti-lymphocyte" and anti-cytokine (e.g. TNF alpha) medications. The initial stages of the disease development have not been successfully targeted: i.e. change of the microbiota ("harmful" vs. "helpful" bacterial flora, change of enterotype), sealing of the epithelial barrier (e.g. mucous, tight junctions), up-regulation of defensins, improvement of the function of the native

immune system (e.g. gene repair to restore antigen presentation and phagocytosis), or modulation of the inflammation by restoration of the intestinal cytokine balance (e.g. locally targeted secretion of anti-inflammatory cytokines by gene delivery or cytokine expressing bacteria, reduction of pro-inflammatory cytokines, e.g. by gene silencing (siRNA)), stimulation of regulatory lymphocytes (T reg). Using nano-sized particles as carriers of genes, small molecules, proteins, etc. many of these treatment approaches may be explored in the future.

WHY WE ARE NOT THE SLAVES OF OUR GENES

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The findings of molecular biology have reopened the disturbing question of whether we humans are merely biochemical machines that are rigidly controlled by genes. This philosophical insult challenges our view of human freedom, individuality and personal responsibility – but so far we have been unable to refute it. Recent biological discoveries may soon change this situation. One of these discoveries is that unpredictable chemical fluctuations within the nanospace of a subcellular compartment may influence development and perhaps also behavior. The second is the fact that the environment may alter our genetic programs and that we can pass on some of these acquired alterations to future generations. And the third is the realization that the immense complexity of living matter may make this matter partly unpredictable.

For nanomedicine, the first of these discoveries is of particular relevance, The physicist Erwin Schrödinger was probably the first to suggest that the hierarchical structure of living matter may impose unpredictable fluctuations in the chemical reactions of a few master molecules onto an entire organism, making that organism unpredictable. Indeed, some of the proteins that read our genes or decode signals from the environment exist in our cells in so few copies that their chemical reactions no longer obey the statistical chemical laws, but are stochastic yes/no decisions. Living cells can amplify such unpredictable binary decisions and make them irreversible. In this way, one and the same genome can generate different variants of an organism even if the environment stays constant. The role of stochastic fluctuations in development and behavior is one of the most fundamental discoveries of modern biology; it shows that the reactions of a few molecules within a cellular nanospace need not mirror those of macroscopic chemical systems.

UNSOLVED PROBLEMS IN NEUROLOGY– ALZHEIMER'S DISEASE AND PARKINSON'S DISEASE

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Alzheimer's disease and Parkinson's disease are the two most frequent neurodegenerative disorders. Because life expectancy is still constantly increasing and age being the most important risk factor the incidence of both disorders will dramatically increase during the next years.

As in most other neurodegenerative disorders aggregation of disease specific proteins are the core features of both disorders. Genetic, biochemical, and transgenic animal data suggest that extracellular amyloid and intracellular tau aggregation and cytosolic α-synuclein aggregation are the initiating points of the pathogenesis in Alzheimer's disease and Parkinson's disease, respectively. In both diseases, propagation of the disorders may occur through a prion-like mechanism. The pathology of the diseases develops and propagates over several years (e.g. 5 – 20 years), before the diseases become clinically manifest. Current data from clinical trials in humans suggest, that removing amyloid plaques in clinically manifest Alzheimer's dementia comes too late to significantly alter the course of the disease. Furthermore, it remains an open question whether the protein aggregates themselves, which are unsoluble, or its soluble precursors in form of oligomers are the toxic mediators of the disease.

Therefore, the following needs should be addressed:

- Very early – or even better – presymptomatic diagnosis of the disease
- Identify treatment strategies and drugs that block the formation of oligomers or of β-sheets
- Identify ways to deliver potential drugs to the brain
- Identify biomarkers that predict the therapeutic efficacy of drugs

NANOMEDICINE - A BIG STEP TOWARDS CURING CANCER

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Development of appropriate nanotechnology based devices and nanocarriers is crucial to achieve substantial improvements on disease drug delivery and imaging tools for biomedical applications. It is clear that in order to achieve higher standards and better technological approaches in this field, is of the most importance to ensure research collaborations among research groups with different biomedical, clinical and technological areas of expertise, and also to provide at the same time a good interactive environment and translational cooperative working through adequate industrial, technological and clinical partnerships. These should ensure an optimal improvement of the current treatment protocols of human disease and will provide better survival, lower health costs and improvement on the overall quality of life of our patients. A ever increasing number of different technologies and nanotechnology approaches is flourishing constantly offering new and promising solutions to clinical needs. Nonetheless, several issues related to sensitivity, specificity, toxicity, efficacy and regulatory issues still need to be considered and properly addressed if we want to ensure their application in the clinical setting. Here we will address some of the central issues that need to be considered for further discussion is this session.

PROTEOMICS AND INTERACTOMICS USING A SMALL NUMBER OF HOMOGENEOUS CELLS: A NEW FRONTIER FOR MEDICAL DIAGNOSTICS

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I will discuss three novel methods to carry out proteomic and interactomic characterization of small, homogeneous populations of cells. These methods are all very sensitive (minimum detection level of at least 100 attogram), are capable of different levels of multiplexing and are, at the moment, at different stages of development.

The first one is an AFM-based technique that works as a sandwich ELISA test, with the detection being based on the careful height measurement of the recognition event between the antigen and the antibody on a very flat surface. The technique has been already applied at the orientation of prion protein on surfaces and is capable of multiplexing on a moderate level.

The second one is a technique based on the mechanical transduction of molecular adsorption and is capable of measuring the mass

adsorbed on the top surface of silicon micropillars by monitoring the changes in their resonance frequency. The pillars are approx. 25 microns high and their top surface is 2x6 microns. With this device we have been able to detect the saturation adsorption (2 10(13) mol per sq cm) and saturation hybridization (40%) in DNA monolayers, where saturation was shown to be reached, in nanomolar solutions, in times that appeared to be by more than a factor of 100 shorter than what reported by others. We explain this discrepancy with the literature by suggesting that when the size of the absorbing surface is on the order of the intermolecular distance of solute molecules, adsorption occurs from a sphere whose radius is more than a factor of 100 shorter than the height of the column from which the molecule adsorbing on a macroscopically extended surface have to make their way to the surface. This phenomenon being of general nature is expected to apply to all adsorptions on nano sized surfaces and objects and it is expected to improve by a factor of more than one hundred the dilution sensitivity of any technique that works at the macro scale.

Finally, we are working on a differential interactomic method that is based on an application of the concept of the Wheatstone Bridge, that is perhaps the most common device measuring electrical resistance. This device is expected to have the smallest minimum detection limit but because it is based on rather sophisticated nanofluidics it is the least developed of the three methods.

This work has been carried out in collaboration with the past and present staff of the SENILab. SENIL is a nanobiomed laboratory of the Synchrotron Light Source Elettra in Trieste Italy run by Dr L. Casalis. The pillar work was carried out in collaboration with drs Lazarino and Melli of the TASC - CNR lab also in Trieste Italy.

THERAPEUTIC TARGETING OF INFLAMMATION IN CHRONIC INFLAMMATORY DISORDERS

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Extensive attention has been given to the concept of nanoparticles as targeted drug carriers to improve the therapeutic index of drugs. However, with respect to their application in the treatment of chronic inflammatory disorders, the literature is still limited and fragmentary, and lacks systematic and comparative studies. Nevertheless, it is clear that particulate carriers can be very functional to improve the therapeutic performance of anti-inflammatory agents, either by introducing a depot (local administration) or by attaining site specific drug targeting (intravenous administration). Large-sized particles are particularly attractive to achieve slow release effects upon local administration. Small-sized nanoparticles are better suited to achieve targeting after intravenous administration. Surface modifications can be introduced to further improve target localization by prolonging the circulation time (passive targeting) and/or by interacting with specific target cell receptors (active targeting).

MEDITRANS represents a multidisciplinary Integrated Project sponsored by the EC (FP6) dealing with targeted nanomedicines. Platform technologies are being developed with broad applicability to disease treatment, as exemplified by the choice for chronic inflammatory disorders (rheumatoid arthritis, Crohn's disease, multiple sclerosis), and cancer as target pathologies. Within the MEDITRANS consortium, significant effort has been made to improve the therapeutic index of glucocorticoids. Glucocorticoids are highly effective anti-inflammatory drugs. However, their use is hampered by their highly unfavorable pharmacokinetic properties, i.e. rapid clearance and a large volume of distribution, which necessitates high and frequent dosing to maintain therapeutic levels at sites of inflammation, which increases the risk for severe adverse effects, especially upon long-term treatment. This lecture will discuss the current status.

NANO-IMMUNE TOXICOLOGY: UNMET NEEDS IN SAFETY PREDICTION

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Immunosafety is a key issue in current R&D of nanomedicines, yet the prediction and prevention of adverse immune effects represent an unmet medical need. The triggered innate and antigen-specific immune responses can be harmful by causing hypersensitivity reactions (HSRs) and by leading to antibody production against the drug (immunogenicity). HSRs can arise as a consequence of complement (C) activation which reactions are called C activation-related pseudoallergy (CARPA). Among numerous symptoms in almost all organ systems, the cardiac complications of CARPA are of particular concern, as they can be fatal in a small percentage of patients. Immunogenicity can be inconsequential or adverse, in the case the antibodies produced against the drugs modulate their pharmacokinetics, and, hence, their action. The presentation will focus on recent progress in the prevention and laboratory prediction of immune reactions to nanomedicines in three areas: 1) prevention of liposome (Doxil) reactions with desensitization using placebo Doxil; 2) prediction of clinical reactions to nanomedicines by in vitro screening for their C activating power, using the sera of patients and SC5b-9, C5a and C3a as endpoints, and 3) ADA (antidrug antibody) ELISA which detects preexisting antibodies against reactogenic nanomedicines (taxanes and monoclonal therapeutics).

PERSPECTIVES OF NANOTECHNOLOGY BASED IN VITRO DIAGNOSTICS IN INFLAMMATION

Laszlo Takaczs

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Molecular medicine aims at testing large number of genomic, proteomic and metabolomic analytes for early detection of disease and for disease management. At the same time, there is in an increasing need to bring diagnosis closer to the bedside and to reduce the time required to do the tests. A potential solution to the paradox is the application of nanotechnology in advanced molecular diagnostics.

Blood protein tests are used in every day disease diagnosis, however no systematic effort has been undertaken to address the majority of blood proteins with immunoassays. Biosystems International (BSI) develops monoclonal antibody libraries directed against the native human plasma proteome. Currently, BSI has over 800 mABs directed against different natural epitops present on plasma proteins of cancer patients and controls.

Here, the combination of monoclonal antibody proteomics and nanotechnology will be presented. Specific examples for cancer and its inflammation relevance will be addressed in the presentation.

DENDRONS/DENDRIMERS: WINDOW TO A NEW NANO-PERIODIC SYSTEM FOR UNIFYING NANO-SCIENCE. IN QUEST OF A COMMON LANGUAGE FOR COMMUNICATION BETWEEN NANOSCIENCE AND NANOMEDICINE

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"Science will continue to advance regardless of disputes over priorities. However, confusion and disagreement over common scientific language and standards can plunge a discipline into chaos. Such was the case for 19th century traditional chemistry before the emergence of Mendeleyev's Periodic Table of the Elements (1869)." Mendeleyev's Dream-The Quest for the Elements"- P. Strathern.(1)

This profound thought reflects on certain critical issues that may currently exist in the contemporary communication between the complementary areas of nanomaterials and nanomedicine. Simple extrapolation of first principles used to underpin traditional chemistry (Figure 1) have been invoked in a proposed new nano-periodic concept for well-defined nanomaterials (i.e., nano-element categories, compounds and assemblies) as described in Figure 2.

A 19th Century Paradigm for Traditional Chemistry

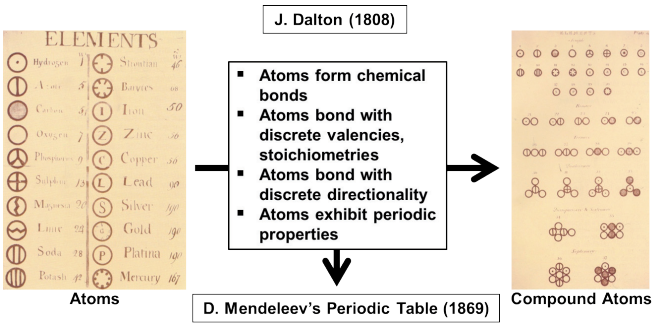

Figure 1. A 21st Century Paradigm for Nanoscience

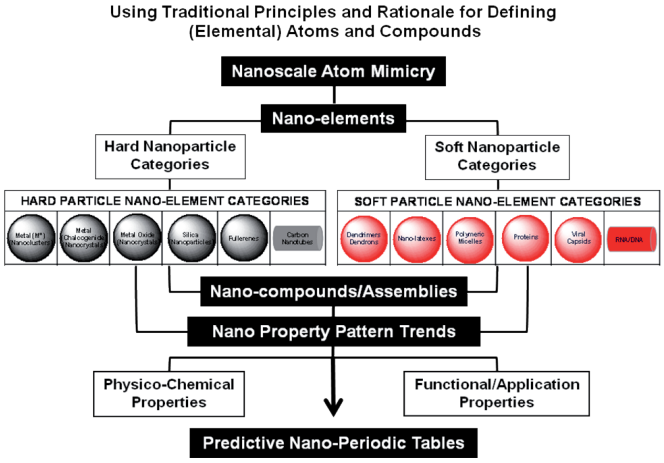

Figure 2. This lecture will introduce these recently proposed nano-periodic principles (2, 3) and illustrate their validity and manifestations in biological nano-systems such as viruses (i.e., Tobacco Mosaic Virus (TMV))(4), as well as synthetic nano-systems such as: dendrimer-based MRI contrast agents (5) and commercial, dendrimer based nano-compounds which are used as cardiological diagnostic nano-devices (i.e., Stratus® diagnostics) presently offered by Siemens (Germany).(6)

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MICROFLUIDIC SYSTEM FOR HIGH-THROUGHPUT SYNTHESIS AND SCREENING OF NANOPARTICLES FOR CANCER THERAPY

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The use of nanotechnology to engineer drug delivery vehicles comprised of controlled release polymers with targeting molecules has the potential to revolutionize drug therapy [1]. Although a myriad of nanotherapeutics have been developed on the bench side, many of them stay at the research stage due to their complexity and difficulty in their optimization. The central challenge for optimization of nanoparticles (NPs) for drug delivery is the ability to reproducibly manufacture and screen a library of NPs with distinct physicochemical properties, from which few are chosen for pre-clinical and clinical studies. We have developed a single-step, controlled procedure to prepare NPs with distinct properties, starting from a well-defined batch of precursors. The procedure uses microfluidic technology, which is ideally suited for the synthesis of monodispersed NPs due to its ability to rapidly mix reagents and provide homogeneous reaction environments. A single controlled nanoprecipitation step using microfluidic rapid mixing ensures reproducible self-assembly and removes variability due to bulk drop-wise mixing [2]. All chemical conjugation steps occur before formulation of the NPs from the polymers, which further minimizes variability. In this system, simply varying the proportions of different precursors results in NPs with different sizes, charge, PEG coverage, and ligand density. Furthermore, our microfluidic technology is amenable to scale up by using parallel streams, thus ensuring that the same NPs prepared on the bench for in vitro testing are the ones used for pre-clinical studies in different animals. This approach is robust and extremely simple in design, making it well-suited for preparing homogeneous NP formulations with distinct properties in an automated, high-throughput fashion.

Our 'proof-of-concept' microfluidic system is made of PDMS, and is composed of a mixing unit and a NP assembly unit (Figure 1). The mixing unit consists on a multi-inlet 2-layer mixer where different well-defined precursors such as polymers of different MW and charge, targeting- ligand-conjugated polymers, drugs, and solvents are mixed at different ratios into a homogenous solution (Figure 1). In the 'assembly' unit, the precursor solution is rapidly mixed with an anti-solvent (i.e. water) using 3D hydrodynamic flow focusing where NPs self-assemble after complete mixing [3]. With this system we can make, a library of NPs of different size, charge, surface chemistry, targeting agent density, and drug loading, by simply varying the flow ratios of the streams entering the mixing unit.

For the NPs to be effective, it is essential for them to avoid immune surveillance, specifically bind to cancerous cells, undergo endocytosis, and release the drug inside the cell [4]. In vitro screening of NPs with varying properties including surface charge, size, targeting ligand density, PEG densities, and chemical composition has the potential to yield valuable insights into the interactions of the NPs at the cellular level. With this aim, we integrated our microfluidic syn-

thesis process with a high-throughput FACS to screen for binding and uptake of different NP formulations (Figure 2A). As a proof-of-concept, here we show preliminary data of high-throughput screening of 12 NP formulation against macrophages (Figure 2B). These results indicate the effect of NP size and demonstrate the capability of our system to combinatorially synthesize and screen NPs against different cell lines.

To show the potential of our microfluidic technology to run parallel streams for in vivo experiments, we fabricated a microdevice with one water inlet and one organic solvent inlet that branch into 16 and 8 streams, respectively. With this type of device the particle throughput is further increased making enough NPs with well-defined properties to inject on animals. Figure 3 shows an experiment, where NPs with different surface chemistries (i.e. PEG coverage) were prepared in a relatively short amount of time and their pharmacokinetic profile in mice was measured. Currently we are working on making a channel with 36 parallel streams for larger animal studies.

Our experiments demonstrate a simple yet powerful technology to optimize polymeric nanoparticles in a high-throughput manner. This technology forms a robust platform for investigating the effects of different physicochemical properties of NPs that will lead to better understanding of the design parameters for polymeric NPs and smoother transition to the clinic. We believe this technology has the potential of being useful for a wide variety of future applications using different drugs, targeting ligands, or different cancer targets, where nanoparticle properties may need to be optimized again for each individual case [5].

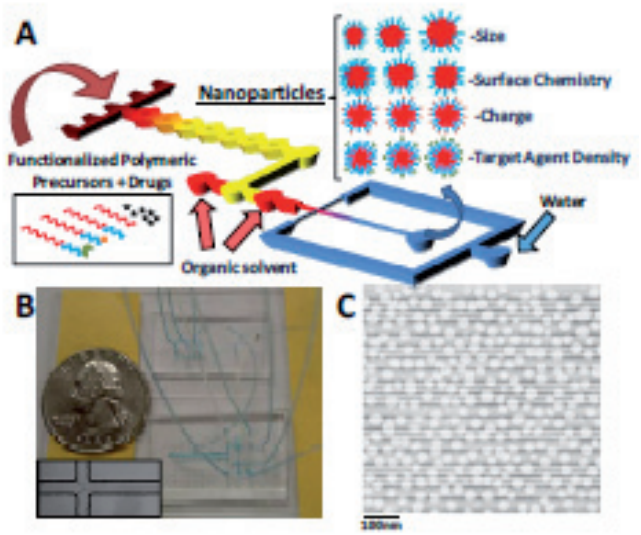

Figure 1. Microfluidic system for the synthesis of polymeric NPs. (A) Schematic of multi-inlet 3D mixing unit linked to the NP assembly unit. NP synthesis is enabled by 3D hydrodynamic flow focusing. By simply varying the flow ratio of the precursors, one can prepare a library of NPs of different size, surface chemistry, charge and targeting ligand density. (B) Photograph of system during operation. (C) TEM image of homogeneous nanoparticles synthesized in the system.

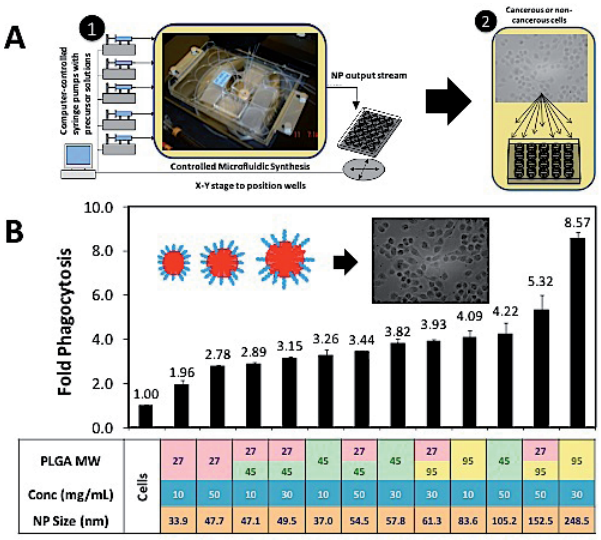

Figure 2. Strategy for high-throughput in vitro screening of nanoparticles. (A) Using our semi-automated microfluidic system we can make tens of NP formulations and incubate them in a 96-well with different cell types. (B) Small demo of screening 12 different nanoparticle formulations against macrophages. NPs of larger size made from high MW polymers are detected more readily by macrophages. Binding and uptake of NPs by macrophages was measured using FACS with a high-throughput sampling robotic arm.

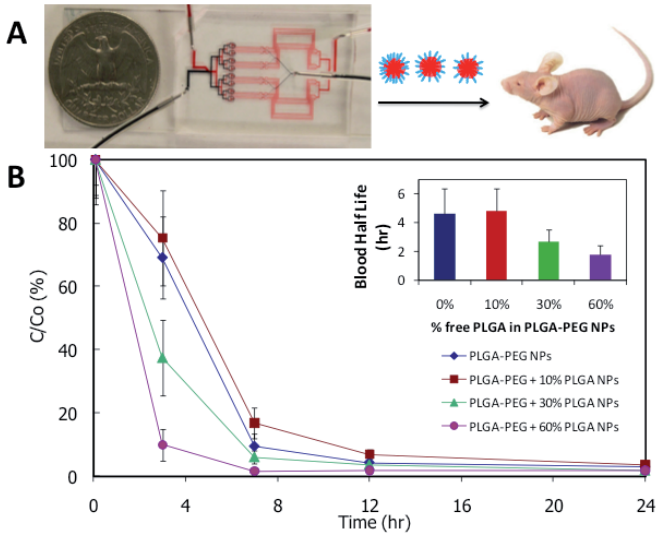

Figure 3. Making NPs at larger scale with parallel stream microfluidic system. (A) Using 8 parallel streams we prepared NPs with different PEG coverage at a rate of 4mg/min. (B) NPs from parallel stream microchannels were administered to mice and blood half-life was measured using a fluorescent reported embedded in the NP core.

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THERMOSENSITIVE POLYMERIC MICELLES FOR TARGETED DRUG DELIVERY

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Core-crosslinked biodegradable polymeric micelles composed of poly(ethylene glycol)-b-poly[N-(2-hydroxypropyl) methacrylamide-lactate] (mPEG-b-p(HPMAm-Lacn)) diblock copolymers have shown prolonged circulation in the blood stream upon intravenous administration and enhanced tumor accumulation through the enhanced permeation and retention (EPR) effect. To fully exploit the EPR effect for drug targeting, a DOX methacrylamide derivative (DOX-MA, Fig. 1B) was covalently incorporated into the micellar core by free radical polymerization. The structure of the doxorubicin derivative is susceptible to pH-sensitive hydrolysis, enabling controlled release of the drug in acidic conditions (in either the intratumoral environment and/or the endosomal vesicles). We showed that the micelles with covalently entrapped DOX had an average diameter of 80 nm and released the drug within 24 hours incubation at pH 5 and 37 oC, whereas only around 5 % release was observed at pH 7.4. DOX micelles showed higher cytotoxicity in B16F10 and OVCAR-3 cells compared to DOX-MA (Fig. 1A), likely due to cellular uptake of the micelles via endocytosis and intracellular drug release in the acidic organelles. Finally, the micelles showed better antitumor activity than free DOX in mice bearing B16F10 melanoma carcinoma (Fig. 1C, D), as well as no acute side effects.

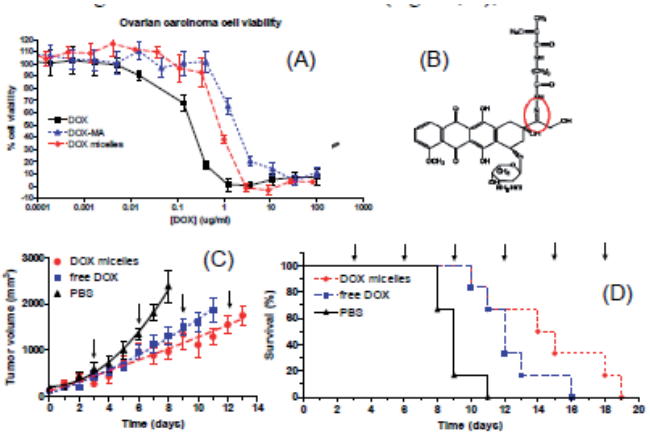

Figure 1. Panel A: % cell viability of OVCAR cells after incubation for 72 h with free DOX, micelles with co-cross-linked DOX-MA carrying a pH sensitive hydrazone spacer (DOX micelles, see structural formula in panel B) and the free DOX-MA. Panel C: Tumor volumes of mice bearing B16 melanoma carcinoma after administration of PBS, free DOX (3 mg/kg), and micelles with co-cross-linked DOX-MA (3 mg/kg); arrows represent i.v. injections. Panel D: Percent survival of mice bearing B16 melanoma carcinoma, after administration of PBS, free DOX (3 mg/kg), and micelles with co-cross-linked DOX-MA (3 mg/kg). Arrows represent i.v. injections.

To ensure tumor cell recognition and uptake of the core-crosslinked micelles, an anti-EGFR nanobody (EGa1) was attached on the surface of empty micelles using disulfide bonds, and the cell association was studied in cancer cells over-expressing EGFR. The conjugation was successful; cellular binding and uptake experiments in EGFR expressing cancer cells (A431 and 14C) showed increased association of the nanobody micelles compared to non-targeted micelles, as well as substantially increased uptake (Figure 2). In conclusion, our results show that mPEG-b-p(HPMAm-Lacn) polymeric micelles with covalently entrapped doxorubicin and an anti-EGFR nanobody on their surface is a system highly promising for the targeted delivery of cytostatic agents.

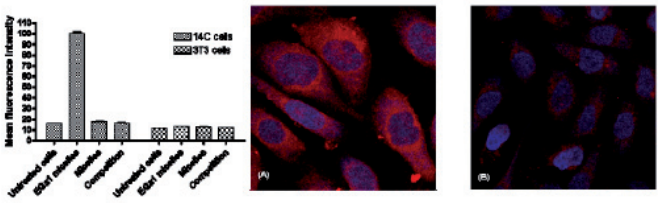

Figure 2. Graph: Mean fluorescence intensity determined by FACS analysis of 14C (EGFR positive) and 3T3 (EGFR negative) cells incubated for one hour at 4 oC with medium (untreated cells), with conjugates of EGa1 to rhodamine-labeled micelles (EGa1 micelles) and non-targeted rhodamine-labeled micelles (micelles) as well as EGa1 micelles containing an 8-fold excess of free nanobody (competition experiment). Pictures: 14C cells incubated for 4 hours at 37 oC with (A) conjugates of EGa1 to rhodamine-labeled micelles and (B) non-targeted rhodamine-labeled micelles. Rhodamine is depicted as red, Draq5 (nuclear staining) as blue.

MULTIPLATFORM NANODEVICES FOR CANCER DIAGNOSTICS

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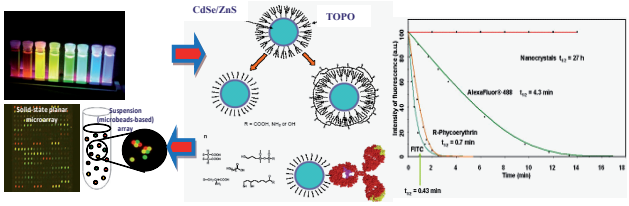
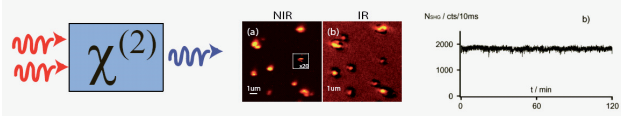
Department of Clinical Medicine, Trinity College Dublin, Ireland

The aim of this work is to develop breakthrough multimodal nanotechnological tools for early detection of rare tumour cells and cancer biomarkers. It is known that 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 super-sensitive "lab-on-a-bead", "lab-on-a-chip" and "lab-on-a-wire" nano-devices, as shown in figure 1 and 2.

The project utilises the expertise of 22 partners, from 9 countries, and it is funded by the European Commission under large scale cooperation programme (FP7 NMP – NAMDIATREAM).

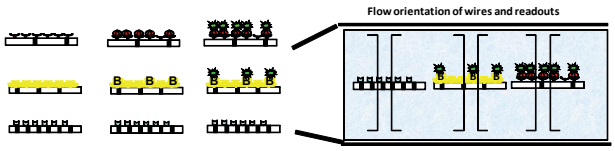


a) Principle of "lab-on-a-bead" based on monodispersed CdSe/ZnS Quantum Dots

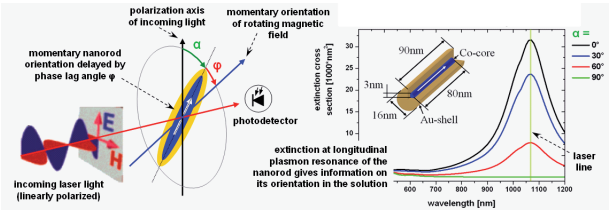


b) Principle of "lab-on-a-bead" based on advanced second harmonic generation

Figure 1: Schematic detection and use of two nanoparticle types used in multimodal imaging and diagnostic



a) Principle of "lab-on-a-wire" and "lab-on-a-chip" based on advanced microfluidics techniques



b) Principle of "lab-on-a-wire" based on plasmon-optical relaxation detection

Figure 2: Schematic detection and use of two types of nanowires used in multimodal diagnostic detection.

The breakthrough techniques will exploit magnetic, plasmonic and photo-luminescent advanced nanoscale material properties for high throughput early diagnosis and treatment of cancer. They allow identification of true "molecular signatures" of specific biomarkers and cancer cells in clinical samples. The validation of these nano-tools will be carried out in compliance with the OECD-regulatory policies in nano-materials.

The evaluation of disease progression during surgical procedures, assessment of tumour heterogeneity for optimised treatment strategies and miniaturised point-of-care biomarker diagnostic tests will be adapted for micro-litre sample volumes, which thereby implies the minimisation of the invasiveness and costs of diagnostic procedures.

Diagnostic devices developed by NAMDIATREAM are based on cost-effective technological solutions implementing optical, non-linear and magnetic properties of nanoparticles enabling to reach a qualitatively new level of detection specificity and sensitivity.

All these will be achieved within a tight safety regulatory and quality assurance control for the development of novel diagnostic, prognostic and monitoring technologies. Thus, risk assessment of the developed nanomaterials is carried out throughout the entire project in compliance with the latest OECD recommendations by all toxicology means and terms, such as bulk material, cytotoxicity, environmental and nanotoxiology.

Relevance to Clinical Medicine and Healthcare. NAMDIATREAM develops nanotechnological toolkits to address 3 of the 4 most frequent cancer types: breast, prostate and lung cancer. These toolkits are validated at both pre-clinical and proof-principle stages as novel probes and devices for detection of cancer molecular targets.

The devices and assay solutions are benchmarked against current clinical diagnostic methodologies and will provide better, faster and cost effective service to the stakeholders and the health service.

Technology Transfer and Translation into Clinical Practise. Through close interaction with the industry partners (SMEs and large multinationals) the innovative technology concepts proposed in NAMDIATREAM, based on super-sensitive nanocarriers (Fig. 1 and Fig. 2) will be adapted for high-throughput readout using advanced flow cytometry, protein microarray and high content screening technological approaches, operating on the principles of molecular, cellular and tissue diagnostics and imaging.

The involvement of several strong industrial partners into the project ensures a significant potential for the translation of the experimental results into clinical and point of care diagnostic and imaging systems.

CONCLUSIONS

The European Commission has determined that nanotechnology is one of the key enabling technologies of the future. The integration of these with highly sensitive biomolecular techniques with cutting-edge detection systems is expected to addresses key issues affecting the competitive position and growth of the European biomedical industry.

Therefore, main effort of this project is focused on bridging the gap between clinical diagnostic of disease and devices available and affordable to the patients.

As the project develops, innovative solutions incorporating cutting edge technological advances will be taken on board enabling further enhancement of the accuracy and reduction of sample volumes and time required for screening and diagnosis of cancer disease. Implications on the healthcare cost and the diagnostic procedure turnover times are evident.

ACKNOWLEDGMENTS

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Further information available at www.namdiatream.eu

THE POWERFUL LINK BETWEEN PHOTODYNAMIC- AND NANOMEDICINE

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Regarding history we can find parallel applications of both Photodynamic Medicine and Nano Technology even though the basic knowledge of each technology was not known at that time as well as their terms of today. At the beginning gold was undoubtedly the material of first choice for ancient nanotechnology. The old romans perfectly synhetized particles of a size of about 70nm, consisting of gold or gold-silver alloy. They did it in order to improve their artwork.

The historical way of light activated medical therapy was very different. In ancient times plants have been widely used through intake and/or local application of their extracts to cure dermatological ailments under influence of sunshine. This is known since about 3500 years first in ancient Egypt and later in India and in China. Thus, the principle of healing with light in combination with herbal extracts in medicine has been established a long time ago.

THE PRINCIPLE OF PHOTODYNAMIC MEDICINE

Photodynamic medicine includes both Photodynamic Diagnosis (PD) and Photodynamic Therapy (PDT) and is a rather new alternative method for clinical detection and therapy of cancer and other ailments. Their basic effects on microorganisms, however, have been known since about 100 years. The use of light in combination with a pharmacological substance (photosensitizer) that can be activated by light is an attractive combination and led to an impressive number of scientific studies up to now. PDT fulfils the requirements for successful cancer therapy such as selective destruction of neoplastic formations and minimal toxicity towards healthy tissue. In the presence of oxygen, the photosensitizer produces cytotoxic reactive oxygen species (ROS) upon light activation at a wavelength matching its absorption maximum and a reaction is initiated by which the photosensitizer is capable of transferring the energy to the desired reactants. By means of their highly oxidative nature, the generated ROS eventually cause the destruction of tumor tissue.

New research avenues aimed at improving Photodynamic Therapy combined with Nanotechnology for better cancer treatment A new project, called Target-PDT (funded by EuroNanoMed) is designed to increase the effectiveness of PDT for treating cancer by developing a novel nanocarrier-based approach. Focusing on PDT against bone cancer and head-and-neck squamous cell carcinoma, a tumor e.g. of the oral cavity, the team of the consortium will study the delivery and targeting of the photosensitizer encapsulated into lipid nanoparticles. For both cancer forms, current treatment regimes often result in low cure rates and show serious side effects or a poor functional outcome. The nanocarriers offer a high payload that will include antibodies targeting specific tumor biomarkers.  PDT has already shown significant potential for improving cancer treatment because it offers a strictly focused application, biocompatibility with other

forms of treatment, the option for repeated use, excellent cosmetic or functional outcomes and fast recovery. Indeed, typically there is a modest enhanced accumulation of the photosensitizer in tumor tissues and an additional selectivity is mainly provided by the confined illumination of the target area.  But the use of PDT has been restrained by limited effectiveness of the photosensitizer on reaching the tumor and the potential damage to healthy cells around the tumor. Improved targeting of the photosensitizer by using targeted nanoparticles prevents damage to the surrounding healthy tissue.  We expect that the nanocarrier-based approach will significantly improve delivery and targeting of the photosensitizer, enhancing concentrations at the tumor site even after systemic application. &#8232.

How to keep ethics in nanomedicine within daily life and work routine

Klaus-Michael Weltring

Director, Network Bioanalytik, Münster, D

Nanotechnology is an enabling technology and as such will accelerate our understanding of diseases at the molecular level. It will help to earlier diagnose diseases, to make implants more biocompatible and lasting, and to target drugs to disease sites more accurately. In other words it will contribute to the development of modern medicine. No more, no less!

Taking this into account it becomes clear that nanotechnology applied to medicine is facing the same ethical and social questions known in daily life of medical ethics for a long time. So, no big deal?

Simulation solutions for the challenges in nanomedicine

Wolfgang Wenzel

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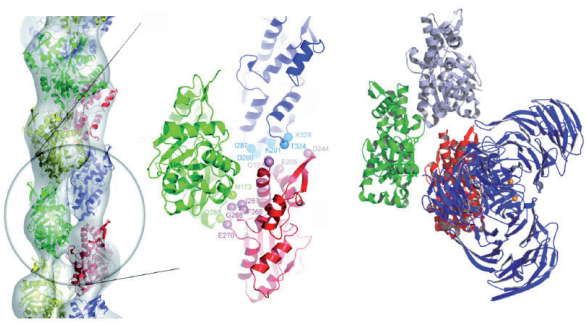
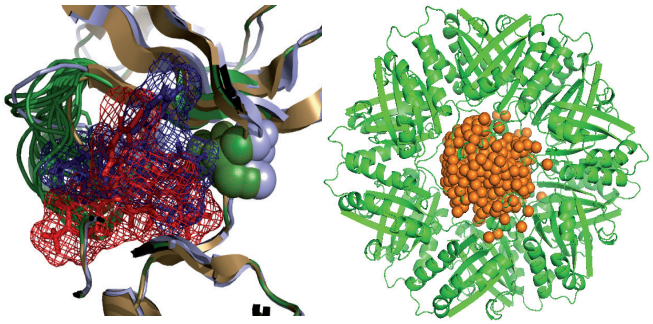
Driven by ever more powerful computational resources, simulation methods have become increasingly important to compliment experimental investigations in many scientific disciplines, including nanomedicine. In this talk I will review simulation methods to conquer this “time-scale gap”, which have allowed significant progress towards understanding and rational design of biological function. Specifically I will report recent developments in drug discovery, including in-silico discovery of nanomolar compounds for blood coagulation; protein modeling and structure prediction, including identification of the genetic causes for human developmental disorders and bio-nano-applications, such as investigations of nanoparticle membrane permeability, design of biocompatible surfaces and protein mediated nanoparticle synthesis.





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Silver nanoparticles - the real “silver bullet” in clinical medicine?

Kenneth Kak Yuen Wong

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The advance in nanotechnology has been setting at an explosive pace. Out of all kinds of nanoparticles used in the filed of nanomedicine, the metallic nanoparticles have shown great promise in terms of biomedical applications. Indeed, the use of silver nanoparticles has especially become more widespread in our society. While many believe that silver can be extremely useful in clinical medicine, firm evidence is still lacking. Furthermore, relatively little research has been done using nanosilver in various disease models. Our group’s current research focus is the understanding of the biological actions of the silver nanoparticles in wound healing and regeneration.

Our previous study has revealed that silver nanoparticles (AgNPs) have potential to promote wound healing by accelerated re-epithelization and enhanced differentiation of fibroblasts. However, the effect of AgNPs to the functionality of repaired skin is unknown. Therefore, we undertook a recent study to explore the tensile properties of healed skin after treatment with AgNPs. Immunohistochemical staining, quantitative assay and scanning electron microscopy (SEM) were utilized to detect and compare collagen deposition, the morphology and distribution of collagen fibers. Our results showed

that AgNPs improved tensile properties and better fibril alignments in repaired skin, with a close resemblance to normal skin. Based on our findings, we concluded that AgNPs were predominantly responsible in regulating deposition of collagen and resulting in excellent alignment in the wound healing process. The exact signaling pathway for which AgNPs have effect on collagen regeneration is yet to be further investigated.

PRESENTATIONTITLE: SHEAR RESPONSIVE NANOSYSTEM FOR CARDIOVASCULAR DISEASE

Andreas Zumbuehl

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Heart attack is the leading cause of global burden of disease and mortality. Ambulatory treatment focuses on intravenous administration of anti-aggregates, anticoagulants, fibrinolytic agents and 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 ensues in complications such as bleeding (fibrinolytic agents) or vasodilation (nitroglycerin) inducing severe hypotension and diminished blood perfusion of the suffering heart.

Here, we show a fresh approach for targeted drug delivery; elevated shear stress, similar to those found in critically constricted coronary arteries, acts as a localized physical trigger for the release of liposome-encapsulated drugs. In controlled in vitro fluorescence release studies, we have found that certain egg-PC-based liposomal formulations led to a preferential release in constricted artery models. Additionally, vesicles containing exclusively the artificial phospholipid Pad-PC-Pad were considerably more sensitive to shear-induced release than mixtures with the natural phospholipid. An explanation of this phenomenum based on the very special geometry of Pad-PC-Pad vesicles will be given.

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.

CLINAM European Foundation for Clinical Nanomedicine

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Conference Proceedings

PART III

Curricula Vitae of the Poster Authors

These are Submitted Individual Posters.

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COLLABORATIONS IN RESEARCH PROJECTS

- Surface Functionalization of SWNT by PEG-PE Lipid-Core Polymeric Micelle for Nucleolin Aptamer Targeted Tumor Delivery of Chemotherapeutic Agents (ongoing), Department of Pharmaceutics, School of Pharmacy, Shiraz
- PAMAM Dendrimer PEGylation for Delivery of Sorafenib to Hep-G2 Hepatocellular Carcinoma (ongoing), Department of Pharmaceutics, School of Pharmacy, Shiraz
- Bioequivalence Study of Generic Lamotrigine 50mg Tablets in Healthy Iranian Volunteers (finished in February 2008), Department of Pharmaceutics, School of Pharmacy, Shiraz
- Development of HPLC method for Determination of Lamotrigine Plasma Concentration (finished in 2004), Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran

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- MCHE English Certificate (2009)
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REFERENCES

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AWARDS, HONORS, DISTINCTIONS

October 2006 Award of young investigators by the Joint Meeting of Egyptian Group of Diabetes-Society of Endocrinology, Diabetes Mellitus and metabolism . June 2010 Certificate of Nanotechnology Training, Radiation Oncology Department, Northwestern University, Feinberg School of Medicine.

Mohammadali Amini

Senior student of Doctor of Veterinary Medicine at Islamic Azad University, Karaj branch (Karaj, Tehran, Iran). I worked my thesis on "Evaluation of immunological responses against chitosan nanoparticles containing FMDv in animal model as a novel mucosal vaccine". Since 2007, I have worked in Biomedical laboratory of Islamic Azad University, Pharmaceutical Biotechnology laboratory of Tehran University of Medical Science and Biotechnology laboratory of Razi Vaccine and Serum Research Institute on in vitro and in vivo investigation of ability of Chitosan nanoparticles as a carrier for mucosal virus delivery, in vitro study of budesonide loaded PLA nanoparticles performance for nebulization and extraction and characterization of chitosan from fungi, which the results has been published and submitted in several international journals. Dou to my research qualification, I was elected as The Best Student Researcher of the year in 2010 at Islamic Azad University-Karaj branch and succeeded to be as a member of National Institute of Elite and Young Researchers Club. To peruse my education, I am looking for a Ph.D. position for a research on Drug and Vaccine delivery systems.
Contacts: NO. 12, 9th alley, Masjed Street, Keshvari Street, Pasdaran Street, Tehran, Iran, 1664844113.Home: (+98) 21 22848984, Cell: (+98) 9122720389, E-Mail: amini918@gmail.com

Mehdi Shafiee Ardestani

Date of Birth: 29.November.1982, Iran, Iran Id. card No.:3289, Mobile Phone: +98-09128267629, Tel/Fax: + 98-21-66953311, Married. Correspondence address: Department of Hepatitis B and HIV and Nanobiotechnology, and R&D, Pasteur Institute of Iran, Tehran, Tehran, Iran, mehdishafieea@aol.com, shafieeardestani@gmail.com, shafieeardestani@yahoo.com

CURRENT POSITION:

Assistant Professor of Pharmaceutical and Radiopharmaceutical Sciences, Pasteur Institute of Iran

EDUCATION

- 10. October 2001 until 25. September 2006: Pharm.D. (Doctorate in Pharmacy). Faculty of Pharmacy, Jondishapour University of Medical Sciences, Ahwaz, Iran
- 30. September 2006 until 15. December 2009: PhD. (Radiopharmaceutical Chemistry), Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

INSTRUMENTATION-TECHNIQUE EXPERIENCES

-Nanomedical experiences especially nano-drug targeting - HPLC - UV/Visible Spectroscopy - IR Spectroscopy - Stereotaxic Surgery - Microdialysis - cannula implantation in the brain and spinal cord for drug delivery purposes -Patch clamp, Current clamp and voltage clamp - The use of Electrical Lesion Maker for demolishing the special area in the brain - [Electrophysiology] - Working with Gamma counter -Iodine/ 99mTc-labeling -Gd-labeling, T1 relaxation time measurment & MRI Imaging -Quality controls of 99mTc radiopharmaceuticals - Vaccine formulation

RESEARCH ACTIVITIES AND EXPERIENCES

A. Dissertations: 1. Title of Pharm.D Thesis: Effects of COX-2 and COX-2 gene expression inhibitors on memory-movement disorders and striatal neurotransmissions in normal and rat model of Parkinson's disease. [Grant no. 84U60] This study was supervised by Professor Hf Moghaddam and Professor AA Hemmati. 2. PhD thesis title: With Supervision by: Dr Amanlou, Professor Alavi and Dr Sadat Ebrahimi. Synthesis, in vitro and in vivo evaluations of Gadolinium-glycosilated compounds as novel cancer contrast media agents for MR imaging

TEACHING EXPERIENCES

- General Chemistry: Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran
- Organic Chemistry: Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran
- Medicinal Chemistry: Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran,
- Instrumentation: Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

AWARDS

- [Yong scientist traveling grant] selected by the editorial board of XXXVI International Congress of Pharmaceutical Sciences. Kyoto, Japan
- Graduation with the First and Best degree in Board Examination of Radiopharmacy-2009
- The Best Iranian Student of the Year 2009 [Receiving the award from the Mr. President]
- First degree in PhD Course of Radiopharmacy Entrance-2006
- The First Young Scientist of Jondishapour University of Medical Sciences, 2005

EDITORIAL BOARD MEMBER: International Journal of Pharmacy and Pharmaceutical Sciences (JAPAN)

ACTIVE-IN PROGRESS RESEARCHES:

- Synthesis & evaluation of DTPA-Levodopa)n=1,2,3-Gd3+ as a novel dopaminergic receptor imaging agent for MRI
- Synthesis & evaluation of Levodopa -Alendronate-Gd3+ as a novel dopaminergic receptor imaging agent for MRI
- Liposome & polymer labeling with Gadolinium for non invasive pharmacokinetic investigation using MRI
- Synthesis of asparagines DTPA-Gd3+ as novel tumor MR imaging agent
- Synthesis of anti-constipation derivatives from thebaine-drived compounds with aryl-maleimides [Grant No: 8077]
- Synthesis & preliminary evaluation of Gd3+-glucose-tamoxifen porphyrin as a novel Breast Cancer MR imaging agent
- Synthesis & investigation of special Gd3+-nano- polymer using the different labeling methods for lymphoma tumor MR imaging {A part of PhD thesis]
- Cancer cell inducing to provide animal models [A part of PhD thesis]
- Synthesis, preclinical & clinical imaging of the novel iodinated ethylen diamine as novel melanoma MR imaging agent
- Evaluation of the Trp-DTPA-Gd complex as a possible cancer MRI agent
- As3+-DTPA-DG; novel anti-cancer agent.
- DTPA-career based design (more than 4 Pharm.D Thesis) &…
- Vaccine Formulations (DTP-Hib), (DTP-HB) Vaccine conjugation, Nano-drug targeting
- Bioconjugation and anti cancer drugs

REFERENCES

Professor Dr. Moghaddam, Department of Physiology & Physiology Research Center, School of Medicine, Jondishapour University of Medical Sciences, Ahwaz, Iran

Dr SE. Sadat Ebrahimi, Associated professor of Medicinal Chemistry, Department of Medicinal Chemistry and Radiopharmacy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

My non native advisors from United States of America (USA): Professor Dr Abbas Alavi, Professor Dr Kenneth N Raymond, Professor Dr Wahl, Professor Dr Rahmim.

Marco Ballestri

Since 1989 I hold a permanent position as a researcher at the Institute for the Organic Synthesis and Photoreactivity of the Italian National Research Council.

My current work is mainly focused on new formulations for anti-cancer drugs delivery systems, based on core-shell nanoparticles. Besides this activity, I am involved in planning and developing novel methods for the preparation of new drugs and prodrugs-nanoparticles conjugates for the photodynamic therapy. I am also currently entailed in a carbon nanotubes project aimed to the preparation of anticancer drugs delivery systems.

Along with these research fields, we are also studying systems for the nanoparticles-incorporation of dyes for photovoltaic and diagnostic applications, thus ranging from medical field to materials. In the past I developed radical reactions kinetics of functional groups reduction. Later, I switched to the preparation of nanoparticles for DNA plasmid and proteins delivery as new vaccine methods.

I am enclosing hereafter some of the most relevant publications on my research activity.

- 1. Samori, C.; Sainz, R.; Menard-Moyon, C.; Toma, F. M.; Venturelli, E.; Singh, P.; Ballestri, M.; Prato, M.; Bianco, A., Potentiometric titration as a straightforward method to assess the number of functional groups on shortened carbon nanotubes. Carbon 2010, 48 (9), 2447-2454.
- 2. Rimessi, P.; Sabatelli, P.; Fabris, M.; Braghetta, P.; Bassi, E.; Spitali, P.; Vattemi, G.; Tomelleri, G.; Mari, L.; Perrone, D.; Medici, A.; Neri, M.; Bovolenta, M.; Martoni, E.; Maraldi, N. M.; Gualandi, F.; Merlini, L.; Ballestri, M.; Tondelli, L.; Sparnacci, K.; Bonaldo, P.; Caputo, A.; Laus, M.; Ferlini, A., Cationic PMMA Nanoparticles Bind and Deliver Antisense Oligoribonucleotides Allowing Restoration of Dystrophin Expression in the mdx Mouse. Molecular Therapy 2009, 17 (5), 820-827.
- 3. Castaldello, A.; Brocca-Cofano, E.; Voltan, R.; Triulzi, C.; Altavilla, G.; Laus, M.; Sparnacci, K.; Ballestri, M.; Tondelli, L.; Fortini, C.; Gavioli, R.; Ensoli, B.; Caputo, A., DNA prime

and protein boost immunization with innovative polymeric cationic core-shell nanoparticles elicits broad immune responses and strongly enhance cellular responses of HIV-1 tat DNA vaccination. Vaccine 2006, 24 (29-30), 5655-5669.

- 4. Chatgilialoglu, C.; Ferreri, C.; Ballestri, M.; Mulazzani, Q. G.; Landi, L., cis-trans isomerization of monounsaturated fatty acid residues in phospholipids by thiyl radicals. Journal of the American Chemical Society 2000, 122 (19), 4593-4601.
- 5. Ballestri, M.; Chatgilialoglu, C.; Clark, K. B.; Griller, D.; Giese, B.; Kopping, B., TRIS(TRIMETHYLSILYL)SILANE AS A RADICAL-BASED REDUCING AGENT IN SYNTHESIS. Journal of Organic Chemistry 1991, 56 (2), 678-683.

György Báthori

Ph.D., M.D., Date of birth: 25 November 1950. Married with three children, Hungarian. Telephone: mobile: 70 554 1345, gyorgy.bathori@gmail.com

EMPLOYMENT HISTORY

2009-Nanomedicina Kutatási Központ, Head of the Applied Liposome Research Laboratorium. 2008-2009 Seroscience Ltd, Budapest. 2007-2008 Solvo Biotechnology, Budaors, Hungary, Project management coordinator. 2005-2007 Solvo Biotechnology, Szeged, Hungary, Head of Adme/Tox Screening Department (Fee for Service Lab). 1989-2004 Semmelweis University, Department of Physiology, Budapest Hungary, Associate professor. 1984-1989 Semmelweis University, Department of Physiology, Budapest Hungary, Researcher. 1975-1984 Semmelweis University, Department of Biophysics, Budapest, Hungary, Researcher. 1972-1975 Semmelweis University, Department of Biophysics, Budapest, Hungary, Research student.

RESEARCH EXPERIENCE ABROAD

- 2002-2004 Thomas Jefferson University, György Hajnóczky`s lab, Guest researcher
- 1998 University of Padova, Department of Biomedical Sciences, Dr. Mario Zoratti's lab, Visiting fellow (CNR - Hungarian Academy of Sciences exchange program)
- 1996-1997 University of Padova, Department of Biomedical Sciences, Dr. Mario Zoratti's lab, Telethon fellowship (Supervisor: Dr. Mario Zoratti)
- 1995 University of Padova, Department of Biomedical Sciences, Dr. Mario Zoratti's lab, Guest researcher
- 1994 University of Padova, Department of Biomedical Sciences, EMBO short term fellowship (Supervisor: Dr. Mario Zoratti)
- 1993 University of Padova, Department of Biomedical Sciences, EEC research fellowship (Supervisor: Dr. Mario Zoratti)
- 1991 University of Trieste, Department of Biochemistry, Biophysics and Macromolecular Chemistry (Supervisor: Prof. Gabriella Sandri)

EDUCATION

- 1998 Semmelweis University, Budapest, Hungary, PhD, Medical Degree, Title of the Thesis: Transport Properties of the Mitochondrial Porin
- 1975 Semmelweis University, Budapest, Hungary, Medical Degree

INVITED LECTURER

20-25 June 1999: NATO Advanced Research Workshop, Pécs, Hungary "Polymer Structure and Transport in Confined Spaces"

PUBLICATIONS

- Number of journal articles: 18
- Cumulative impact factor: 74,971 (without congress abstracts)
- Number of independent citations: 316
- Patent application: 1

TEACHING EXPERIENCE

- biophysics and general physiology for medical students
- FEBS advanced course on "Biochemistry of Membrane Transport" (1989, Budapest)

SKILLS AND COMPETENCES

- Liposome related Competencies: Preparation and working with artificial membranes as liposomes and planar bilayers (BLM), isolation and reconstitution of mitochondrial porin and some other proteins into liposomes. Experienced with fluorometry, confocal microscopy. Biotechnological applications of liposomes as immuno-adjuvants, drug vehicles for intracellular delivery, liposome based artificial erythrocites.
- Non Liposome related: Mitochondrial biochemistry and physiology, single channel analysis,raft and caveolar membrane fraction isolation , multidrug transporters.

Computer literacy: MS Office (Word, Excel, PowerPoint, MS Project EPM, MS Access, MS Groove) Curve fit and evaluation applications (Graphpad PRISM, Microcal Origin, Sigmaplot), Minitab (for 6 Sigma), Quality Comapgnone (for 6 Sigma) Bioinformatics (MACAW, ClustalX, Genedoc)

Project management knowledge: PMBOOK (Project Management Institute), also interested in 6 Sigma, PM course, organized by Szinergia Kft., Monitoring of outsourced projects

LANGUAGES

English - fluent, Russian - good, German - weak, Italian - beginner

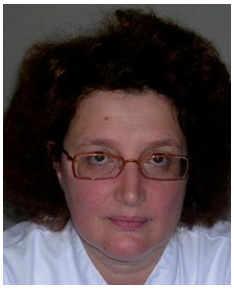
MEMBERSHIPS IN SCIENTIFIC SOCIETIES

Hungarian Biophysical Society, Hungarian Biochemical Society, Hungarian Society of Physiology, International Adjuvant Group, Hungarian Chamber of Physicians

Rodrigo Berté

I was born in Cuiabá - Mato Grosso - Brasil, I´m 24 years old and I graduated in Ciencias Físicas e Biomoleculares at the Instituto de Física de São Carlos - Universidade de São Paulo. I developed a project for 2 years in graduate which the title was: Collisions in cold samples of Na: preparation and characterization of reference cells. These cells were used to make an active control in dye laser frequency, used in the study of cold samples of sodium.

Nowadays I´m working in my Master´s degree project, in which we seek to functionalize AgNps with Antimicrobial Peptides and test the activity of this nanocompound against strains of multi-resistant bacteria. We see this project as being of great importance, because it is urgent to a developing and economically unequal country like Brasil to have applied science as a tool to fight against poverty.



Lina Bezdetnaya

(spouse Bolotine), Nationality: French. Professional address: Tél : 03 83 59 83 53 Fax : 03 83 59 83 78 E-mail : l.bolotine@nancy.fnclcc.fr. http://www.cran.uhp-nancy.fr/ (thematic Group "Engineering for Health") http://www.alexisvautrin.fr/

DIPLOMAS: 1980: Master Degree in Biophysics, Russian State Medical University, Moscow, Russia; Qualification: physician-biophysicist (1980)

- 1988: PhD Biological Sciences, speciality Biophysics, Institute for Physical Chemistry, Moscow, Russia. Accreditation for Supervising Research (Habilitation à Diriger les Recherches); Faculty of Medicine, Université Henri Poincaré, Nancy I, France

PROFESSIONAL EXPERIENCE

- 1988- 1996 Assistant Professor, Department of Biological and Medical Physics, Russian State Medical University, Moscow
- 1993-1996: Invited Professor, Faculty of Medicine, Nancy, University Henri Poincaré, Nancy I, France,
- 1996-1998: Associate Professor, Faculty of Medicine, Nancy, University Henri Poincaré, Nancy I, France , Associate Senior Lecturer, Faculty of Medicine, Nancy, Université Henri Poincaré, Nancy I, France, Senior Researcher, Centre Alexis Vautrin, Nancy, France

- Since September 2007: Associate Professor–Hospital Practioner, University Henri Poincaré, Centre Alexis Vautrin ; Section CNU, 47-02 cancerology/radiotherapy, Responsable of the Unit "Research" of the Centre Alexis Vautrin; Supervisor of 8 thesis since 2004, Jury member in 11 PhD theses and 2 HDR theses (2004-2008)

ADMINISTRATIVE ACTIVITIES

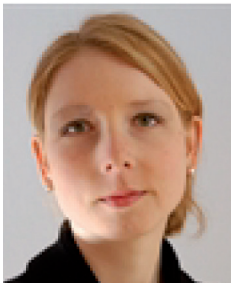
- Steering committee of the French Society of Photobiology (since 2005, renewed in 2008)
- Steering committee of the Center Alexis Vautrin (2008-present)
- Steering committee of the Head and Neck Optical Diagnosis Society (UCL, London) (since 2009)
- Appointed Expert at AERES (Evaluation Agency for Research and High Education), march 2009
- Appointed Expert at the Ministry of Foreign and European Affaires of France (September 2009)
- Grant holder of the international Convention of CNRS with the Institute of Bioorganic Chemistry (2006-2007, N 19123); Grant holder of the international program ECO-NET 2008-2009.
- Grant holder of the research contract with the Ligue Against Cancer (Department 54) (2003- 2011); Co-participant in the contract with the National institute of Cancer (INCa) (2007-2008)

SCIENTIFIC PRODUCTION

More than 55 papers in peer reviewed journals
More than 100 communications in national and international conferences or meetings

SELECTED PUBLICATIONS (2007-2010)

- S Marchal, A François, D Dumas, F Guillemin, L. Bezdetnaya : Relationship between subcellular localisation of Foscanâ and caspase activation in photosensitised MCF-7 cells. Br J Cancer, 2007, 96, 994-951.
- MA. D'Hallewin, D. Kochetkov, Y. Viry-Babel, E. Werkmeister, D. Dumas, S. Gräfe, V. Zorin, F. Guillemin, L. Bezdetnaya. Photodynamic Therapy with intratumoral administration of lipid-based mTHPC in a model of breast cancer recurrence. Lasers Surg Med. 2008 40, 543-54
- H-P. Lassalle, D. Dumas, S. Gräfe, M-A. D'Hallewin, F. Guillemin, L. Bezdetnaya. Correlation between in vivo pharmacokinetics, intratumoral distribution and photodynamic efficiency of liposomal mTHPC . J Control Release, 2009 134 :118-24
- 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. 2010 25;4(5):2531-8.
- Garrier J, Bressenot A, Grafe S, Marchal S, Mitra S, Foster TH, F. Guillemin, L. Bezdetnaya. Compartmental targeting for mTHPC based photodynamic treatment in vivo: correlation between efficiency, pharmacokinetics and regional distribution of apoptosis. Int. J. Radiation Oncology Biol. Phys. 2010, 78 : 563-71



Ines Block

Dr. rer. nat., CPR: 170779-3496, Oppermannsvej 5, 5230 Odense M, Denmark, Phone: +45 50388707, Mail: iblock@health.sdu.dk. Date of birth: July 17th, 1979 (Bremerhaven, Germany)

SCHOOL EDUCATION: July 1999: High school graduation ("Abitur")

EDUCATION AND POSITIONS

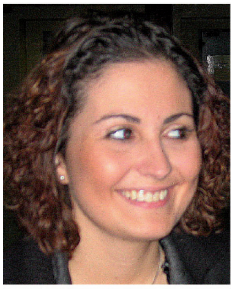
- October 1999–March 2005: Studies of Biochemistry, Ernst-Moritz-Arndt-University of Greifswald, Germany
- August 2002–February 2003: Research internship at the Chemistry Department of the New Mexico State University in Las Cruces (New Mexico, USA), Research field: Development of electrochemical biosensors
- 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 Medical Biotechnology Center 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 novel biochip format for profiling the molecular fingerprint of cancer stem cells

Diana Boraschi

Institute of Biomedical Technologies, CNR, Area della Ricerca di Pisa, Pisa, Italy. Diana Boraschi is an immunologist that built her experience both in academic institutions (Italian National Council for Nuclear Energy, National Cancer Institute in Bethesda, MD, Mario Negri Institute in Milan, Italy, University of Michigan Medical School, Ann Arbor, MI) and industrial settings (the vaccine company Sclavo in Siena, Italy, the pharmaceutical company Dompé in L'Aquila, Italy). She is presently Research Director at the Italian National Research Council in Pisa. She has served as Director of Fellowships at the Human Frontier Science Program Organization in Strasbourg, France, and as external expert evaluator for the research programmes (5FP, 6FP, 7FP) of the EU Commission in Brussels, Belgium. She is author of 135 peer-reviewed research articles in immunology, editor of ten books, and inventor in eight patents, in addition to numerous monographic and divulging publications.
Diana Boraschi studies the mechanisms of innate defence responses, focussing in particular on the role of macrophages and inflammatory cytokines in the effector phase of defence reactions against infections and tumours. Her main interests are the receptors of the IL-1R/TLR family and their cytokine ligands (IL-1 and IL-18). A fragment of IL-1 endowed with immunostimulatory activity is now defined as the "Boraschi loop". She is currently studying the role of inflammation in the pathogenesis of diseases (from autoimmune syndromes to degenerative diseases such as ALS), with particular emphasis on abnormalities in the activation of macrophages. Within the study of the initiating mechanisms causing disease, she has recently addressed the possible impact of nanoparticles from different sources in triggering pathology-related inflammation.



Anita Boscaini

28/03/1983 - Negrar (Verona), Italian, Phone +39-45-8126457, E-mail: "boscaini, anita" <anita.boscaini@gmail.com>, Institution: Verona University, phD student, 2009-2011, Graduate school in Translational Biomedicine; phD thesis on Nanomedicine. Master Degree in Industrial Biotechnology, achieved on 18/12/07 with graduating mark 110/110, at Padua University with specialization in "Immunological biotechnology and production of recombinant proteins". Thesis title: "Production and Purification of Immunotoxin directed against the PSMA (Prostates Specific Membranes Antigen)". Bachelor Degree in Industrial Biotechnology, achieved on 27/07/08 with graduating mark 102/110 at Padua University. Thesis title: "Analysis of NadA immunoreattivity through ELISA assay and Dot Blot"

PUBLICATIONS

- Vincenco Amendola, Moreno Meneghetti, Stefania Fiameni, Stefano Plizzi, Giulio Fracasso, Anita Boscaini and Marco Colombatti. SERS labels for quantitative assays: application to the quantification of gold nanoparticles uptaken by macrophage cells Analytical Methods, DOI: 10.1039/c0ay00660b IN PRESS
- Boscaini A., Fracasso G., Anselmi C., Cingarlini S., Giglio B., Zanini S., Selvestrel F., Mancin F., ReddiI E., Papini E., Colombatti M. (2010) Tumor Targeting with Guided Silica Nanopartilces for Prostate Cancer Therapy. In Nanotechitaly 2010, 20-22 Octobe,Venice, p 246.
- Fracasso G., Amendoola V., Anselmi C., Marcolongo G., Cingarkgarlini S., Cremonese G., Figini M., Boscaini A., Meneghetti M., Colombatti M. (2009). Investigation of Properties of Anti-Prostate Specific Membrane Antigen-targeted Gold Nanoparticles as Drug Carriers in Tumor Therapy. In: 2nd Targeted Tumor Therapy. Berlin, 31 Marzo-03 Aprile/2009, p. 62-62
- Boscaini A., Fracasso G., Amendola V., Anselmi C., Marcolongo G., Cingarlini S., Cremonese G., Figini M., Meneghetti M., Colombatti M. (2009). Anti-prostate specific membrane antigen targeted gold nanoparticles in tumor therapy and diagnosis. In: 51st Annual meeting of the Italian Cancer Society. Milan, 23-26/11/2009, p. 75-75

Wolfgang Bost

Fraunhofer IBMT, Ultrasound, Ensheimer Strasse 48, St. Ingbert, 66386, Germany, wolfgang.bost@ibmt.fraunhofer.de

Wolfgang Bost received the diploma in Physics from the Saarland University in 2008. He is currently working toward the Ph.D. degree in Physics at Saarland University in cooperation with the Fraunhofer Institute for biomedical research in Sankt Ingbert. His currently research interests include biomedical ultrasound imaging, especially for high resolution optoacoustic and acoustic imaging.

Helle Christiansen

University of Southern Denmark, Molecular Oncology, J B Winsloew Vej 25, Odense 5000, Denmark, hchristiansen@health.sdu.dk. Jan 2010-present: Head of High Throughput Screening Robotic Platform at the Molecular Oncology Unit inst.of Molecular Medicine University of Southern Denmark. Sept 1st 2008-present: Post doc at The Molecular Oncology Unit (Prof Jan Mollenhauer) inst. of Molecular Medicine University of Southern Denmark.

- March–August 2008: Scientific Assistant at the Clinic for Molecular and Endocrinological Treatment (KMEB), Odense University Hospital
- March 2005–June 2008: Ph.D. student at the Clinic for Molecular and Endocrinological Treatment (KMEB) and the Centre for Experimental Bioinformatics (CEBI), Biochemistry & Molecular Biology (BMB), University of Southern Denmark. Supervisors: Professors Jens S Andersen (CEBI) and Moustapha Kassem (KMEB)
- February 2004–March 2005: Research Assistant the Clinic for Molecular and Endocrinological Treatment (KMEB) and the Protein Research group, Biochemistry and Molecular Biology (BMB) at University of Southern Denmark
- July 2003–April 2004: Research in and translation of patent applications in molecular biology, biochemistry, physics, medicine and chemistry. Lingtech A/S Vesterbrogade 4, 1620 Kbh.V.
- M.Sc-project 2002/2003: Supervisor: Professor Peter Højrup, The Protein Research-group, & Molecular Biology University of Southern Denmark and Professor James D Crapo MD, Duke University, Durham, NC (North Carolina) USA.
- 2003: Instructor in 4 week lab course Analytical Protein Chemistry at University of Southern Denmark
- 2002 Novo Nordisk Scholarstipend for M.Sc students, "Novo Nordisk Scholarship Program in Biotechnology and Pharmaceutical Sciences" kr. 61000.

STAYS/VISITS

Fall 2008 Max Planck Institute Dresden: Instrument Center for Robotics, inst. for Molecular Genetics. Fall 2009 Max Planck Institute for infection biology, Berlin (Chariteplatz), Research Center for genome-wide screens and automated assays via robotic platforms.

Reza Ahangari Cohan

Nationality: Iranian, Address: Pasteur Institute of Iran, 12 Farvardin Ave., Tehran, Iran, E-mail: Cohan_r@yahoo.com

EDUCATION: Ph.D., Pasteur Institute of Iran, Pharmaceutical Biotechnology (2006-now). Pharm. D., Tabriz University of Medical Science, Pharmacy (2000–2006).

PROFESSIONAL EXPERIENCE: Immuno-Chemical Techniques, Molecular Cloning Techniques, Chromatography Techniques, Cell Culture Techniques, Bioinformatics (Modeling, Molecular Dynamics Simulation)

PUBLICATION: Nouri Inanlou D., Yakhchali B., Khanahmad H., Gardaneh M., Movassagh H., Ahangari Cohan R., Mahdian R., Zeinali S. (2010), β-Globin Gene-targeting with Integrase-Defective Lentiviral Vectors. Biotechnology Letters, 32 (11) pp: 1615-1621.

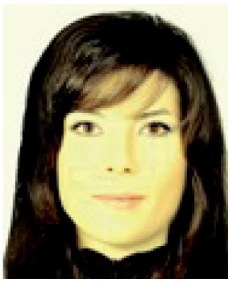
Marganit Cohen-Avrahami

E-mail address: marganit7@gmail.com. Ph.D student in chemistry. Thesis title: liquid crystals applications for pharmaceutical purposes, supervised by Prof. Nissim Garti and Dr. Abraham Aserin, the Institute of Chemistry, the Hebrew University of Jerusalem..

M.Sc. Thesis title: microemulsion-based drug delivery systems and creating polymorphism, supervised by Prof. Nissim Garti and Dr. Abraham Aserin, the Institute of Chemistry, the Hebrew University of Jerusalem.

PUBLICATIONS

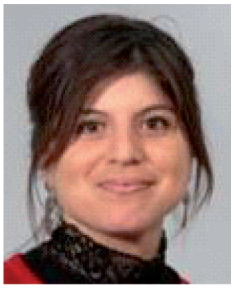
I. Improved solubi lization of Celecoxib in U-type nonionic microemulsions and their structural transitions with progressive aqueous dilution; Journal of Colloid and Interface Science, Volume 299, 2006, Issue 1, Pages 352-365. II. Crystallization of Celecoxib in Microemulsion Media; Journal of Dispersion Science and Technology, Volume 28, 2007, Issue 8, Pages 1228-1235. III. HII mesophase and peptide cell-penetrating enhancers for improved transdermal delivery of sodium diclofenac; Colloids and Surfaces B-Biointerfaces, Volume 77, 2010, Issue 2, Pages 131-138. IV. Sodium Diclofenac and Cell Penetrating Peptides Embedded in HII Mesophases-Physical Characterization and Delivery; submitted to the Journal of Physical Chemistry B.



Elina Esmaeilzadeh-Gharedaghi

This is Elina Esmaeilzadeh- Gharedaghi MS.c. student of Medical Nanoechnology at Teheran University of Medical Science. I have worked my thesis on "Optimization the condition of preparation chitosan nanoparticles by sonication method with Artificial Neural Networks (ANN)" (supervisor: Dr. Amir Amani) in laboratory of pharmaceutical biotechnology (pharmacy School of Teheran University of Medical Science). Because of my deep enthusiasm in drug and gene delivery systems, since last two years, I worked on "Preparation of chitosan nanoparticles with ultrasound

method for siRNA delivery in cancerous cell lines". In this project, I have investigated different parameters affecting synthesis of chitosan/siRNA nanoparticles using sonication method. As the result, the optimum parameters affecting size, PDI and loading efficacy of particles for silencing EGFP gene in HECH cell line has been estimated. Since Apr 2010, I have also cooperated in a project about "production and characterization of budesonide loaded poly (lacticacid) nanoparticles for nebulization". The results of mentioned researches have been published and submitted in several international journals. Contacts: Permanent Address: #30, 2nd Bahar alley, Sarv-e-Gharby St., Sarv Square, Saadatabad, Tehran, Iran, Postal Code: 19988-93551, Home: (+98) 21 2208717, Cell: (+98) 91221444263, E-Mail: esmaeilzade@razi.tums.ac.ir



Cinzia Esposito

Cinzia Esposito has studied Pharmaceutical Sciences in Naples where she also got her PhD. She then moved to the USA to work in the field of Membrane Biophyiscs at the Department of Chemistry, University of Pennsylvania, Philadelphia, PA. Presently she is working at the Department of Biophyiscal Chemistry of the Biozentrum, University of Basel. Her interest focuses on understanding the mitochondrial metabolism using novel techniques to monitor cellular impedance, the extracellular acidification and oxygen consumption rate, respectively.

Ilise Feitshans

Prof. Ilise L Feitshans JD and ScM is a USA Citizen, a bi-lingual lawyer with a Masters of Science in Public Health from the Johns Hopkins University, Member of the Bar of the Supreme Court of the United States and Former Member of the Faculty, Columbia University School of Law in the City of New York, and as a student, Legal Intern, Office of the Solicitor of the US Department of Labor for OSHA, (during the time of the Benzene case). She currently serves as a Visitin gScinetist at the University of Lausanne In Switzerland, and a Faculty Member a and a Doctoral Candidate in International Relations "Forecasting Nano Law" at the Geneva School of Diplomacy, Geneva Switzerland. Ilise is a Member of the Board of Directors of the International Safety Resources Association, ISRA, for whom she prepared detailed comments to NIOSH, in response their 2011 request for Public Comment regarding recommended exposure limits for carbon nanotubes fand nanofibers. Ilise is the Author, DESIGNING AN EFFECTIVE OSHA COMPLIANCE PROGRAM (Westlaw) and Bringing Health to Work (Emalyn Press), ilise@prodigy.net 41 79 836 3965 USA 917 239 9960

Luisa Fiandra

PROFESSIONAL PREPARATION AND APPOINTMENTS: Graduate studies (Natural Sciences-University of Milan, Italy); specialization in Transport Physiology. PhD in Animal Biology (University of Milan, Italy). 2003-2010 Postdoctoral fellowship (University of Milan, Italy) on Cell Biology and Transport Physiology. 2010-present Researcher in "L. Sacco" University Hospital of Milan, Italy, on the project "Development of novel nanostructured materials for the diagnosis of breast cancer, inflammatory bowel disease (IBD) and modulation of antiretroviral therapy (HAART) in HIV"

SIGNIFICANT TRAINING AND WORKING EXPERIENCES:

- 2000 Training on electrophysiological techniques applied to epithelia (Univ. of Lecce, Italy)
- 2003 School of Physiology organized by the Italian Physiology Society on "Transmembrane transport in cells and epithelia" (Univ. of Lecce, Italy)
- 2004-2005 Research activity on separation of the different cell types from crustacean hepatopancreas and transport experiments in the isolated cells (Univ. of North Florida, USA)
- 2010 Theoetical and practical course on "Nanoparticles: characterization and biological interactions" (Univ. of Milano Bicocca, Italy)

Expert in transport physiology with a specific competence in permeability processes through intact epithelia and cellular membranes, my present field of research is the study of fluorescent tracers biodistribution in animal models. A particular interest in cancer diagnosis is currently pursued by in vivo detection of targeted nanoparticles by CCD camera or confocal microscopy analysis.
The results of my research are reported in 16 peer-reviewed publications and have been presented in 37 congresses (23 national and 14 international).

Victoria Firstova

Dr., SRCAMB, Infection immunity, Biologov,1 fl.211, Obolensk, RU-142279, victoria1@mail.ru
Firstova Victoria, immunologist, graduated from State University, Dnepropetrovsk, Russia in 1992. She worked in Russian Research Anti-Plague Institute \"Microbe\" 1993-2005. Since 2005, she is work in State Research Center for Applied Microbiology and Biotechnology, Obolensk, Russia.
Since 2007 Firstova Victoria is the Head of infection immunity sector, immnobiochemistry department. In recent year she focused on toxicity of nanomaterials.

Pieter Gaillard

Dr., to-BBB technologies BV, Niels Bohrweg 11, NL-2333 CA Leiden, gaillard@tobbb.com.
Pieter obtained a MSc degree in biomedical sciences from Utrecht University, and a PhD degree in pharmacology from Leiden University at the lab of Prof. Douwe Breimer. During his time as post-doc at the Academic Medical Centre in Amsterdam he managed the scientific group focusing on ocular angiogenesis.
With his extensive pharmacology and neuroscience experience he is globally recognized as an expert in the blood-brain barrier research field. In 2001 he co-founded to-BBB, where he currently holds the position of CSO, up to 2008 he also held the position of CEO.

Pernille Lund Hansen

Work place: The Molecular Oncology Unit, Institute for Molecular Medicine, Faculty of Health, University of Southern Denmark Job title: PhD student, Project Title: Systematic analysis of candidate genes involved in the cancer stem cell phenotype

EDUCATION: Cand. Scient in Biomedicine (2002-2009). Title of Master Thesis: Osteogenic differentiation in 3D-osteospheres: Novel approach for studying in vitro bone formation. Supervisor: Proff. Moustapha Kassem Title of Bachelor Thesis: Osteogenic differentiation of human bone marrow derived mesenchymal stem cells immortalized by telomerase and grown as 3D multicellular spheroids. Supervisor: Proff. Moustapha Kassem

PUBLICATIONS

- Parameters in three-dimensional osteospheroids of telomerized human mesenchymal (stromal) stem cells grown on osteoconductive scaffolds that predict in vivo bone-forming potential.
- Pernille Lund Rasmussen, Jorge S. Burns, Kenneth H. Larsen, henrik D. Schrøder, Moustapha Kassem Tissue Engineering, Part A. 2010 Jul;16(7):2331-42.
- The pattern recognition molecule Deleted in Malignant Brain Tumors 1 (DMBT1) and synthetic mimics inhibit liposomal nucleic acid delivery Pernille Lund Rasmussen, Stephanie Blaich, Caroline End, Steffen Schmidt Jesper B. Moeller, Uffe Holmskov, and Jan Mollenhauer Chemical Communications, Sep. 7 2010.

AWARDS: 2008-2009 Novo Scholarship Programme in Biotechnology and Pharmaceutical Sciences.

Margarethe Hofmann-Amtenbrink

Margarethe Hofmann-Amtenbrink started as Assistant for Metallography in industry. She then studied foundry technology and materials science and received her PhD in materials science at Max Planck Institute Stuttgart and Technical University Berlin, Germany. In 1987 she started her own business (Mat Search Consulting Hofmann) in Switzerland. Among various consulting activities for industry she became CEO of the Swiss governmental Priority Program for Materials (PPM 1991-1994), CEO of the Swiss Association for Materials Technology, SVMT and is still CEO of the Foundation for Rare Metals, ESM. From 2003 to 2009 she was chairperson of the Biotechnology Advisory Board of the AO Foundation Davos, Switzerland and as such responsible for a research program of about 1 Mio per year. Since about 10 years she is involved in research projects on nanoparticles for biomedical applications and initiated two European Research Projects (FP5 and FP7) for which she was/is the scientific coordination. She is member of several Advisory Boards in Germany and Switzerland and individual member of the Swiss Academy for Engineering Sciences, SATW, Switzerland and has become member of ETP Nanomedicine. M. Hofmann-Amtenbrink has published several reports in the field of technology assessment and innovation and published book chapters and articles in the field of biomedical nanotechnology.

Contact: Margarethe Hofmann-Amtnebrink Dr.-Ing., Mat Search Consulting Hofmann, Ch. Jean Pavillard 14, CH-1009 Pully Switzerland, Tel 0041 21 729 01 55, e-mail: mhofmann@matsearch.ch, URL: www.matsearch.ch

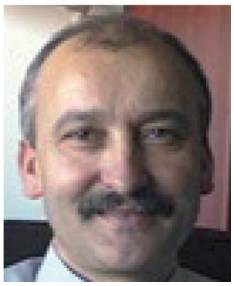
Yong Hu

EDUCATION AND WORKING EXPERIENCE

2010.7-Present, Alexander von Humboldt fellowship in Duisburg-Essen University. 2006.3-Present, Associate professor in Department of Materials Science and Engineering, Nanjing University, China. 2005.12-2006.3, Research fellow A in Singapore-MIT Alliance, Singapore. 2004.12-2005.11, Research staff in Department of Polymer, Nanjing University, China. 2003. 11- 2004.12, Postdoctor in John Hopkins Singapore. National University of Singapore. 2002.7-2003. 11, Postdoctoral position in Department of physics, Nanjing University. 1997.7-2002.6, Ph. D in Department of Polymer, Nanjing University, China. 1993.9-1997.7, BS in Department of Chemistry, Nanjing University, China.

RESEARCH INTEREST

- Synthesis and characterization of biodegradable nanoparticles and their application in drug delivery system and cell imaging.
- Bio-mineralization of silica and calcium phosphate nanomaterials



Cornel Iancu

Date and place of birth: 19 June 1953, 1991-2007 Surgical University Hospital no.3, Specialist General Surgery (second degree physician), General Surgeon (first degree physician) (1996), Associate Professor (2002) Clinical Activity, Teaching Activity, Research Activity. 2001-2006 Surgical University Hospital no.3, General Manager, Hospital Manager. 2006-2009 Surgical University Hospital no.3, Professor UMF Cluj Napoca Head of Surgical Department at Surgical University Hospital Cluj-Napoca, Clinical Activity, Teaching Activity, Research Activity

ACADEMIC TITLES: Professor in General Surgery, (main expertise: colon, liver and pancreatic cancer surgery-more than 1500 resections for cancers) Ph D in Medical Sciences

FOREIGN LANGUAGES: French, English

SHORT PUBLICATION LIST

- Iancu C, Mocan L, Bele C, Catoi C, Tabaran F, Stiufiuc R, Simon S, Stir A, Matea C, Iancu D, Zaharie F, Biris AR, Mocan T "Enhanced laser thermal ablation for in vitro liver cancer destruction by specific delivery of multi wall carbon nanotubes functionalized with BSA."(International Journal of Nanomedicine, in press)
- Alokita Karmakar, Cornel Iancu, Lucian Mocan, Dana Todea Iancu, Teodora Mocan, Ashley Fejleh, Philip Fejleh, Yang Xu, Enkeleda Dervishi, Samuel.L.Collom, Thikra Mustafa, Fumiya Watanabe, Zhongrui Li, Alexandru R. Biris, Mariya Khodakovskaya, Dan Casciano, Alexandru.S. Biris "Carbon Nanotubes Conjugated with EGF for In Vitro Specific Targeting and Enhanced Destruction of Pancreatic Cancer Cells by Photo-Thermal Ablation" (Nanoletters 2010, under external review)
- Mahmood M, Karmakar A, Fejleh A, Mocan T, Iancu C, Mocan L, Iancu DT, Xu Y, Dervishi E, Li Z, Biris AR, Agarwal R, Ali N, Galanzha EI, Biris AS, Zharov VP."Synergistic enhancement of cancer therapy using a combination of carbon nanotubes and anti-tumor drug" Nanomedicine 2009 Dec;4(8):883-93
- Mahmood M., Casciano D.A., Mocan T., Iancu C., Xu Y, Mocan L, Todea-Iancu D., Dervishi E., Li Z., Biris AR, Abdalmuhsen M., Ali N., Biris AS. "Cytotoxicity and Biological Effects of Functional Nanomaterials Delivered to Various Cell Lines": Journal of Toxicology and Applied Pharmacology, 2010 Jan;30(1):74-83.
- Iancu C, Ilie IR., Georgescu C, Ilie R, Biris AR, Mocan T, Mocan C, Zaharie F, Todea-Iancu D., Susman S, Rus Ciuca D., Biris AS. "Applications of Nanomaterials in Cell Stem Therapies and the Onset of Nanomedicine". Particulate Science and Technology, 2009, 27(6) , 562-574
- Osian G, Procopciuc L, Vlad L, Iancu C, Mocan T, Mocan L. C677T and A1298C mutations in the MTHFR gene and survival in colorectal cancer. J Gastrointestin Liver Dis. 2009 Dec;18(4):455-60.
- Ionescu D, Iancu C, Ion D, Al-Hajjar N, Margarit S, Mocan L, Mocan T, Deac D, Bodea R, Vasian H. Implementing fast-track protocol for colorectal surgery: a prospective randomized clinical trial. World J Surg. 2009 Nov;33(11):2433-8.

Tore Geir Iversen

Tore-Geir Iversen is a senior scientist and Project Leader at the Centre for Cancer Biomedicine, The Norwegian Radium Hospital in Oslo, Norway. He earned his PhD at the Norwegian University of Science and Technology (NTNU), Trondheim in 1995, at that time studying microbial genetics. He joined the group of professor Sandvig in 1997, then studying endocytosis and intracellular transport of different protein toxins. In 2006 he turned his focus into studying how nanoparticles are endocytosed and transported in cells. His group

investigates fluorescent nanoparticles (e.g. iron oxide particles and quantum dots) both with respect to their use as relevant intracellular probes for the routing of ligands in live cells, and their effects on cellular transport pathways. They were the first to demonstrate that accumulation of nanoparticles within endosomes could induce changes in the normal intracellular transport of the cell. Current research interests also include more applied biological studies about nanoparticles and the criteria required for their clinical use in therapy and imaging.

Aleksandra Maria Jaskot

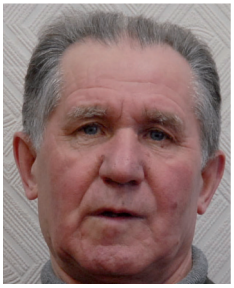
Nationality: Polish. Date of birth: 1st of September 1987. Address: Elsdyrløkken 112F, 5210 Odense NV, ajaskot@health.sdu.dk, Mobile: +45-501 07 195, CPR: 010987-3446

EDUCATION: 2004-2006 Secondary School no. II in Gliwice, Poland. Class with extended Biology, Chemistry and Physic. 2006-2010 Medical University of Silesia in Katowice. Poland. Main field of the studies: Biotechnology (Department of Pharmacy) Area of studies: Biochemistry, Molecular Biology, Biology, Microbiology, Proteomics, Genetics. June 2009 Internship in the Department of Molecular Biology of the Oncology Institute in Gliwice. Responsibilities: Cell culture of the mouse melanoma cell line (B16F10, B16FA and B78-H1). Test of drugs with anticancer properties. Handling and taking care of laboratory animals. January-June 2010 Erasmus student on University of Southern Denmark in Odense. Area of study: Biochemistry and Molecular Biology. Individual study activity : Introduction into functional genomics methods in cancer research. Since August 2010 Master student in Molecular Bioscience at the University of Southern Denmark in Odense. Master project in Jan Mollenhauer group in Institute of Molecular Medicine, University of Southern Denmark.

LANGUAGE SKILLS: English - fluent in speaking and writing (due to employments in Ireland and England during summer holidays), German - basic, Danish - basic, Polish - native language

Miklós Kellermayer

Miklós Kellermayer is the chairman of the Department of Biophysics and Radiation Biology and Vice Rector for Education and International Affairs at Semmelweis University, Budapest, Hungary. Trained as a medical doctor and having had international research experience in single-molecule biophysics, he currently focuses on nanobiotechnology, biomolecular mechanics, cytoskeletal nanobiology, protein folding and misfolding. He supervises the Nanoscience Network at Semmelweis University and runs a Nanobiotechnology and In Vivo Imaging Center that houses state-of-the-art instrumentation that allows imaging and manipulation from single molecules to small-animal organisms. Author of four books and more than fifty research papers. Member of the European Commission Expert Advisory Group on Nano, Materials and Productions.



Vasily Petrovich Kholodenko

Dr., FGUN State Research Center for Applied Microbiology & Biotechnology, Rospotrebnadzor, SRCAMB, Moscow region,Obolensk, RU-142279, vpkhol@mail.ru

EDUCATIONAL INSTITUTION

Moscow State University, Biology & Soil Faculty Place of employment, position: FGUN State Research Center for Applied Microbiology & Biotechnology; Senior Expert in the field of biological security. Academic degree, title, specialty to Uphold a Thesis: Sc.D (Biol), Microbiology.

SCOPE OF SCIENTIFIC ACTIVITY

Research on microbial biodegradation of different xenobiotics (oil hydrocarbons & polyaromatic hydrocarbons; organophosphorous compounds, dimethylhydrazine, etc). Investigation of the impact of space flight conditions on physiology- biochemistry characteristics of oil hydrocarbon-degrading microorganisms; participation in some programs of the Ministry of Science & Technology RF, Russian Space Agency, and manager of three ISTC projects.

Scientific advances: Russian publications: 120; Foreign publications: 20; Patents: 12.

CURRENT SCIENTIFIC ACTIVITY

I am a principal specialist in the field of biological security at the State Research Center for Applied Microbiology & Biotechnology; member of the Russian Nanotechnology Society Board; Professor at the Pushchino State University, Deputy dean at the Nanobiosafety Educational Center; involved in the Federal program aimed at assessing nanomaterial safety for humans and the environment.

Yvonne Klapper

Born on: August 29th, 1985, Karlsruhe

Since 09/2010 PhD-Thesis at Carl Gustav Carus-Institute and Institute of Applied Physics, Kalsruhe Institute of Technology (KIT). 10/2005-06/2010 Diploma in physics, specialized in biophysics, KIT. 07/2009-06/2010 Diploma thesis at Institute of Nanotechnology, KIT: “Characteristics of Biomolecules in Membranes and aqueous solutions”. 08/2008-05/2009 Exchange semester at National University of Singapore (NUS). 10/2006-07/2008 Additional Studies “Cultural Sciences” at Center for Cultural and General Studies, KIT. 02/2006-04/2006 Research internship at University of New Mexico, Albuquerque, USA. 09/1996-07/2005 High School Privates Gymnasium St.Paulusheim in Bruchsal. 09/1992-09/1996 Primary School in Kraichtal

AWARDS AND FELLOWSHIPS

- Research Travel Scholarship of Karlsruhe House of Young Scientists, KHYS (2011)
- Baden-Württemberg Stipendium (2008-2009)
- Ferry Porsche Award (2005)
- Lions Award (2005)

Petra Kocbek

Petra Kocbek, Ph.D., received her B.Sc. degree in Pharmacy in 2004, and her Ph.D. in Pharmaceutical Nanotechnology in 2008, both from the University of Ljubljana, Faculty of Pharmacy (Slovenia). During her postgraduate study and after graduation she worked as an assistant in Pharmaceutical Technology, and in 2011 she became assistant professor in Pharmaceutical Nanotechnology at her home faculty. The results of her research work were presented at numerous scientific and professional meetings at home and abroad, as well as published in several scientific journals.

Her area of interest are nanosized systems for delivery of small molecular weight drugs as well as biomacromolescules, especially focused on nanosuspensions and polymeric nanoparticles for targeted drug delivery.

Gergely Tibor Kozma

Date of birth: 23rd June 1977

EDUCATION

1995-2000: Technical University Budapest
Professional qualifications: 2000: Biologist engineer (MSc)
Academic qualifications: 2004: Semmelweis University, PhD

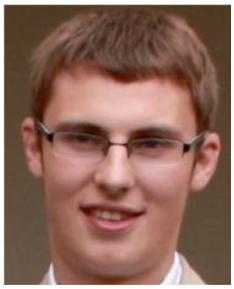
PRESENT AND PREVIOUS POSITIONS

- 2010–: Institute of Pathophysiology-Semmelweis University (Budapest), senior research associate
- 2008–2010: Soft Flow Hungary Ltd., research fellow
- 2007–2008: Marie Curie Research Training Networks-EUrythron research group (Rome), post-doc researcher
- 2003-2007: Hungarian Academy of Sciences-Semmelweis University 1st Department of Pediatrics, (Budapest), firstly assistant research than research fellow
- 2000-2003: Department of Genetics Cell, and Immunobiology-Semmelweis University (Budapest), PhD student

Linguistic ability: English, Italian, Hungarian (Native language)
Activities in higher education: 2001-2003: leading Medical Cell Biology and Genetic practices for Hungarian students in Semmelweis University. Research interests: Immunology, Molecular Biology, Cell biology, Molecular pathomechanisms of allergic and pseudo-allergic diseases.

Juliana Kristl

Ph.D., Professor Julijana Kristl become Doctor of Pharmaceutical Sciences at the University of Ljubljana in 1988, and did PostDoc at University in Geneva. She has expertise in formulation, characterization and biological evaluation of new drug delivery systems, biopolymers, and nanomedicine. Her research activity has resulted in more than 140 scientific papers published in national and international journals, numerous research projects for pharmaceutical industry, invited lectures and reports at scientific meetings. Her international scientific collaboration includes membership in the SFD, CRS, APGI and APV, memberships in the editorial boards and referee for 27 scientific journals.



Vytautas Kulvietis

Current position: Junior research associate, Laboratory of Biomedical Physics, Scientific Research Centre, Institute of Oncology, Vilnius university (2007-current). P. Baublio st. 3b, LT-08406 Vilnius, Lithuania. Contacts: E-mail: Vytautas.kulvietis@vuoi.lt; Address: Franko st. 8-7, LT- 08431 Vilnius, Tel.: (370) 69833844, Nationality: Lithuanian

EDUCATION

- PhD studies, Biophysics, Vilnius university (2009- current);
- Master of Biophysics, Faculty of Natural Sciences, Vilnius university (2007-2009);
- Erasmus studies at University of Copenhagen, Denmark (2008, 30 ECTS);
- Summer school, University of Luton, Great Britain (2004).

SCIENTIFIC INTERESTS: Biomedical optical imaging; nanomaterials biodistribution in vivo; fluorescence spectroscopy.

PROJECTS

- "Nanoparticle distribution in organism, penetration across placental barrier and effect on embryogenesis", No. MIP-10440. Sponsored by the Research Council of Lithuania, 2010-2011.
- "Multifunctional nanoparticles for specific non-invasive early diagnostics and treatment of cancer" No. 2004-LT0036-IP-1NOR. Sponsored by Norwegian financial mechanism and Lithuanian government, 2008-2010.

LANGUAGES: Lithuanian (native), English, German, Russian.

Dong Soo Lee

M.D.Ph.D., Dept of Nuclear Medicine, Seoul National University Hospital, 28 Yungundong Chongnogu Seoul, 110-744 Korea. Tel: 82-2-760-2501 Fax; 82-2-745-7690 Email: dsl@plaza.snu.ac.kr
Current appointment (position and institution): - President of Korean Society of Nuclear Medicine (2010-2012) - President of Korean Society of Human Brain Mapping (2010-2012) - Vice-President of Korean Society of Cognitive Science (2010-2011) - President of PET Association of Korea (since 2008 April) - Professor and Chairman, Department of Nuclear Medicine, Seoul National University College of Medicine; Interdisciplinary Programs for Cognitive and Brain Science, Seoul National University; Interdisciplinary Program of Radiation-Applied Life Sciences - Professor, WCU Department of Molecular Medicine and Biopharmaceutical Sciences, Seoul National University

FIELD OF SPECIALIZATION : Nuclear Medicine (Neurology and Cardiology) and Molecular Imaging.

SHORT SCIENTIFIC BIOGRAPHY: - Published 256 articles in SCI journal (1999-2010). (as the first or corresponding author for over 60 of these articles) - Editor of Journal of Nuclear Medicine, Editor of European Journal of Nuclear Medicine and Molecular Imaging since 2002, Editor of Journal of Nuclear Cardiology since 2010, Editor of The Open Journal of Nuclear Medicine since 2008 - Fellow of American College of Cardiology.

Xiaochun Li-Blatter

Biozentrum, Biophysical chemistry, CH-4056 Basel, xiaochun.li-blatter@unibas.
Xiaochun Li-Blatter is working at group Anna Seelig, biophysical chemistry department, Biozentrum, university of Basel. She has published 10 publications since 2000. Together with Professor Anna Seelig, they have studied P-glycoprotein substrate binding principle, and investigated the mechanism of P-glycoprotein inhibition. Xiaochun Li-Blatter has studied chemistry before.

Caroline Loew

Current activity: since 05.2011: PostDoc in biophysical chemistry at the Biozentrum of Basel University
Education: 06.2007: Master and Engineer Diploma in chemistry and physical chemistry, Ecole Nationale Supérieure de Chimie de Paris, France. Additional Experience: 2007-2011: PhD student in biophysical chemistry at the Biozentrum of Basel University . Membrane binding of β-amyloid peptides (Prof. J.Seelig).

PUBLICATIONS

- Comprehensive Kinetic Screening of Palladium Catalysts for Heck Reactions, D.G. Blackmond, T. Schultz, J.S. Mathew, C. Loew, T. Rosner, A. Pfaltz, Synlett Letter, 2006, 1, pp 1-5
- Near-UV molar absorptivities of acetone, alachlor, metolachlor, diazinon and dichlorvos in aqueous solution, V. Feigenbrugel, C. Loew, S. Le Calvé, P. Mirabel, Journal of Photochemistry and Photobiology A:Chemistry, 2005, 174, pp 76-81

Abram M. Madiehe

Ph.D. (Biochemistry and Molecular Biology). Date of Birth: December 30, 1967, Citizenship: South African. Address: 32 Aloe Crescent, Vredelust, Kuilsriver, 7560, South Africa, Tel (w): + 27 21 959 2913, Fax (w): +27 21 959 3311, Mobile: +27 82 323 58 28, e-mail: amadiehe@mrc.ac.za

PROFESSIONAL EXPERIENCE

- Presently: Lab Head, Diabetes Research Group, Medical Research Council, South Africa
- Member of the DST/MINTEK Nanotechnology Innovation Center Biolabels Unit at the University of the Western Cape.
- 2003-2007 Specialist Scientist, Medical Research Council, Tygerberg, SA
- 2001-2002 Postdoctoral Research Associate, University of Georgia, USA
- 1997-2000 Graduate Research Assistant, Louisiana State University, Baton Rouge, USA

EDUCATION & SELF DEVELOPMENT

- 2009 Certificate in Essential IP Management, presented by DNA-biotec (Pty) Ltd. New Venture Creation – Creating start-ups from science and technology based business ideas, presented by Venture Solutions cc.
- 2008 Management Development Programme, University of Stellenbosch Business School, Bellville
- 2005 Diploma in Project Management (cum laude), Peninsula Technikon
- 1995-2000 Doctor of Philosophy (Biochemistry & Molecular Biology), LSU & Pennington Biomedical Research Center (PBRC), Baton Rouge, Louisiana, USA; Fulbright Fellow, Dissertation: 'Glucocorticoid regulation of the leptin receptor signaling system in the rat', supervised by Dr. David A. York, Ph.D.
- 1992-1995 M. Sc. (cum laude), Biochemistry, University of the North, Pietersburg, SA, Project: 'Induction of Apoptosis (programmed cell death) in lithium treated HL-60 cells', supervised by Prof. Errol M. Tyobeka
- 1991-1992 B. Sc. Honours (cum laude), Biochemistry, University of the North, Pietersburg, SA, Project: 'Effects of acridine derivatives on the growth of HL-60 promyelocytic leukemia cells', supervised by Prof. Errol M. Tyobeka.
- 1987–1991 B. Sc., B aggregate. University of the North, Pietersburg, SA, Majoring in Biochemistry, Microbiology and Physiology

SCIENTIFIC SKILLS

- Molecular Biology. DNA cloning and bacterial cell culture; Reverse Transcription Polymerase Chain Reaction (RT-PCR); Polymerase Chain Reaction (PCR); Real-Time PCR; DNA sequencing; Mammalian DNA and RNA extractions; Northern and Southern Blot analysis; Primer extension analysis; Ribonuclease Protection Assay (RPA); SDS-PAGE and Western blot analysis; Recombinant protein expression and purification from E. coli; Isoelectric focusing and 2-dimensional gel electrophoresis
- Cellular Biology. Tissue culture of primary hepatocyte cells; Established cell lines (HL-60, U937, K562, 3T3-L1); Cellular fractionation
- Protein Chemistry. Nuclear and cytosol extractions; SDS-PAGE analysis; Western blotting & Detection assays (chemiluminescence, colorimetric); Electrophoretic mobility shift assays; Immunoprecipitations; Size exclusion chromatography; Affinity chromatography Immunohistochemistry
- Animal Work. In vivo studies in rats: icv / ip / im applications, behavioural feeding studies, glucose tolerance test, insulin sensitivity test; Surgeries in rats: icv canulation, adrenalectomy. Dissections: hypothalamus, hippocampus, cortex, liver, brown and White adipose tissues, kidney. Body composition analysis; Fecal lipid analysis
- Assays. Determination of serum hormones via radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA)

TEACHING EXPERIENCE

- 02/2008-present: Currently teaching Bioethics and Obesity/Diabetes Honours modules in the Department of Biotechnology at the University of the Western Cape
- 08/99-05/00: Graduate Teaching Assistant, Louisiana State University, Baton Rouge, USA
- 01/91-06/95: Graduate Teaching Assistant, University of the North, Pietersburg, SA
- 01/91-12/92:Tutor, University of the North, Pietersburg, SA
- 07/87-06/88: Teacher, Higher Primary School in Ikageng/Potchefstroom, SA

Serena Mazzucchelli

Post-doc at the Nanobiotechnology lab at the Department of Biotechnology and Biosciences, faculty of Sciences (University of Milano Bicocca) since January 2010.

EDUCATION

PhD in biological sciences on October 2009. "Studies on the mechanism and physiological role(s) of the interaction of ataxin-3 with tubulin", tutor prof. Paolo Tortora, Department of Biotechnology and Biosciences, Faculty of Sciences (University of Milano Bicocca).

- Degree in Biology obtained on 20.10 2006 at the University of Milano-Bicocca, Italy with an experimental thesis on "Studies on the physiological roles of human protein CGI-58, a molecule associated to Lipid droplets". Qualification: 110/110 cum laude.
- Bachelor Degree in Biological Science obtained on 01.10.2004 at the University of Milano -Bicocca, Italy with experimental thesis on "N- and C- terminal sostitution of leucine with acidic residues points out hydrophobic interaction crucial for stability of Carboxipeptidase from S. solfataricus". Qualification: 110/110 cum laude.
- High school leaving qualification at Liceo Classico Statale "Giovanni Pascoli", Gallarate (VA) Italy, in 2000.

PUBLICATIONS

E. Occhipinti, S. Mazzucchelli, et al.; Nanoscale., (2011), 3(2), 387-90. - S. Ronchi, S. Mazzucchelli, et al.; AIP conf. Proc. (2010), 1275, 102-105. - S. Mazzuchelli, M. Colombo, et al.; ACSnano (2010), 10 , 5693-702. - C. Morasso, M. Colombo, S. Mazzucchelli et al.; Adv. Funct. Mater. Pub. on line (doi: 10.1002/adfm.201001274). M. Colombo, S. Mazzuchelli, et al.; Pharmacological Research, (2010), 62, 150-65. S. Mazzucchelli, et al.; Int. J. Biochem. Cell. Biol., (2009), 41(12), 2485-92.

COMPETENCES

Expression of hetherologous protein in E. coli using commercial expression systems, protein purification, protein assay, enzymatic assay, elettrophoretic techniques, western blot, dot-blot, immunoprecipitation, FPLC separation, GST-pull down, cell culture from human sample or commercial cell lines, cellular fractionation, immunofluorescence, cytofluorimetry, molecular biology techniques and functionalization of hybrid nanoparticles for biomedical applications.

Teodora Mocan

Date of birth: 28.7.1977, MD, University of Medicine and Pharmacy, Cluj-Napoca, Romania, 2008- Asistant Professor, Physiology Department, Teaching Activity, Research Activity. Foreign languages: French, English

SHORT PUBLICATION LIST

- Iancu C, Mocan L, Bele C, Orza AI, Tabaran FA, Catoi C, Stiufiuc R, Stir A, Matea C, Iancu D, Agoston-Coldea L, Zaharie F, Mocan T. Enhanced laser thermal ablation for the in vitro treatment of liver cancer by specific delivery of multiwalled carbon nanotubes functionalized with human se-

rum albumin. Int J Nanomedicine. 2011 Jan 17;6:129-41.
- Karmakar A, Iancu C, Mocan L, Todea Iancu D, Mocan T, Fejleh A, Fejleh P, Xu Y, Dervishi E,.L.Collom S, Mustafa T, Watanabe F, Li Z, Biris AR, Khodakovskaya M, Casciano D, Biris AS "Carbon Nanotubes Conjugated with EGF for In Vitro Specific Targeting and Enhanced Destruction of Pancreatic Cancer Cells by Photo-Thermal Ablation" (Nanoletters 2011, under external review)
- Mahmood M, Karmakar A, Fejleh A, Mocan T, Iancu C, Mocan L, Iancu DT, Xu Y, Dervishi E, Li Z, Biris AR, Agarwal R, Ali N, Galanzha EI, Biris AS, Zharov VP."Synergistic enhancement of cancer therapy using a combination of carbon nanotubes and antitumor drug" Nanomedicine 2009 Dec;4(8):883-93
- Mahmood M., Casciano D.A., Mocan T., Iancu C., Xu Y, Mocan L, Todea-Iancu D., Dervishi E., Li Z., Biris AR, Abdalmuhsen M., Ali N., Biris AS. "Cytotoxicity and Biological Effects of Functional Nanomaterials Delivered to Various Cell Lines": Journal of Toxicology and Applied Pharmacology, 2010 Jan;30(1):74-83.
- Iancu C, Ilie IR., Georgescu C, Ilie R, Biris AR, Mocan T, Mocan L, Zaharie F, Todea-Iancu D., Susman S, Rus Ciuca D., Biris AS. "Applications of Nanomaterials in Cell Stem Therapies and the Onset of Nanomedicine". Particulate Science and Technology, 2009, 27(6) , 562-574.
- Mocan T, Clichici S, Agoşton-Coldea L, Mocan L, Şimon Ş,Ilie IR, Iancu C, Biriş, AR, Mureşan A, "Implication of oxidative stress mechanisms in toxicity of nanoparticles. Acta Phys. Hung, 2010, 27(3), 247-255.
- Simon S., Biris AR, Lupu DM, Misan I, Clichici S, Mocan T, Biris AS, Dispersion of carbon nanotubes by single –strand wrapping for advanced biomedical applications, Journal of Physics: Conference Series, (2009),182, 12079.
- Osian G, Procopciuc L, Vlad L, Iancu C, Mocan T, Mocan L. C677T and A1298C mutations in the MTHFR gene and survival in colorectal cancer. J Gastrointestin Liver Dis. 2009 Dec;18(4):455-60.
- Ionescu D, Iancu C, Ion D, Al-Hajjar N, Margarit S, Mocan L, Mocan T, Deac D, Bodea R, Vasian H. Implementing fast-track protocol for colorectal surgery: a prospective randomized clinical trial. World J Surg. 2009 Nov;33(11):2433-8.

Urszula Narkiewicz

Prof., West Pomeranianian University of Technology, Institute of Chemical and Environment Engineering, Pulaskiego 10, 70-322 Szczecin, Poland, phone (48) 914494687, 4730 , Fax : (48) 914494686, e-mail : urszula.narkiewicz@zut.edu.pl.
Born 11.10.1952 in Goleniów.

EDUCATION

- Faculty of Chemistry and Chemical Engineering, Technical University of Szczecin, 1971-1976
- PhD thesis : 1986
- Post-graduated studies: French Studies of Management of Industrial Systems, "mastère" diploma, certified by French "Grandes Ecoles" 1996
- Habilitation thesis:2000

PROFESSIONAL CAREER

Assistant: 1976-1981, Senior assistant: 1981-1986, Lecturer: 1986, Professor assistant: 1986-2003, Professor of Szczecin University of Technology- 2003, Full Professor 2008.

SCIENTIFIC INTERESTS

Chemical technology, catalysis, surface science, nanomaterials
Scientific papers: more than 70 original, full text scientific papers. In general-more than 180 scientific publications.
Technological applications: 8 patents, 5 industrial applications.
Projects: Head of 6 projects granted by State Committee for Scientific Research or by Ministry of Science and High Education, 1993-2008

FUNCTIONS IN ORGANISATIONS AND INSTITUTIONS

- Polish representative at the Mirror Group of the European Technology Platform for Nanomedicine, 2006 -.
- Polish expert in COST DC_MPNS Materials, Physical and Nanosciences-2006-. Member of the Executive Committee of the European Materials Research Society, 2007 -.
- Polish expert in Programme Committee COOPERATION-NMP of FP7, 2006-2009
- Head of the Chair of Inorganic Chemical Technology, 2009-
- Vice-President of the Polish Materials Research Society, 2009 -
- Member of the Group for Nanotechnology and Nanoscience at the Polish Ministry of Research-2006 (April to September)
- Member of the Group for Strategic Programs of Scientific Research and Development at the Polish Ministry of Research, 2008-2009
- Member of the Nanomaterials' Section of the Committee or the Materials Science of the Polish Academy of Science, 2007 - …
- Member of the Board of the Polish Materials Research Society, 2004-2009
- Dean's representative for International Educational Co-operation (Faculty of Chemical Engineering, West Pomeranian University of Technology), 2008-…

STAGES NATIONAL AND ABROAD

- 1987- Police S.A. (chemical factory), 6 months, ammonia synthesis plant
- 1989 r. - 8 months, postdoc, Laboratoire Maurice Letort, CNRS, Nancy, France
- 1994 r. - 4 months-stage in the Laboratory of Heterogeneous Catalysis, University I Nancy, France
- 1996 r.-1 month, stage in the field of production management, Université Catolique Louvain-La-Neuve, Belgium
- 2001-2 weeks, stage in the field of accreditation and certification of quality management systems, centre CEWAC, Liège, Belgium

PRIZES AND DISTINCTIONS

Prizes for scientific activity: 3 prizes of the Minister of National Education (team prize, II degree). 11 prizes of the Rector of the Szczecin University of Technology

SPREADING OF KNOWLEDGE

- Chair of the Symposium I at the E-MRS Fall Meeting 2004
- Chair of the Symposium B at the E-MRS Fall Meeting 2006
- Conference Chair of the E-MRS Fall Meeting 2008
- Conference Chair of the E-MRS Fall Meeting 2010
- Guest editor of the special issue of „Catalysis Today" and „Reviews on Advanced Materials Science"

5 IMPORTANT PUBLICATIONS

- U. Narkiewicz, N. Guskos, W. Arabczyk, J. Typek, T. Bodziony, W. Konicki, G. Gąsiorek, I Kucharewicz, A. Anagnostakis, XRD, TEM and magnetic resonance studies of iron carbide nanoparticle agglomerates in a carbon matrix, Carbon 42 (2004) 1127
- U. Narkiewicz, W. Arabczyk, W. Konicki, A. Pattek-Jańczyk, Nucleation of the Fe3C in the process of the methane reaction with the nanocrystalline iron, J. Mater. Res. 20(2) (2005) 386
- U. Narkiewicz, W. Arabczyk, W. Konicki, Studies of the kinetics of the carbon deposit formation in the decomposition of methane on nanocrystalline iron, Fullerenes, Nanotubes and Carbon Nanostructures, 13 (2005) 99
- U. Narkiewicz, D. Moszyński, M. Brosławski, Thermal diffusion of potassium on a modified iron surface, Appl. Surf. Sci. 252(3) (2005) 833
- U. Narkiewicz, I Pełech, Z Rosłaniec, M Kwiatkowska and W Arabczyk, Preparation of nanocrystalline iron–carbon materials as fillers for polymers, Nanotechnology, 18(40) (2007) 5601

LIST OF THE MOST IMPORTANT SCIENTIFIC ACHIEVEMENTS

- participation in an implementation of a technology of production of fused iron catalysts (applied in Polish ammonia plants)
- description of the mechanism of deactivation and regeneration of iron catalyst for ammonia synthesis
- development of an inexpensive and effective method of synthesis and purification of carbon nanomaterials
- development of a method of preparation of magnetic nanometali encapsulated in carbon
- description of kinetics of synthesis and hydrogenation of nanocarbons.

Tracey Newman

Dr. Tracey Newman is a neurobiologist who has worked extensively with in vitro and in vivo model systems to understand the cascade of events that occurs during neuronal injury as a consequence of inflammatory brain disease. She obtained her PhD in 1998 for work on the continuum between ageing and the pathology of Alzheimer's disease. She was appointed to a lectureship in the University of Southampton in January 2010, where she is also the deputy director of the Masters in Medical Science. For the last 4 years she has led the Southampton team on a FP6 funded project (NANOEAR) to develop drug-loaded nanoparticles for targeting neurons of the inner ear. The aim of this work is to treat age-related deafness and to improve the integration of cochlear implant electrodes to produce better sound resolution in implanted patients. This work is now leading to a series of publications including, Zhang Y et al., Hear Res. 2010 Oct 1;269(1-2):1. Roy S et al., Int J Pharm. 2010 May 10;390(2):214. Johnston A et al., J Nanoparticle Res 2010 12(6) 1997. Anderson et al., J Nanoneuro 2009 1(2) 78. She is leading a project to develop an innovative microfluidic device for the in vitro study of neurons, with the intention of developing a test-bed for nanoparticle toxicity in neurons. This work is funded by an NC3Rs/MRC research grant. She is interested in understanding how bothengineered and anthropogenic (e.g. as a byproduct of combustion) nanoparticles interact with the nervous system. She is currently working with Prof G Poppy (University of Southampton) to establish a multidisciplinary team to investigate the impact of diesel-derived nanoparticles on the nervous system using a model insect, the honeybee. They are awaiting the outcome of a Leverhulme Trust application. She also holds pilot funding with Dr Y Cheong (University of Southampton) to generate a system for the enhanced detection and diagnosis of endometriosis-through the use of biocompatible organic nanoparticles.

Martin G. Nussbaumer

martin.nussbaumer@unibas.ch, Phone 061 267 38 40. Date of birth: November 27th 1985, Citizen of Wallisellen ZH, Switzerland

EDUCATION

Since 07/2010 PhD student in the group of Dr. Nico Bruns, Department of Chemistry, University of Basel

- 2008-2010 Master of Science, Major in Nanosciences with Minor in Biology, University of Basel (final grade: 5.6) Master thesis: Conjugation of Antibodies to Polymer Vesicles for targeted Drug Delivery Purposes
- 2005-2008 Bachelor of Science, Major in Nanosciences, University of Basel
- 2004-2005 Military education to lieutenant in NBC defence (ABC Abwehr)
- 1998-2004 Grammar School in Köniz (Mathematics, Physics)
- 1992-1998 Primary School in Wabern

PUBLICATION

Biocompatible Functionalization of Polymersome Surfaces: A new Approach to Surface Immobilization and Cell Targeting using Polymersomes S. Egli, M.G. Nussbaumer, V. Balasubramanian, M. Chami, N. Bruns, C. Palivan, W. Meier, J. Am. Chem. Soc., 2011, 133 (12), pp 4476-4483

AWARDS AND PRIZES

Prize for the best poster presentation at the International Nanoscience Conference, August 22, 2010; The Netherlands

Anamaria Ioana Orza

Office Address: Department of Organic Chemistry, Nanoscience, anamariaorza@gmail.com. Home Address: 21 Decembrie 1989 Street, no.5, Cluj-Napoca. Date of Birth: September 30, 1986. Place of Birth: Sighetu-Marmatiei. Nationality: Romanian. Marital Status: Not Married

EDUCATION: 2008-present: PhD student (Nanotechnology) Babes- Bolyai University, Faculty of Chemistry and Engineering Chemistry

RESEARCH PROJECT: Synthesis and Physical-Chemical characterisation of some metallic nanostructurated compounds. Aplications in electronics and medicine

RESEARCH EXPERIENCE: Preparation of various nanostructures base nanoparticles, UV-VIS, FTIR, TEM, NMR, I-V characterisation of these nanostructures.Cell culture tests

CONFERENCE: National: ''Natural and Synthesized products applied in medicine '', 25 november 2009, Cluj Napoca, Romania
Poster Presentation: ''Collagen supported metallic nanowires'' , Anamaria Orza, Stela Pruneanu, Liliana Olenic, Adrian Florea, Mircea Diudea

POSTER PRESENTATION:''Biomolecules with applications in molecular electronics'', Stela Pruneanu, Liliana Olenic, Anamaria Orza, A.Houtlon, B.R.Horracks

INTERNATIONAL: RomPhyschem14, 2-4 july 2010, Bucharest, Romania. Poster Presentation,'Single-step synthesis of gold nanowires using biomolecules as capping agent/template. Applications for tissue engineering'', A. Orzaa*, C. Tomuleasab,c, O. Soritaub, A. Floreac, S. Pruneanud, L. Olenicd, M. Diudea.
7th International Conference on Applied Mathematics (ICAM7) in Nano-ERA'', 1-4 septembrie 2010, Cluj-Napoca Romania
Oral Presentation,'' Highly Efficient Gold Nanoparticles Drug Delivery for in Vivo Therapy of malignant gliomas''Anamaria Orza, Olga Soritau, Ciprian Tomuleasca

WORKSHOSP: III European Workshop in Drug Synthesis, 23rd to 27th May, 2010 Siena, Italy

POSTER AND ORAL PRESENTATION: ''Synthesis Characterization and Synergetic Effect of Gold Nanoparticle-Cisplatin/ Doxorubicin/ Capecitabine Vectors on Hepatic Cancer Stem Cells ,Anamaria Orza1, Ciprian Tomuleasca2,3, Olga Soriţău2, Olenic Liliana4, Stela Pruneanu4, Mircea Diudea1

PAPERS:

- Morphological and electrical characteristics of amino acid–AuNP nanostructured two-dimensional ensembles, Anamaria Orza, Olenic Liliana, Stela Pruneanu, Florina Pogacean, Alexandru Biris, Chemical Physics, Volume 373, Issue 3, 3 August 2010, Pages 295-299
- Enhanced LASER thermal ablation for in vitro liver cancer destruction by specific delivery of multi wall carbon nanotubes functionalized with human serum albumin, Cornel Iancu ,Constantin Bele, Cornel Catoi, Anamaria Orza, Flaviu A. Tabaran Rares Stiufiuc, Ariana Stir, Cristian Matea, Dana Iancu1, Florin Zaharie, Teodora Mocan, Lucian Mocan, Accepted at International Journal of Nanomedicine
- Conductive Collagen-based Gold Nanoparticles for Placental-derived Mesenchymal Stem Cells Differentiation, Anamaria Orza1*, Olga Soritau2, Adrian Florea3, Liliana Olenic4 Submitted to NanoLetters
- 2004-2008: Babes- Bolyai University, Faculty of Chemistry and Engineering Chemistry, BSc (Chemistry) Degree - Result: A. Final Year Research Project: Title: Synthesis, functionalization and characterization of some terpyridines metal complexes. Analyses results: H1-NMR, C13-NMR, MASS Spectroscopy. University Babes- Bolyai, Faculty of European Studies, Management Studies
- 2000-2004 King Ferdinand High School Leaving Certificate: 'A' grades in higher level Mathematics and Chemistry.

Adkhamjon Paiziev

Citizenship: Uzbekistan, Date of Birth: 18/10/1950. Institution and address: Institute of Electronics Uzbek Academy of Science, Durmon Yuli str. 33, Tashkent 100125, Uzbekistan, Professional phone: .(998-71)2623719, Professional Fax: (998-71)2628767 adkhampaiziev@gmail.com

DEGREES (DATES AND INSTITUTIONS)

- Ph.D in Physical-Mathematical Sciences (1994), Institute of Electronics Uzb. Acad. Sci., Tashkent, Uzbekistan
- M.Sc. in Theoretical Physics (1972), Tashkent State University, Tashkent , Uzbekistan

QUALIFICATIONS

- 2009-present time, Head of Structural Analysis, Group Human and plant cells physics and physiology, Arifov Institute of Electronics Uzb. Acad. Sci.
- 2009-Visiting professor, Cell biology, Plant genetics and physiology, Hebrew University Jerusalem (Israel)
- 2005- 2006 Cell biologist, Cell wall morphology, Wageningen University, Lab. Cell Biology , Wageningen, The Netherlands
- 2005-2006 Cell physics, Mathematical modelling of cell wall, 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 Head of Research Group, 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)

FIELD OF INTEREST

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

COURSES WHICH MAY BE TAUGHT BY DR. PAIZIEV:

Physical methods in medicine - Agrophysics - Optical microscopy of cells - fiber morphology - Nondestructive testing

Virginia Pensabene

Virginia Pensabene received her Laurea degree in Electronic Engineering from the University of Pisa in 2005 (final-discussed thesis on nanomodification of microelectrode surface for improving adhesion of neural cells). On January 209, she received a Ph.D. in Humanoid Techonologies from the University of Genova, with a fellowship fund by the Italian Institute of Technology, discussing the thesis "Biointerfaces: from nanostructured substrates to novel nanoparticles for cell therapy". In 2006 she joined the CRIM Lab of the Scuola Superiore Sant'Anna in Pisa, as assistant researcher until October 2009 working on design and development of micro and nanodevices for biomedical applications, including nanoparticles for cell therapy and ultrathin films as suturing patch for mini invasive surgery. Part of this work was carried on in the framework of European projects related to robotic surgery, dealing with adhesion and sticking phenomena of devices in the gastrointestinal tract and inside the abdomen. She is with the Center for Micro-BioRobotics of the Italian Institute of Technology since March 2010, as Post-Doc, with her main research focused on innovative soft robots and microcomponents for drug delivery and therapy inside the human body.

COMMUNICATIONS TO NATIONAL CONFERENCES

- "Characterization of carbon nanotubes interaction with extracellular matrix by an in vitro test bench", V.Pensabene, S. Tognarelli, A. Menciassi, P. Dario, I Congresso Nazionale di Bioingegneria, Pisa, Italy, 2008.
- 'Biocompatibility and Functionality of PLA Nanosheets', L. Ricotti, S. Taccola, V. Pensabene, V. Mattoli, A. Menciassi, P. Dario, Congresso Nazionale di Bioingegneria, Torino, Italy, 2010.

INTERNATIONAL PATENTS

- V Raffa, A Menciassi, V Pensabene, G Ciofani, P Dario, Title:" METODO DI ELETTROPORAZIONE NON INVASIVA MEDIATA DA NANOTUBI DI CARBONIO E DISPOSITIVO PER ATTUARE TALE METODO" (method and device for non-invasive electroporation mediated by carbon nanotubes) PCT/IB2007/054754, 2007.
- P.Valdastri, V.Pensabene, A.Misuri, A.Mazzeo, S.Scapellato, Title:" DISPOSITIVO DI SUPPORTO DI SENSORI E/O ATTUATORI FACENTI PARTE DI UNA RETE DI NODI DI MISURA E/O ATTUAZIONE"(Support device for sensors and/or actuators taht can be part of a wireless network of sensors/actiators, PCT B30/0225 2008 A 32, 18 April 2008.

Xiomara Pérez Gutiérrez

Full Researcher and Head of the Group for Research of Liposomes as Drug Delivery System at the Center for Pharmaceuticals Research and Development, Havana, Cuba. Member of the Cuban Society of Pharmaceutical Sciences. Bachelor in Pharmaceutical Sciences, University of Havana, Institute for Pharmaceutical and Food Sciences, 1990-95, Golden Bachelor Diploma for outstanding graduation.

SPECIAL AND GRADUATE EDUCATION RELATED TO LIPOSOMES:

Liposomal Technology: biomedicine and biotechnology applications. Faculty of biology UH, 1996. Liposomes in Latin-America. National University of Quilmes, Buenos Aires, Argentina, 2004. 1st School of Nanotechnology, Liposomes in Latin-America 2. National University of Quilmes, Buenos Aires, Argentina, 2006.

SCIENTIFIC RESEARCH EXPERIENCE

Development and validation of stability-indicating assay methods for more than 20 Cuban generic products and their stability studies. Director of two National Research Projects (Cuban Ministry of Science, Technology and Environment): "Liposomal formulations for topical use as advanced drug delivery systems", 1996-2000; "Liposomal Formulation of a steroidal anti-inflammatory", 2002-2006.

MORE IMPORTANT PUBLISHED PAPERS:

- "Statistical comparison between two methods for cholesterol quantification from MLV liposomal dispersions". Rev Cubana Farm, Vol. 34, special issue, jun/2000, págs. 264-267.
- "Characterization and preclinical study of the clobetasol propionate in liposomes". Abstract Book, IV Spanish-Portuguese Conference on controlled drug delivery sept/2000.
- "Assisted microwaves extraction combined to liquid scintillation detection in determining clobetasol propionate label with tritium". Radiocarbon. LSC 2001, Advances in Liquid Scintillation Spectrometry. Proceedings of 2001 Karlsruhe Conference.
- "Liposomic Formulation of clobetasol propionate". Patent Cooperation Treaty (PCT) International Publication No.: WO 02/07702 A2 (2001)
- "Application of some validation criteria in the cholesterol quantification from liposomes". Rev Cubana Farm, Vol. 38, Suplemento 1, jun/2004, págs. 305-311.
- "Increased thymolytic action from liposomal clobetasol". Rev Cubana Farm, Vol. 38, No. 2, 2004.

PATENTS

"Liposomic formulation of clobetasol propionate". Granted in: Cuba (2006), Mexico (2006), Europe (2006), Australia (2006), China (2007); India (2009) and Canada (2009).

Simona Pinzaru

Dr. Simona Cinta Pinzaru is associate professor at Babes--Bolyai University, Molecular Spectroscopy Department from Cluj-Napoca, Romania. She received her PhD in Physics at Babes-Bolyai University in 1998. She has carried out doctoral and postdocs studies in the Institut für Physikalische Chemie from University of Würzburg, Germany. She conducted various national and international research projects focussed on applied vibrational spectroscopy in biological and medical field. She received the Scientific Excellence Award in 2010 from Babes-Bolyai University. Recent research topic covers the applied Raman spectroscopy techniques in medical field, particularly in Raman cancer diagnostic.

Denis N. Prodius

Dr., Institute of Chemistry, Academy of Sciences of Moldova, Academiei str. 3, MD-2028 Chisinau, Republic of Moldova, Phone: (3732) 73 97 22 Fax: (3732) 73 99 54, E-mail: denis.prodius@gmail.com, denis.prodius@kit.edu. Born: October 03, 1977. Nationality: Rep. of Moldova. Birthplace: v. Beleavinti/Briceni, Moldova

- (1999-2002): PhD student, Institute of Chemistry, Academy of Sciences of Rep. of Moldova
- (1994-1999): M.S. in Chemistry, Faculty of Chemistry, State University of Moldova, Chisinau
- (1994-2000): M.S. in Technology, Faculty of Foodstuff Technology, Technical University of Moldova, Chisinau
- 15 June 2007: PhD in Inorganic Chemistry, http://www.cnaa.acad.md/en/thesis/6180/

HONOURS AND AWARDS

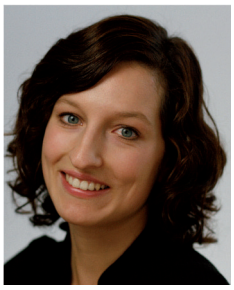
- 2004 Silver Medal Award - International Exposition INFOINVENT-2004, November 10-13, Chisinau; 2006-2010 President of Council of young scientists of Institute of Chemistry ASM;
- 2007 The encouragement "Yu. T. Struchkov 2007 Prize" - for the best study in the field of crystal chemistry or application of X-ray diffraction analysis for the solution of chemical problems (young scientists from the C.I.S. or Baltic states);
- 2009 "Best young scientist of the year in Republic of Moldova" Award; 2010 Alexander von Humboldt Fellowship (Germany, Host: Prof. A.K. Powell, KIT);
- 2010 Gold Medal Award - International Expo - Brussels Eureka Innova 2010, November 18-20

THE AREA OF SCIENTIFIC INTERESTS:

- coordination chemistry, especially:
- Synthesis and investigation of mono- and polynuclear iron (manganese) compounds, mixed valence iron (manganese) carboxylate clusters; Biological active coordination compounds;Nanoparticles and nanocomposite materials.

PUBLICATIONS: (total 32, inclusive 6 patents*): * - http://v3.espacenet.com/results?sf=q&DB=EPODOC&IA=Prodius+Denis&PGS=10&CY=ep&LG=en&ST=quick.

LANGUAGE KNOWLEDGE: Romanian (g), Russian (ex), English (g), Italian (w), Ukrainian (w), German (w).



Angela Riedel

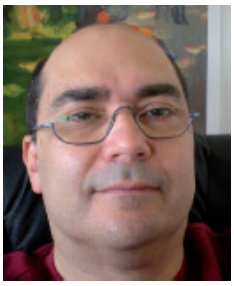
Diploma in Molecular Biotechnology, PhD student Group for Molecular Oncology, Institute for Molecular Medicine, University of Southern Denmark, J.B. Winsløws Vej 25/1, 5000 Odense-C, Phone: +45-6550-3977, Fax: +45-6550-3950, ariedel@health.sdu.dk

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: 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

Anjali Roeth

Anjali Roeth studied medicine and physics at the RWTH Aachen University in Germany. She graduated in medicine in 2005 and earned her diploma in physics for her thesis on "Focussing of USPIO (Ultrasmall Superparamagnetic Iron Oxides) in Prostate Cancer for Optimization of Magnetic Targeting Strategies" at the Institute for Applied Medical Engineering at the RWTH Aachen in 2008. She then joined the Department of Surgery at the Aachen University Hospital as a surgical resident, first under Prof. Dr. Dr. V. Schumpelick and since 2010 under Prof. Dr. U. Neumann. Her medical doctoral thesis was on "Investigation of Molecular Reactions after Central and Peripherous Lesions of Nerve´s in the Rat with Special Regard Towards Neurodap1" at the Department of Neuropathology at the Aachen University Hospital. Besides her work as a resident she is currently working on her Ph.D. thesis on focussing of magnetic nanoparticles (MNP) in cancerous tissues. She is member of the European Society for Nanomedicine since 2010.



Zoltan Rozsnyay

Degree MSc, PhD. Date of Birth 28.01.1961. HU-1124 Budapest, Zolyomi lepcso 7, rozsnyayz@t-online.hu. Function senior scientist. Place of work Semmelweis University, Budapest, Hungary, Phone 36 20 825 9693, Fax 36 12 100 100

PROFESSIONAL ACTIVITIES

Current:Head of Human Nanoimmunotoxicology, 2007-present, Dept. of Nanomedicine, Semmelweis University, Budapest, Hungary.

Previous: Senior Scientist (1999-2004) Pfizer, France; Senior scientist (1996-1999) DKFZ, Germany; Postdoc (1992-1996) Sandoz/Novartis, Austria; Graduate and Ph.D. student (1985-1992), L. Eötvös University, Hungary

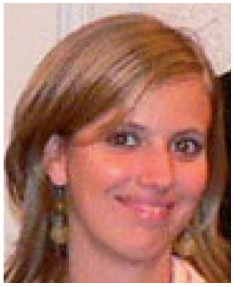
Reto Sauder

Department of Biophysical Chemistry, Biozentrum, University of Basel, Klingelbergstrasse 50/70, CH - 4056 Basel, Switzerland, E-Mail: reto.sauder@unibas.ch

EDUCATION

Bachelor of Science in Biology (Major in Molecular Biology), Biozentrum, University of Basel 2003-2007

- Master of Science in Molecular Biology (Biophysics), Biozentrum, University of Basel 2007-2009
- PhD in Biophysical Chemistry, 2009-present



Priscila Schilrreff

PhD Student (Degree in Biotechnology), Nanomedicine Research Program, Department of Science and Technology, Av. Roque Saenz Peña 352, Bernal, Buenos Aires, Argentina, pschilrreff@unq.edu.ar

Priscila Schilrreff was educated at Quilmes National University (UNQ), Argentina where she obtained her degree in Biotechnology (2007). She is currently developing her doctoral research in Basic and Applied Science, at the Nanomedicine Research Program (NRP) at UNQ, Buenos Aires, Argentina. National Scientific and Technical Research Council (CONICET) awarded her with a type I (three years, 2008-2011) and type II (two years, 2011-2013) doctoral fellowship. Her research work is supervised by Maria Jose Morilla, PhD and Eder Romero, PhD, PNM director. Her doctoral research project is focused on the design of Megamers (core-shell nanoparticles) capable of crossing the mucosal epithelial barrier. Her contribution has been published as first/second author in International Journal of Pharmaceutics. In addition, this project was presented (poster format) in NanoBio-Europe 2010 Congress, Germany (2010), XLII National Congress of Pharmaceutic Sciences, Mexico (2009), First workshop on artificial organs, biomaterials and tissue engineering, Argentina (2009).

She is an Instructor Professor of Chemistry (2008) at the Department of Science and Technology, National University of Quilmes, Buenos Aires, Argentina. She also participated in the Nanomedicine School in Latinoamerica (2008, 2010) as professor and organizer.

Helmut Schmid

Affiliation and official address: Fraunhofer Institute Chemical Technology (ICT), Joseph-von-Fraunhofer-Str. 7, D-76327 Pfinztal (GERMANY). Date and place of birth: June, 1st, 1955, Aistaig/Neckar, Germany. Nationality: German

EDUCATION: 1986: Dipl.-Chem. (University Karlsruhe)

CAREER/EMPLOYMENT: 1986: Scientific Assistant (Theoretical and Mathematical Group) at ICT. 1987: Scientist (Theoretical and Mathematical Group) at ICT. 1992: Scientist (Polymer Chemistry) at ICT. 1996: Head of Gas Generator Development, Product Area Energetic Systems at ICT. 1998: Lecturer / Reader at Berufsakademie Mannheim in the field of Mechanical Engineering, 1998. 2002: Head of Nanotechnology Special Branch, Product Area Energetic Systems at ICT.

SPECIALIZATION (SPECIFY): (i) main field Nanotechnology, Gas Generator Development. (ii) other fields Thermoanalysis, Kinetics, Interior Ballistics, Processing Technology. (iii) current research interest Nanotechnology, Gas Generator Systems

AWARDS: European Excellence in Research Award 2008

FELLOWSHIPS, MEMBERSHIP OF PROFESSIONAL SOCIETIES

- Member of Organization Committee of GEFTA (Gesellschaft für Thermische Analyse), 1990
- Deputy member of Airbag 2000+ Program Committee, 2000
- Member of ETC-Concepts and Propellants Group responsible for GE/US-Data Exchange by order of German Federal Minister of Defense, 2000
- Consultant of Ministry of Construction, Dubai
- Consultant of DECHEMA Working Group "Mikrobielle Materialzerstörung und Materialschutz"
- Consultant of GfKORR Working Group „Mikrobiell beeinflusste Korrosion"
- Consultant of Public-Health-Office, Baden-Württemberg
- Consultant of Public-Health-Office, Bremen
- Consultant of Hygiene Medicine, Brothers Hospital, Trier
- Consultant of Medical Office, German Armed Forces, Munich
- Consultant of German Federal Environmental Agency, Berlin
- Member of International Biodeterioration Research Group (IBRG), Working Groups: Plastic, Polymer Dispersion, Functional Fluids, Paint,
- Consultant of Foundation Risk Dialog, Waldenbuch
- Member of Scientific Advisory Board of Institute of Textile Technology and Process Engineering, Denkendorf
- Member of Advisory Bord of Bioni C.S. Company, Oberhausen
- Consultant of VDI, (PtJ, BMBF), Department Nanotechnology (Building, "NanoTecture"),
- Düsseldorf
- Member of AIST, Association for Iron & Steel Technology, Warrendale, USA
- Member of The American Ceramic Society, Westerville, OH 43081, USA
- Consultant of Prussian Palaces and Gardens Foundation, Berlin-Brandenburg, Textile Restoration
- Consultant of EMPA / eawag, Swiss Federal Institute of Aquatic Science and Technology
- Consultant of European Nanotechnical Association (ENA)
- Member of American Academy of Nanomedicine (AANM)
- German Representative and Member of International Academy of Nanomedicine (IANM)
- Consultant of European Foundation for Clinical Nanomedicine (CLINAM), Basel, Switzerland
- Member of European Society for Nanomedicine (ESNAM), Basel, Switzerland
- Member of German Standards Committee (DIN) "Bautenbeschichtungen" (NA 002-00-15 AA
- Member of German Standards Committee (DIN) „Probenahme, Konditionierung und Prüfung von Beschichtungsstoffen mit Be-

zug auf CEN/TC 351/WG 2, Innenluft“ (NA 002-00-15-02 AK

- Member of German Standards Committee (DIN) „Auswaschung von Bioziden aus Beschichtungen und Putzen für architektonische Zwecke im Außenbereich“ (NA 002-00-15-01 AK

Number of papers in refereed journals / other publications: Approx. 50. Number of communications to scientific meetings/scientific reports: Approx. 100. Inventions/Patents: Approx. 25

Steffen Schmidt

PhD student at University Of Southern Denmark, Institute for Molecular Medicine, group of Molecular Oncology and Lundbeckfonden Center of Excellence in Nanomedicine NanoCAN

- 03/2004-03/2008: Study of Biotechnology at Mannheim University of Applied Sciences; Academic degree: Dipl. –Ing.
- 10/2007-03/2008: Graduand at Sanofi-Aventis Pharma, Frankfurt am Main, Germany; Department: Target-to-Lead Biology
- 05/2007-09/2007: Research assistant at Mannheim University of Applied Sciences, Mannheim, Germany; Institute of Molecular- and Cell biology
- 10/2006-03/2007: Trainee at Sanofi-Aventis Pharma, Frankfurt am Main, Germany; Department: S&MA Genomic Sciences
- 03/2005-08/2005: Trainee at the German Cancer Research Centre (DKFZ.) Heidelberg, Germany; Department: Molecular Genome-analysis

Dariusz Smolen

Institute of High Pressure Physics, Polish Academy of Science, Sokolowska Street 29/37, 01-142 Warsaw, Poland, Phone: +48 22 876 03 16, Email: dsmolen@unipress.waw.pl, dareksmolen@wp.pl

EDUCATION: 07/2010-currently: Institute of High Pressure Physics, Polish Academy of Science, PhD student. 10/2008-currently: Warsaw University of Technology, Management Department. 10/2005-06/2010: Warsaw University of Technology, Chemistry Department, M.A. thesis: Synthesis of aluminum nitride nanopowder.

EXPERIENCE

- 10/2009-currently: Institute of High Pressure Physics, Polish Academy of Science, Laboratory of Nanostructures, Responsible for: hydroxyapatite nanopowder synthesis
- 10/2009-06/2010: Warsaw University of Technology, Chemistry Department, Young Assistant, Responsible for: aluminum nitride nanopowder synthesis gallium nitride nanowires synthesis
- 10/2009-12/2009: Physics Institute, Polish Academy of Science, Training in Transmission Electron Microscopy Group
- 07/2008-09/2008: L’Oreal Manufacturing UK, Training in Quality Department
- 07/2005-08/2005: Instituite of Organic Chemistry, Polish Academy of Science, Training in the labolatory of stereocontrolled organic synthesis and supramolecular chemistry (award for High School achievements)

PATENT APPLICATIONS

S. Podsiadlo, D. Smolen, P. Dominik, W. Adamkiewicz, K. Trocewicz, P-389659, Synthesis of aluminium nitride nanopowder.
S. Podsiadlo, P. Dominik, W. Adamkiewicz, D. Smolen, P-390477, Synthesis of gallium nitride nanowires.

PUBLICATIONS: P. Dominik, J. Gola, D. Smoleń, S. Podsiadło, Non catalytic growth of gallium nitride nanowires by sublimation sandwich method on (001) Al2O3, Journal of Materials Science and Engineering, 2010, 4, 53-56

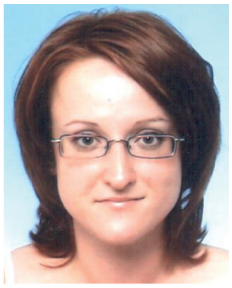
OTHER ACHIEVEMENTS: L’ORÉAL „INGENIUS“ contest - International final in Paris, 2008. Finalist of the Polish L Chemistry Olympiad, 2004.

Akhter Sohail

Dr. Sohail Akhter is a young researcher working in area of nanotechnology based drug delivery system. Presently working as Senior research Fellow under CSIR at Nano-medicine Research Lab, Department of Pharmaceutics, Faculty of Pharmacy, Hamdard University, New Delhi-110062, India. He has completed his M.Pharm in 2008 at the age of 25 years from Hamdard University (Delhi, India). He has 2 patents and published more than 15 papers in the reputed International journals. He also has life time membership of Indian Pharmacy Graduate Association (IPGA). Recently, Dr. Sohail successfully completed a major research project (3 years) of DBT, Govt of India, related to application Nanocarrier in drug targeting for ophthalmic disorder, in brain and cancer while working under DBT research fellow. He is also serving as invited manuscript reviewer for various international journals.

ACHIEVEMENTS

- Secure 99.70 percentile in Graduate Aptitude test in Engineering (GATE) 2006, conducted by Indian Institute of Technology (IIT) kharagpur.
- Secure 98.56 percentile in Graduate Aptitude test in Engineering (GATE) 2007, conducted by Indian Institute of Technology (IIT) kanpur.
- Participated in ‘International Symposium on guiding principles for pharmacist-HIV / AIDS in India and update on newer drug development for HIV / AIDS and in Ocular pharmacology held at AIIMS, New Delhi
- Registered pharmacist under Delhi Pharmacy Council having registration no. 017436.
- Secure first prize in Pharma quiz in Rx fest 2006 organized by faculty of pharmacy Jamia Hamdard, N.Delhi-62, India.
- Life time membership of Indian pharmacy Graduate Association (IPGA).



Denisa Stránská

Ing., Rubínová 1183, 46006 Liberec 6,Czech Republic, telephone: +420 608 633 690, denisa.stranska@faf.cuni.cz

EDUCATION: 1999-2004: Institute of chemical technology in Prague, Organic and pharma chemistry. 1993-1999: Secondary school, Kojetín 2009-present: PhD.student, Charles University, Faculty of Pharmacy, Theme: Nanofibers membranes as a drug carrier

PUBLICATION

Kvíčala J., Stránská D., Krupková A., Baszczyński O.: Hydroboration of 1,1’-Bi(cyclopent-1-ene) and 3,3’-Biindene: Experimental and Theoretical Study, Collection of Czechoslovak Chemical Communications, 71, 11-12, pp. 1611-1626. Author or co-author of many patent applications.

Cristina Surdu-Bob

RESEARCH INTERESTS: Development of plasma based technologies for medical applications including cancer therapy and biocidal surfaces. Thin film deposition by plasma for metal, nitride, oxide and compound coatings. Surface analysis using XPS, AFM, XRD, Raman, TEM, profilometry. Plasma based synthesis of micro and nanospheres.

EDUCATION: Year 1995 - Graduated from Faculty of Physics, University of Bucharest, Physics Engineer Year 2003 - Doctoral Degree

from Aston University, Electronic Engineering & Applied Science Dept., Surface Science Group- Birmingham, UK

WORK EXPERIENCE

- Current place of work: National Institute for Lasers, Plasma and Radiation Physics (INFLPR), Bucharest, Romania. Low Temperature Plasma Laboratory. Head of Plasmacoatings Group
- April. 2003-Oct. 2008: Postdoctoral Researcher, INFLPR
- Jan.1999-April.2003: PhD Student. (including 6 months maternity leave), Aston University, Electronic Engineering & Applied Science Dept., Aston Triangle, B4 7ET - Birmingham, UK and co-funding by Marconi Ltd., UK.
- Oct. 1996-Jan. 1999: Graduate Researcher, Low Temperature Plasma Laboratory, INFLPR.

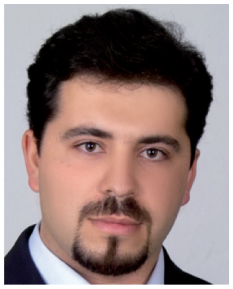
PATENTS

- 1. Issued patent. Technique for thin film deposition from gaseous precursors using electrical arc plasmas with hot cathode; Patent No.: 1041285/2004 (Romania), Authors: G.Musa, C.C.Surdu-Bob
- 2. Patent pending. Technique for the synthesis of spherical metal nano and micro-particles, patent pending. C.C. Surdu-Bob, M. Badulescu; Deposition No. A/00754/2009
- 3. Patent pending. Echological, antimicrobial, permanent and large spectrum effectivity composite and equipment for the production of this composite A/01267/2010

Farzaneh Tajdini

Faculty member of Veterinary Medicine School, Islamic Azad University (Karaj, Alborz, Iran). Already I have 5 undergraduate DVM students in my laboratory (Laboratory of microbiology and Biotechnology). I have worked on clinical aspect of Mycosis disease in cattle. However, recently I have focused on biotechnology aspect of Saprophyte fungi. In this area, I have succeeded d to extract and characterize chitosan from fungi and use it as a new carrier for vaccine and drug delivery systems.

Contacts: Address: School of Veterinary Medicine, Islamic Azad University, Moazen Boulevard, Gohardasht, Karaj, Alborz, Iran. Tel: (+98) 9122720389, tajdinifarzaneh@yahoo.com & farzaneh.tajdini@kiau.ac.ir.



Ali Mohammad Tamaddon

Pharm.D, Ph.D, Ass. Professor, Pharmaceutical Nanotechnology and Cellular Delivery Lab, Department of Pharmaceutics, Faculty of Pharmacy Shiraz University of Medical Sciences, PO Box: 71345-1583, Shiraz, Iran, Tel:+98-711-242-4127 (Ext. 257), Fax: +98-711-242-4126, Email: amtamadon@sums.ac.ir, amtamadon@gmail.com http://pharmacy.sums.ac.ir/en/departments/pharmaceutics/ali-mohammad-tamaddon.html

EDUCATIONAL RECORDS

- Research Fellowship (2005): PROTHETS project, Partner #8, UMR CNRS 8121, Vectorologie et Transfert de Genes, Institut Gustave Roussy, France.
- Ph.D (2000-2006): Shaheed-Beheshti University of Medical Sciences, School of Pharmacy, Tehran, Iran.
- Pharm.D (1994-2000): Esfahan University of Medical Sciences, School of Pharmacy, Isfahan, Iran.

ACADEMIC APPOINTMENTS

- 1.Dec 2006 - Present: Assistant Professor, department of pharmaceutics, school of pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran. (1st Appointment) 2.Oct 2007 - Present: Chairman of Shiraz University Nanotechnology Committee, Shiraz University of Medical Sciences, Shiraz, Iran.
- 3.Apr 2008 – Present: Vice-president, Department of Pharmaceutics, Shiraz University of Medical Sciences, Shiraz, Iran.
- 4.Dec 2008 – Present: Assistant Professor, department of pharmaceutical biotechnology, school of pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran. (2nd Appointment) 5.Oct 2009 – Present: Head of Pharmaceutical Nanotechnology and Cellular Delivery Lab, Graduate School of Modern Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran.

RECENT PUBLICATIONS:

- 1.Marie Villemeur, Ali Tamaddon, Jean-Rémi Bertrand, Claude Malvy. Comparative Activity and Specificity of Antisense Oligodeoxynucleotides and Small Interfering RNA in an in vitro Ewing Sarcoma Model. The Open Nanomedicine Journal, 2009; 36-45.
- 2.Tamaddon AM, Niknahad H. Pharmaceutical Biomaterials. Iranian Journal of Pharmaceutical Sciences, 2009; 1-2.
- 3.Peymani P, Tamaddon AM, Jaberipour M, Shahbazi MA, Hamidi M. Formulation of chitosan nanoparticle for P53 gene delivery in tumor cells. Iranian Journal of Medical Hypotheses and Ideas, 2008; 15(2).
- 4.Tamaddon AM, Shirazi FH, Moghimi HR. Modeling cytoplasmic release of encapsulated oligodeoxynucleotides from cationic liposomes. International Journal of Pharmaceutics, 2007; 336(1): 174-182.
- 5.Tamaddon AM, Shirazi FH, Moghimi HR. Preparation of oligodeoxynucleotide encapsulated cationic liposomes and release study with models of cellular membranes. DARU, 2007; 15(2): 61-70.
- 6.Toub N, Bertrand JR, Tamaddon AM, Elhamess H, Hillaireau H, Maksimenko A, Maccario J, Malvy C, Fattal E, Couvreur P. Efficacy of siRNA nanocapsules targeted against the EWS-Fli1 oncogene in Ewing sarcoma. Pharmaceutical Research, 2006; 23(5):892-900.

Bogdan Ionel Tamba

M.D. PhD., Str. Bogdan Dragos, Nr. 18 bis, Roman 611075 Romania , Tel: 0040 744 635 724; Fax: 0040 233 743 860, Email: bitamba@mail.umfiasi.ro

EXPERIENCE: Actual position (since 2005): Assistant Professor, Department of Pharmacology and Algesiology, Faculty of Medicine, University of Medicine and Pharmacy "Gr. T. Popa" Iasi, Romania

- 2010-2013: Partner coordinator for the FP7 project AMI4EUROPE - Advanced, Cross-Disciplinary & Integrated Medical maging for all Europeans through a Network of Regional Clusters and Development Strategies
- 2010-2011: Invited expert in research management for national POSDRU EU funded projects
- 2010 - 2011: Director of IASP grant - initiative for improving education in developing countries
- 2007-2008: National Scientific Research Council $ 20.000 grant for finalising the PhD thesis on trace elements and their implication on pain
- 2004-2008: Member of 5 research projects financed by the National Scientific Research Council, totaling $ 400.000. The main research areas are new drug development, nanotechnologies, pain, inflammation.
- 2004-2008: Clinical research associate at Pharma Consulting Ltd., Iasi, Romania, - bioequivalence trials, prospectus research, documentation for on the market release and National Drug Agency approvals.
- 2006-2008: Project vice manager of "Platform for physiopharmacological and clinical studies on the mechanisms of nononcologic and oncologic pain." financed by the Romanian government and the European Union, totaling aprox. $ 4 million for 24 months.
- 2001-2002: Visiting student researcher at the Molecular Medicine Department, Salamanca University (project on genetics of cancer)
- August 2000 IFMSA research scholarship on Irritable Bowel Syndrome at the Digestive Research Unit, Vall D'Hebron Hospital, Barcelona, Spain

- Summer 1996: Soros Foundation scholar: 5 weeks academic program focused on English, Science-Fiction and Astronomy at Choate Rosemary Hall College, Wallingford, CT, USA

EDUCATION

- 2010-PhD in Pharmacology "Gr.T.Popa" University of Medicine and Pharmacy, Iasi, Romania
- 2010-Board certified specialist in Clinical Pharmacology
- 1998-2004 "Gr.T.Popa" University of Medicine and Pharmacy, Iasi, Romania, Faculty of Medicine, Medical Doctor (MD) Degree, National Award of Merit (1998/1999)
- 2001-2002 Universidad de Salamanca, Spain, Faculty of Medicine, European Union Scholar
- 1994-1998 "Roman Voda" Highschool, Roman, Concentration: Physics-Chemistry, Graduated Summa Cum Laude (ranked 5th / 270)
- 1998 January-SAT II scores: Math I-750/800 top 2% in the world, Physics-800/800, top 5%, Chemistry-770/800, top 6%,
- 1998 February-ACT composite score-34/36, top 2%

ADDITIONAL EDUCATION

2007 August-1 week PTPI leadership seminar in Berlin, Germany. 2006 March-1 week European Union training of trainers in Human Rights Education, Plovdiv, Bulgaria. 2005 October-1 week PTPI leadership seminar in Berlin, Germany. 2005 March-1 week European Union contact making seminar for youth NGO's, Warsaw, Poland 1999 December-1 week European Union training session on NGO's, Budapest, Hungary.

SCIENTIFIC ACTIVITIES

Member of: the International Association for the Study of Pain (IASP), Member of the American College of Clinical Pharmacology (ACCP), Member of the EACPT Education sub Committee and Wider group. Member of European Federation of IASP Chapters (EFIC), Treasurer of the Algesiology Association of Romania (AAR), Scientific and organizing committee's member of the Medicines' Day National Conference for the XIVth to the XXth editions.

PRIZES AND AWARDS

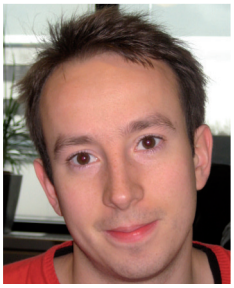
- 2010-IASP financial aid receipient for the 13th World Congress on Pain, Montreal, Canada
- 2010-EACPT bursary for the 8th EACPT Summer School, Dresden, Germany
- 2009-EACPT Young Investigator Bursary (European Association of Clinical Pharmacology and Toxicology)
- 2008 May-IIIrd prize award for the best poster at the "Medicines' Day" XVIIth National Conference, Romania
- 2006 August-"Outstanding leadership award" by People to People International, World Wide Conference, Sidney, Australia
- 2005 May-1st prize award, Medicines' Day, XIVth edition, Iasi, Romania
- 2003 November - Session Award on Clinical Neurology, the 14th European Students Conference, Berlin, Germany
- 2003 May - Session Award on Diabetes/Endocrinology, the 14th European Students Conference, Berlin, Germany
- 2003 May-Special Award on Clinical Neurology, the 7th International Medical Students' and Young Doctors' Meeting, Timisoara, Romania
- 2002 April-1st prize award, the 8th Cardiology Congress for Students, Salamanca, Spain

EXTRACURRICULAR ACTIVITIES

- 2003-2007-European Youth Coordinator of People to People International
- 2000 October-PR secretary of the Medical Students Association affiliated to the Medics' and Naturalists' Society, Iasi, Romania
- 1999-present date: member of People to People International
- 1996-6th Speaker at the "Karl Popper" National Debate Camp

SKILLS

Foreign languages: English-fluent, TOEFL test (10/1997) points 637/680, top 10%. Spanish - fluent. French-intermediate.
Computer: excellent skills in all major office and non office applications. Project management: advanced skills. Communication skills: created educational material, held presentations at scientific meetings/conferences.



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
Others: Member of Young European Biotech Network Organization.

Rudolf Urbanics

Function: Head of in vivo lab.. Degree 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, 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 of 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.

Zhidao Xia

Dr., College of Medicine, Centre for Nanohealth, Institute of Life Science, Singleton Park, Swansea, SA2 8PP, UK, z.xia@swansea.ac.uk

Dr. Xia is a lecturer of regenerative medicine at Swansea University, UK. He obtained his medical degree in China with 12-years clinical experience in orthopaedics and traumatology. He obtained a Ph D at Oxford University in 2005. He specialised in mesenchymal stem cells and skeletal tissue engineering, nanotoxicity of wear metal particles and worked cross-disciplinarily between medicine, engineering and material sciences. He was awarded a Wellcome Trust VIP fellowship in 2003, and carried on working as a post-doc research scientist in Oxford University between 2005 and 2007. He was granted a five year Botnar Fellow (Senior Research Fellow) in 2007 and established a research group of skeletal tissue regeneration in Oxford. In 2010 he moved to Swansea University to take up a Lectureship in the School of Medicine. He is establishing a regenerative medicine group.

Fabian Zehender

Division of Biophysical Chemistry, Biozentrum, University of Basel, Klingelbergstr. 50-70, CH-4056 Basel

EDUCATION:

2008-present: University of Basel, Biozentrum: PhD student in biophysics. 2002-2007 Albert-Ludwigs-Universität Freiburg: Diploma studies of chemistry

Matthias Zwick

PhD student, Department of Biophysical Chemistry, Biozentrum, University of Basel, Klingelbergstr. 50-70, 4056 Basel, Switzerland

EDUCATION: 2002-2007 Diploma studies in biochemistry, University of Regensburg, Germany. Ssince 2008 PhD student, Biozentrum, University of Basel.

CLINAM European Foundation for Clinical Nanomedicine

4th European Conference for Clinical Nanomedicine

The Great Strides towards the Medicine of the Future

May 23-25, 2011 - Congress Center Basel, Basel, Switzerland

Conference Proceedings

PART IV
Abstracts of the Posters

These are Submitted Individual Posters.

ENHANCEMENT OF DOXORUBICIN CHEMO-SENSITIVITY OF HER-2 POSITIVE BREAST CANCER CELLS BY DEHYDRATION-REHYDRATION STABILIZED LIPOPLEXES AGAINST SURVIVIN MRNA TRANSCRIPTS

Samira Sadat Abolmaali*, Ali Mohammad Tamaddon, Mojgan Neekravesh, Hossein Niknahad

Nanotechnology and Cellular Delivery Lab., School of Pharmacy and Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz 71345, Iran. *presenting author

INTRODUCTION

Drug resistance, as the function of efflux transporters or apoptosis inhibitors, still remains the main restriction factor for chemotherapy [1]. Survivin, an antiapoptotic protein specifically found in tumor cells that acts by Caspase-3 inhibition, was shown recently as an interesting therapeutic target [1]. To inhibit Survivin expression at mRNA level, antisense technology was applied in the present study.

For cellular delivery of antisense molecules, they formed complexes with cationic nanosized liposomes and were further stabilized by dehydration-rehydration method. Finally, we aimed to increase chemo-sensitivity of doxorubicin in HER-2 positive breast cancer cells (SKBR-3) in comparison with those of HER-2 negative (MCF-7).

METHODS

Nanosized homogeneous DOTAP liposomes with different helper lipids of DOPE, DOPC, DPPG and cholesterol in 1:1 mole ratio was prepared in HEPES buffer (pH=7.5) by thermobarrel extruder through double stacked 100 nm polycarbonate filters above critical temperatures [2]. They were characterized according to their particle size distribution, total lipid recovery and physical stability. Following the incubation of the nanoliposomes and antisense agent for 15 min at room temperature, the degree of complexation were determined by ethidium bromide exclusion assay. Due to shelf-life and complete culture medium instabilities of the lipoplexes, either the lipoplexes or the nanoliposomes were freeze-dried following 10% sucrose addition and reconstituted in either distilled water or antisense solution, respectively prior to application [3, 4]. Physico-chemical characteristics of the resulted particles were studied regarding their size distribution, the degree of complex formation, and extracellular stability modeled in heparin sulfate supplemented medium [5]. The potential of the lipoplexes and those of dehydration-rehydration stabilized to transfect different cell lines (SKBR-3, MCF-7) and the level of cellular association of FITC-labeled antisense agent was studied by flow cytometry in comparison with the commercialized vector, Metafectene reagent, after 4 hour incubation at 37°C. Doxorubicin cell growth inhibition was studied after 4 and 24 hour incubation at different concentrations and following 4 hour incubation with the stabilized lipoplexes of antisense in comparison with those of a scrambled control by MTT assay.

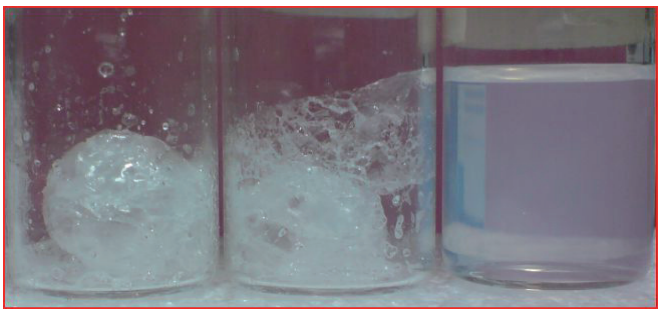


Fig. 1. Freeze-dried nanoliposomes (DOTAP/DOPE, 1:1) and the corresponding lipoplexes prior to and after reconstitution

RESULTS

Nanoliposomes of the different formulations had a favorable particle size distribution (50-150 nm). They had lipid recovery percentages around 85% following extrusion and sterile membrane filtration varied depending on their lipid compositions.

Upon incubation with the antisense agent, the degree of complex formation was determined almost 70-80% (N/P ~ 2.5) which decreased rapidly following incubation with either phosphate buffered saline or complete culture medium. To overcome such instabilities including their limited shelf-life encouraged us to stabilize the nanoliposomes or the complexes formed at the optimum N/P by dehydration-rehydration method. The liposomes comprising DOPE and DOPC not only were superior regarding preservation of the initial particle size distribution following dehydration-rehydration, but also enhanced the stability of the liposomes in extracellular matrix. This may imply that nanoencapsulation takes part in better protection of the antisense molecules during dehydration-rehydration process, but this requires further investigation.

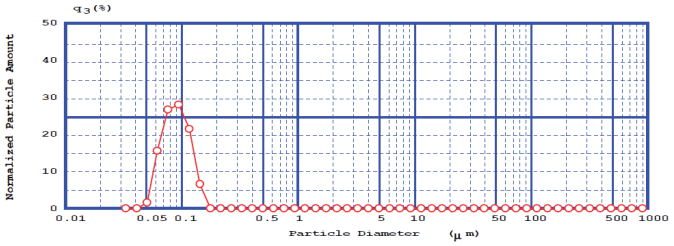


Fig. 2. Particle size distribution of dehydration-rehydration stabilized nanoliposomes (DOTAP/DOPE, 1:1).

It was revealed in the flow cytometry experiment that about 90% of the cells were transfected with fluorescence intensities comparable with those of the parent lipoplexes prior to dehydration-rehydration. The fluorescence intensity was significantly higher in SKBR-3 than MCF-7 cells.

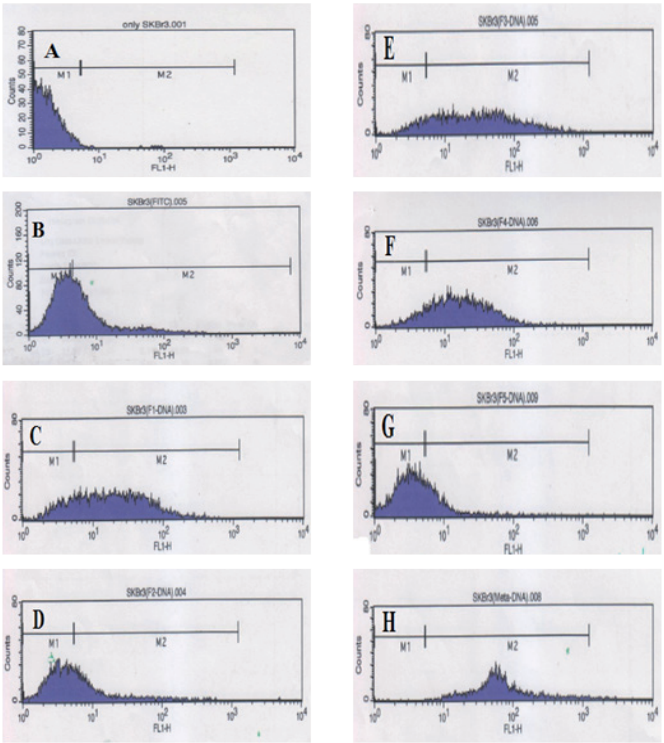


Fig.3. Flow cytometric histograms of the cell associated fluorescence intensities. A: untreated cells, and cells treated with B: free antisense agent, C to G: dehydration-rehydration nanoliposomes of different lipid compositions, and H: freshly prepared Metafectene complex. .

IC_{50} for the cell growth inhibition associated with the stabilized antisense lipoplexes were calculated in range of 0.5-1 μM and significantly lower in SKBR-3 than MCF-7 cells. Pre-treatment of the resistant SKBR-3 cells with the stabilized lipoplexes lowered IC_{50} of doxorubicin to the same level of the sensitive MCF-7 cells.

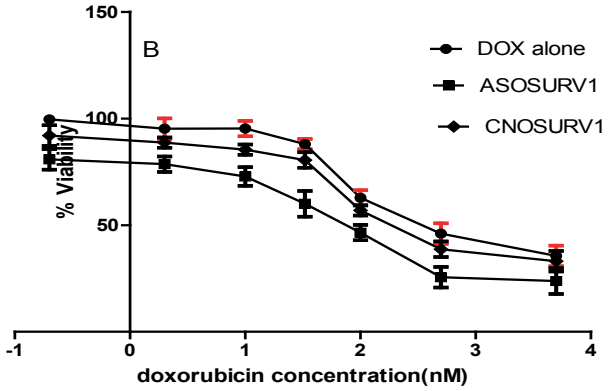


Fig.4. MTT assay of doxorubicin cell growth inhibition alone (●), and as pre-treated by antisense loaded nanoliposomes (■) and those of scrambled control sequence (♦).

CONCLUSION

The developed gene nanocarrier could sensitize the cells to doxorubicin chemotherapy. Now we are going to run an experiment to evaluate such synergistic effect in animal model. It's expected the combination of both chemotherapeutic and antisense modalities into a unique nanocarrier may benefit the patients with more therapeutic effectiveness and less chemoresistance in future.

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EFFECTS OF FLAVONOID COATING OF FE3O4@ TIO2 NANOCOMPOSITES ON DNA SCISSION

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Reactive oxygen species(ROS) generated at the surface of nanomaterials caused by titanium dioxide photoactivation can cause in vitro plasmid DNA cleavage.(1) Similarly to the DNA in vitro, cellular DNA can sustain damage in cells that contain TiO2 materials and are exposed to radiation or white light. While cells are able to cope with some quantity of ROS and protect the integrity of nuclear and mitochondrial genomes, it is possible to overwhelm the cellular antioxidant machinery and induce DNA cleavage in situ. This in turn, can lead to cell cycle arrest, senescence or cell death. Overpowering cells by induction of ROS is the basis of many of the anti-cancer treatments and forms a basis for potential therapeutic uses of TiO2 nanoparticles, nanoconjugates and nanocomposites.(2) Recently, there has been an interest in using the unique photocatalytic properties of titanium dioxide coupled with the ability to conjugate biomolecules to nanoscale titanium dioxide as a novel method for delivering therapeutic or diagnostic agents to malignant cells.(3,4,5)

The conjugation of various flavonoids to Fe3O4@TiO2 nanocomposites may alter the multifunctional properties of these nanocomposites on cancer cells. Fe3O4@TiO2 is a core shell nanocomposite with an iron oxide core with titanium dioxide shell.This type of nanocomposite is useful in that the Fe3O4 core will permit MRI imaging of the nanoparticles and might be useful in diagnostic imaging. We investigated the effects of conjugating the 2 flavonoids, quercetin and catechin to Fe3O4@TiO2 nanocomposites on the ability of resultant nanoconjugates to cleave DNA in vitro and in cells.

Nanoconjugates prepared by conjugating quercetin and catechin to Fe3O4@TiO2 nanocomposites have been tested through two separate groups of experiments. First, nanoconjugates have been mixed with plasmid DNA and illuminated with white light. Plasmid scission in this case was monitored by gel electrophoresis (Figure 1). Second, HeLa cells were incubated with the same nanoconjugates and irradiated. Cleavage of genomic DNA was followed as an increase in the number of 53BP1 foci in cells which indicate sites of double strand DNA breaks (Figure 2). Modulation of nanocomposites with quercetin or catechin lead to alterations in their DNA scission behaviour. Surprisingly, in vitro and intracellular data did not show the same trends in increase/decrease of DNA scission with different nanocomposite modulations. This suggests that coating of nanoparticles modulates not only their ROS producing capacity but also their intracellular localization and/or other factors that are brought to bear when nanocomposites have surface coating. Moreover, this data indicates the necessity to test each new nanoparticle formulation in different experimental setups.

The ultimate goal of the work is to develop a theranostic agent that would cleave DNA in an inducible and sequence specific manner that could be used for imaging and treating cancer cells simultaneously in the patient.

Figure 1. Gel Electrophoresis

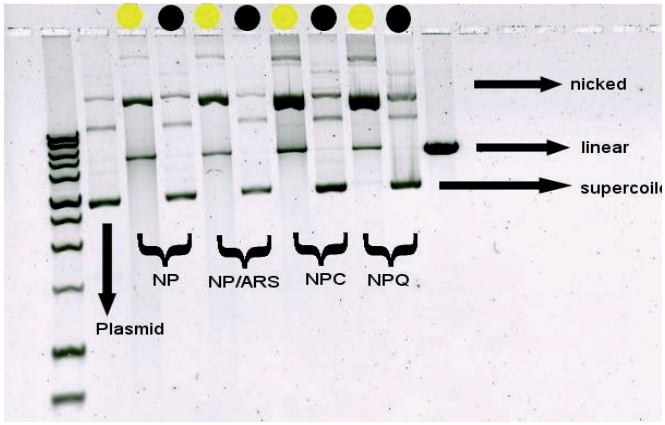
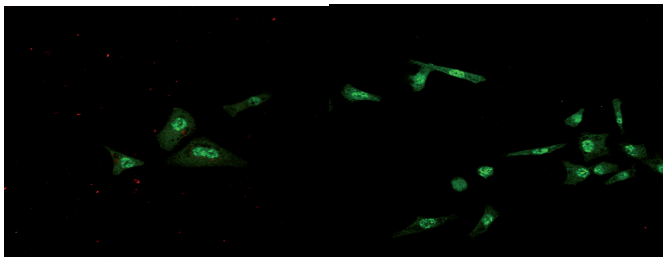
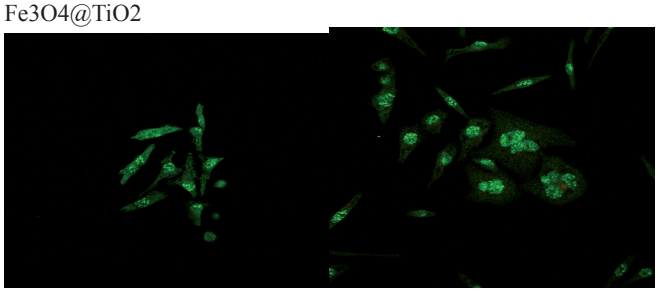


Figure 2. DNA strand breaks(green nuclear foci by 53bp1 stain following incubation of HeLa Cells with different nanoconjugates and exposure to 2Gy radiation



HeLa cells incubated with Fe3O4@TiO2

HeLa cell incubated with NP-ARS



HeLa cells incubated with NP-C

HeLa cell incubated with NP-Q

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PRODUCTION, CHARACTERIZATION AND IN VITRO DEPOSITION OF BUDESONIDE LOADED POLY(LACTIC ACID) NANOPARTICLES

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INTRODUCTION

Budesonide, a potent corticosteroid with high glucocorticoid receptor affinity and prolonged tissue retention, is already used for the treatment of chronic inflammatory respiratory disorder in the form of a pressurized metered-dose nasal inhaler, an orally administered dry powder inhaler and suspension for nebulizer. The aim of this study was to design a stable PLA-budesonide nanosuspension for nebulizer, capable of sustaining budesonide release.

METHOD

Preparation of Nanoparticles: For preparation of nanoparticles, 2.5 mg of budesonide were dissolved in 2 ml chloroform containing 15 mg of PLA. The solution was added to the gelatin 0.5% drop wise under sonication using an ultrasonic probe (Lab Sonic®, B.Broun, Germany) operating at 65% amplitude for 75 second. Finally, 100 µl of a 200mg/ml aqueous solution of Tween 80 was added to the solution and sonicated by bath sonicator at 40°C for 30 min (sample 1). The suspension was centrifuged at 2000 g for 2 min and the elicited supernatant was considered as another sample (sample 2). Also, Sample 3 was obtained by filtration of suspension through 0.45µm syringe filter.

Characterization of nanoparticles: The mean particle size of nanoparticles were determined by photon correlation spectroscopy (PCS), using a Zetasizer (Malvern Instruments, United Kingdom).

In-vitro aerosol characterization: Aerodynamic particle size distribution was measured using an Anderson 8-stage cascade impactor for 10 min at a flow rate of 28.3 l/min. After nebulization with a Beurer Medical jet nebulizer, the amount of budesonide deposited on each stage was analyzed using HPLC.

RESULTS AND DISCUSSION

Table 1 indicates the results of aerosol characterization of the samples. The mean particle size of the samples 1, 2 and 3 were 435.8, 309.4 and 158 nm, respectively.

Table 1. Mass median aerodynamic diameter (MMAD), Geometric standard deviation (GSD) and Fine particle fraction (FPF) of nebulized PLA-budesonide nanosuspension (Sample1,2) and Commercial budesonide suspension (CBS).

	Particle size (nm)	MMAD	GSD	FPF (%)
Sample 1	435.8	2.93	2.77	70.67
Sample 2	309.4	2.16	2.5	77.03
Sample 3	158	1.4	3.49	83.76
CBS	--	4.6	2.7	54.32

Fig. 1 shows the the amount of drug (%) in each stage of cascade impactor. The fine particle fraction was taken as cumulative amount of particles <5 µm. Results demonstrated that samples1, 2 and 3 had a higher FPF (ie. 70.67% and 77.03% and 83.7% respectively) in comparison to that of commercial budesonide suspension (CBS) (ie. 54.32%). Moreover, significantly lower MMAD values of samples1, 2 and 3 compared to that of CBS demonstrated the suitability of PLA_budesonide nanosuspension for deep lung delivery.

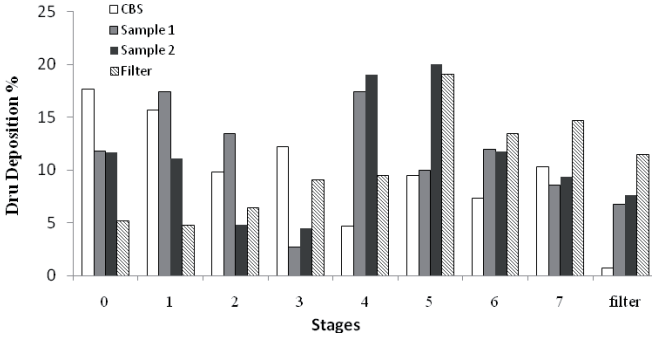

Fig. 1. In vitro deposition profile of Sample 1,2 and CBS

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NOVEL NANO-SIZE GLUCOSE LOADED GADOLINIUM BASED DUAL ANTICANCER AND MOLECULAR IMAGING AGENT

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BACKGROUND

The difficulties in the use, preparation and cost of radioactively-labelled glycosylated compounds led to the research and development in this present study of a new gadolinium-labelled glucose that does not have a radioactive half-life or difficulties in its synthesis and utilization.

METHODS

Based on the structure of fluorodeoxyglucose (18FDG), a new compound consisting of a D-glucose (1.1 nm) conjugated to a well-known chelator DTPA was synthesized, labelled with Gd3+ and examined *in vitro* and *in vivo*. **Results**: This novel compound demonstrated not only a less costly and an excellent imaging capability, but it also showed anti cancer effects on treated cells. Our results demonstrated that the new compound Gd3+ -DTPA-DG (GDD, with GDD conjugate aggregation of ~8 nm at 0.02 mg/ml concentration, Figure 1) had the ability to decrease the tumour cell numbers in HT1080 and HT 29 cell significantly. Application of GDD on cancer cells increased the level of TNF-α as well. It, however, did not alter the blood glucose level. Interestingly, no toxicological findings were obtained on normal human kidney cells. **Discussion**: In conclusion, dual application of GDD in both imaging and treatment (Figure 2) of tumour cells could be a remarkable advantage for using such compound in cancer treatment and diagnostics concomitantly.

(Complete version has been accepted and in press in : International Journal of Nanomedicine)

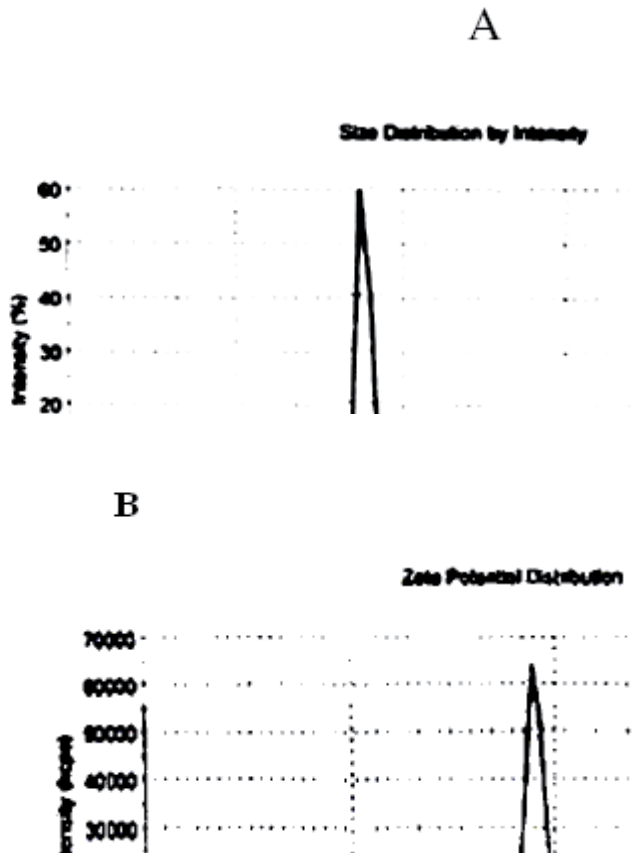

Figure 1. Size and zeta potential distribution diagrams. Size (A) and zeta potential (B) distributions of the GDD at concentration (0.02 mg/ml) in D.D.W

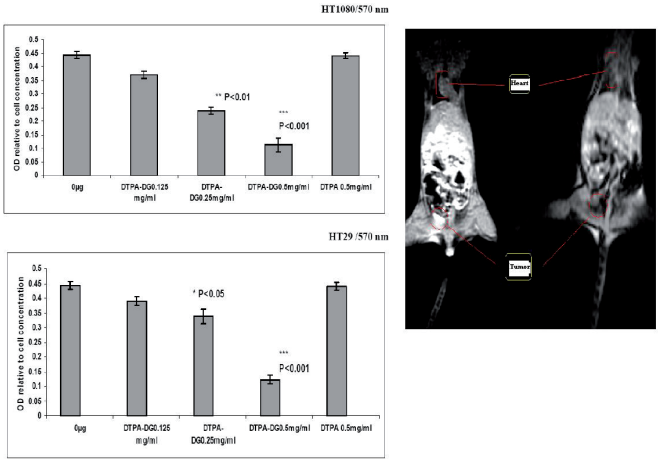

Figure 2. The data show dual anti cancer and molecular imaging applications for nano size agent GDD.

NOVEL SULPHONATES-CORE-SHELL-NANOPARTICLES CONJUGATES: A NEW AND VERSATILE DRUG DELIVERY SYSTEM. IN VITRO AND IN VIVO STUDIES

Marco Ballestri,[a] Giovanna Sotgiu,[a] Andrea Guerrini,[a] Michelandrea De Cesare,[b] Giovanni L. Beretta,[b] Franco Zunino,[b] Michele Laus,[c] Katia Sparnacci,[c] Claudio Monasterolo[a] and Greta Varchi.*,[a]

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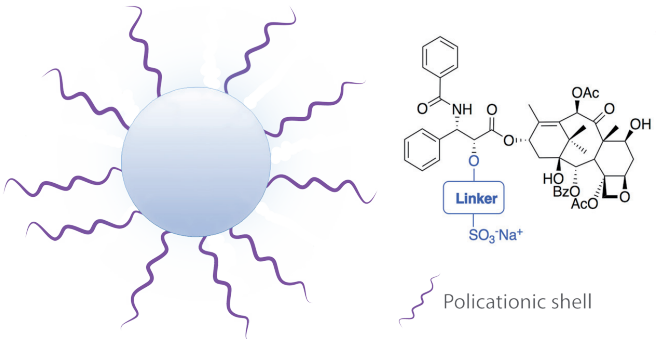
Nanotechnology1 is enabling technology that deals with nanometer sized objects. One of the most attractive and promising application of nanotechnology is the use of nanomaterials in medicine. Nanomedicine offers numerous possibilities to significantly improve medical diagnosis and therapy. At this aim, we explored the viability of a novel conjugated nanoparticles-based system. Specifically we studied the attachment, based on ionic interactions, of different anionic sulphonate organic salts to positively charged poly(methylmethacrylate) (PMMA)-based core-shell nanoparticles having an high density of ammonium groups on their shells.2 We evaluated drugs-polymers conjugates, consisting of camptothecin and taxol conveniently tailored analogues. In vitro (human non-small cell lung cancer carcinoma NCI-H460 cell line) along with in vivo (human ovarian carcinoma IGROV-1 xenograft) studies were performed in order to assess the effectiveness of the polymer-drugs conjugates. The administration of the nanoparticles by slow infusion is necessary to improve the efficacy and relevant to this point is the good tolerability of this novel drug formulation.

The available data support the interest of these nanoparticles system as a promising formulation for the delivery of negatively charged drug molecules.3



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Varchi, G.; Sotgiu G.; Ballestri, M.; Laus, M:; Sparnacci, K.; Monasterolo, C.; Guerrini, A.; De Cesare, M.; Beretta, G. L.; Zunino, F. Chem. Comm. 2011, submitted for publication.

PREPARATION AND IMMUNOTOXICOLIGICAL EVALUATION OF SMALL SIZE LIPOSOMES

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During the last decade intravenous liposomal drug formulations became accepted and approved by the regulatory agencies. The mean advantage of liposomal nanoformulations is the strong reduction of adverse effects of the highly toxic cytostatic treatment. For instance liposomal form of Doxorubicin (Doxil, Caelyx) has far more less side effects and therefore reduced cost of the treatment. However nanodrugs display a new, fortunately rare but serious life threatening adverse effect – the Complement Activation Related Pseudo Allergy (CARPA). As a consequence of the nanodrug induced complement activation the reactive patients suffer a strong pulmonary circulation disturbance that may result in fatal outcome. Our institute is actively researching the factors influencing the CARPA reactivity of liposomal formulations. These factors are surface charge, lipid composition, aggregation and size. Here we report the effect of the size on the CARPA reactivity in in vitro tests and in animal model.

METDODS

We prepared liposomes by extrusion methods from ethanol solved lipid mixture (hydrogenated soy lecithin, cholesterol, pegylated phophatidyl ethanolamine). For developing appropriate size liposomes we used Design of Experiments method (DOE). The size of the liposomes was measured by DLS method (Malvern Instruments, ZetaSizer NS). Data were evaluated by MINITAB software package.

For in vitro test we used human sera from clinical samples. After incubation sera with various size liposomes the produced SC5b-9 soluble complement activation product has been measured by ELISA assay.

The effects of various size liposomes on circulation were studied in porcine model. Pulmonary and systemic circulation pressure plus heart rate were monitored for evaluate the circulatory response.

RESULTS

We found that

- the lowest size of liposomes what can be produced by extrusion method is 65 nm.
- smaller liposomes displayed less complement activation
- smaller liposomes displayed less reactivity in porcine model.

CONCLUSION

The size of the liposomes might be a factor in CARPA reactions.

SYNTHESIS AND CHARACTERIZATION OF AG-NPS FUNCTIONALIZED WITH ANTIMICROBIAL PEPTIDES.

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Silver Nanoparticles (AgNps) exhibit a well-known antimicrobial activity [1]. Recent studies show that some peptides, isolated from a variety of organisms, have the same effect [2]. In this project we performed the synthesis of AgNps(Fig. 1)[3] and its characterization using spectroscopic techniques and Dynamic Light Scattering (DLS). The AgNps were functionalized with antimicrobial peptides and the nanocomplex was characterized by chromatographic and spectroscopic techniques including Fourier-Transfor Infrared and UV-Vis spectroscopies. Further studies will focus on the activity of the nanocompounds against a multi resistant bacteria like MRSA (Multi-Resistant *Staphylococcus aureus*).

We acknowledge support from CAPES, INEO, CNPQ, FAPESP and NANOBIOMED.

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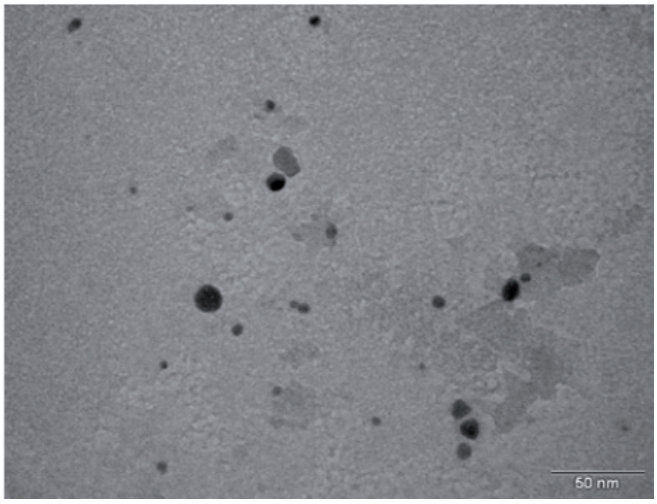

Fig.1 – Silver Nanoparticles

MTHPC-BASED BIODEGRADABLE NANOPARTICLES FOR CANCER TREATMENT

Bezdetnaya L, Lassalle H, Garrier J, D'Hallewin MA, S Marchal, Guillemin F

Centre de Recherche en Automatique de Nancy, Nancy Université, CNRS, Centre Alexis Vautrin, Vandœuvre-lès-Nancy, France.

A serious limitation of photodynamic therapy (PDT) is the absence of specific cancer targeting, resulting in an excessive tissue destruction, which can provoke life threatening situations. To partially overcome these constraints, incorporation of the active compound into liposomal nanoparticles can be proposed. Embedding of active drugs in liposomes favours passive targeting of tumors through Enhanced Permeability Retention (EPR) effect. Liposomal formulations of mTHPC in conventional (Foslip®) and pegylated (Fospeg) liposomes enable a more selective and faster accumulation of drugs in the tumors, with a faster clearance, together with at least similar to

liposomes-free mTHPC therapeutic efficacy. Best Foslip® - photoinduced response was obtained from the study of spatial intratumoral Foslip distribution rather than from bulk pharmacological tissues pharmacokinetics. Comprehension of the transport kinetics of the photosensitizer from the vessels towards extravascular structures is essential for optimizing clinical protocols. We have observed different redistribution patterns of mTHPC to plasma components according to liposomal composition (conventional vs pegylated liposomes) in vitro. The visualization of these processes in real time in vivo using the chick chorio allantoic membrane (CAM) model corroborate with in vivo results. Future directions consist in aptamer-mediated targeted delivery of chlorin types photosensitizers to tumor tissues.

This study was supported by the French National Ligue against Cancer and Biolitec AG (Jena, D).

DEVELOPMENT OF EXPANDED HIGH-THROUGHPUT SCREENING OPTIONS FOR NUCLEIC ACID-BASED NANODRUGS VIA CELL LYSATE BIOCHIPS

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Short interfering RNAs (siRNAs) represent an attractive option for the next generation of nanodrugs, because their selectivity of inactivation of a particular gene grants remarkable opportunities for both personalized cancer medicine and the targeted elimination of cancer stem cells, which are otherwise difficult to eradicate. Typically, potentially suitable siRNAs are recovered from automated genome-wide screens, assessing the differential killing activity for cancer cells versus normal cells in a total of about 120,000 individual cell experiments per screen.

Except for killing of cancer cells or cancer stem cells, forcing them into targeted differentiation is a conceivable therapeutic concept. However, measurement of differentiation processes in robotic high-throughput screens remains a challenging task. Present solutions comprise the use of reporter cells or of automated cell imaging, which have in common that only one or few parameters per screen can be determined.

Our goal is to use cell lysate biochips as tool to readout protein markers at large scale and in a high-throughput fashion. This may allow for gaining 100- to 1000-fold more data points from a genome-wide siRNA screen and would drastically accelerate the development of nucleic acid-based nanodrugs.

Here, we report on the printing and testing of first prototype chips. To this end, placing 15,000 samples on a chip of the size of a microscopic glass slide has successfully been accomplished and test marker proteins could be detected in the lysate of as few as 2 cancer cells per spot, which comprises a volume of about 350 pl (see Figure 1). Ongoing work aims at further increasing the sensitivity of the approach, expanding the set of marker proteins that can be detected, and integrating this approach into automated high-throughput screens for nucleic acid-based anti-cancer nanodrugs.

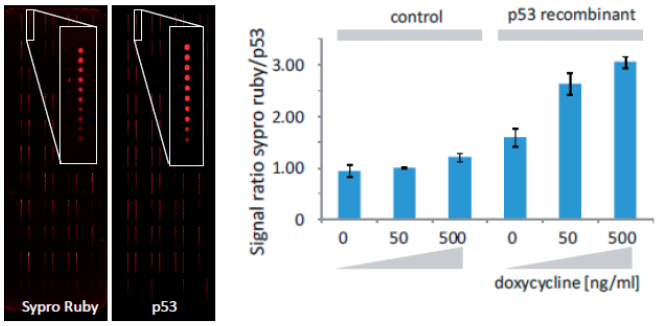

Figure 1. RPPA after staining with a p53 specific antibody and Sypro Ruby. Each lysate was spotted in a row with 1 to 16 depositions with a set off of 450 µm (left). Each concentration was applied to the slide twice per row. Spot sizes ranged between 160 to 200 µm. Per deposition 350 pl lysate are transferred, which is equivalent to the protein content of about 2 cells. The diagram to the right shows the Sypro Ruby-normalized p53 signal, which was determined via the RPPAs, suggesting an about 2.5-fold upregulation after induction with doxycycline compared to the equally treated isogenic control cells.

EXPLORING THE INTERACTION OF NANOMEDICINES WITH THE HUMAN IMMUNE SYSTEM. ASSESSING IMMUNOSAFETY AND INVESTIGATING PREVENTIVE/THERAPEUTIC TARGETING.

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INTRODUCTION

Within the interaction of nanomedicines with the human body, a particular importance assumes the interaction with the immune system, which is the system devoted to identifying and neutralising dangers. While it is expected that foreign materials can be recognised as such by the immune system and thereby eliminated, in the case of nanomaterials the small size may hamper recognition and consequent elimination. This possibility may represent an advantage, in the case of nanomedicines that should reach their target without being destroyed by immune reactions, but it may also represent a danger if persistent nanomaterials cause unwanted tissue damage.

In this perspective, three major immunological issues must be considered when administering nanomedicines to human patients.

- immune-mediated destruction/rejection, i.e., we should avoid the possibility that the immune system recognises the nanomedicine as a foreign dangerous entity thus initiating a defensive reaction leading to elimination of the nanodrug.
- immunosafety, i.e., we should make sure that nanomedicines do not affect the normal course of the immune response and/or do not induce unwanted immune/inflammatory reactions.
- immune targeting, i.e., we should learn how to exploit the encounter between the nanomedicine and the immune system for delivering signals and drugs specifically to immune cells.

IMMUNE-MEDIATED DESTRUCTION

If nanomedicines are recognised by the immune system, the first possible consequence is the destruction of the particles by specialised immune cells. The first and most potent mechanism of defence is that brought about by blood or tissue phagocytes, which take up and destroy the foreign material. Surface derivatisation with molecules that modulate recognition and engulfment by phagocytes is a strategy that may yield interesting results. As an example, human blood monocytes were exposed for 2 h to fluorescent PLGA nanoparticles (NP), either pristine or surface-modified with sialic acid (Nau5Ac).

In the case of pristine NP, monocytes could phagocytose NP in a dose-dependent fashion (Figure 1, upper panel). Conversely, the surface modification of the NP caused an optimal recognition and uptake already at the lowest dose (Figure 1, lower panel). Thus, NP surface modifications may direct and modulate recognition by the immune cells. This possibility should be exploited according to characteristics and targets of the nanodrug.

IMMUNOSAFETY

The possible effects of nanomedicines on the immune responses are a major health issue that should be addressed with appropriate and reliable tools. Possible interference of NP with the normal functions of the immune system may lead to pathological consequences. To assess immune-related risk of developing diseases, robust and representative assays should be designed and implemented.

Reliable nano-immunosafety assays should respond to a series of requisites:

- rapid and easy to perform (e.g., in vitro assays);
- taking into account the physico-chemical characteristics and behaviour of NP (e.g., optical interference with assay readouts);
- 1. predictive of the NP effects in vivo in human beings (e.g., using human primary cells in vitro);
- 2. robust and reproducible (e.g., standardised with cell lines and selected reported genes).

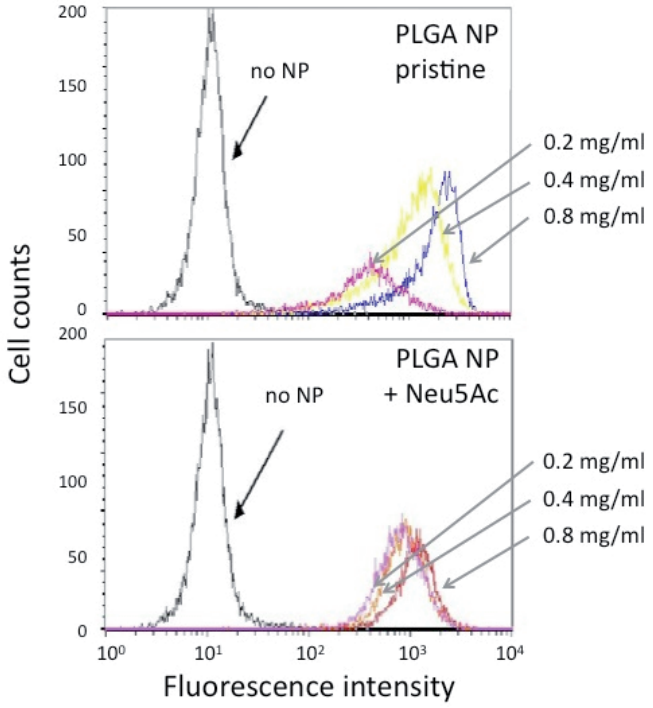

FIGURE 1: Surface-dependent phagocytosis of PLGA NP by human blood monocytes

To address these points, a human model of innate/inflammatory defence response has been set up *in vitro* that recapitulates the different phases of the defence response, from recruitment and initiation, to development of inflammation, resolution, repair and re-establishment of homeostasis. The model is based on human primary blood monocytes exposed in culture to sequential changes of microenvironmental conditions (chemokines and cytokines, temperature, bacterial-derived molecules, etc.) for 48 h. Genome-wide transcriptomic analysis and multiplex proteomic detection of inflammatory factors have been used for profiling the kinetics of response in the absence of interfering molecules ("physiological" innate defence response) and defining "signatures" that describe its evolution (Figure 2). These signatures will then be used for monitoring, in comparative experiments, the possible interference and alterations caused by nanomedicines. As a final step, analogous models of "pathological" inflammation (*e.g.*, as in chronic inflammatory and autoimmune diseases) are being set up and validated in order to define pathology-related molecular signatures. These, in turn, will be used as reference benchmarks for evaluating whether the NP-induced changes can be associated to a pathology-related signature, thus allowing us to predicting risk.

IMMUNE TARGETING

By considering the interaction between nanomedicines and the immune system (an interaction that can most likely occur, as in the above case of blood monocytes), it is possible to exploit this "natural" targeting (see Figure 1, upper panel) and improve it (see Figure 1, lower panel) in order to achieve an optimal delivery to immune cells. In the case of monocytes/macrophages, the first concept that comes to the mind is that of vaccination. In fact, targeted delivery of vaccine antigens to antigen-presenting cells (macrophages, dendritic cells) is easily achievable by loading the NP with the antigens that, upon *in vivo* delivery, will be readily taken up by monocytes or tissue macrophages. In this view, it is of particular relevance the fact that the NP can also play another key role in immunisation, besides antigen delivery, *i.e.,* that of adjuvanticity. Amplification of the immune response and more efficient induction of protective immunity and immunological memory is obtained, in current vaccines, by the use of "adjuvants", *i.e.*, substances that cause a mild innate reaction which in turn allows rapid and effective initiation of adaptive immunity. It is becoming clear that the major mechanism of adjuvanticity is the activation of an intracellular protein complex called the "inflammasome", whose role is that of cleaving and activating the immunostimulatory cytokine IL-1β. Inflammasome activation can be induced by a range of stimuli, but one of the most effective is the intracellular production of ROS through SOD1 activation. The most effective means for stimulating ROS production and activating the inflammasome are the phagocytosis of bacteria, of monosodium urate crystal particles, of aluminium hydroxide nanoparticles (alum). Thus, the design of new vaccination strategies can take advantage of the particulate form of NP and of their tropism for antigen-presenting macrophages for the concomitant delivery of the vaccine antigen and the triggering of adjuvanticity through inflammasome activation.

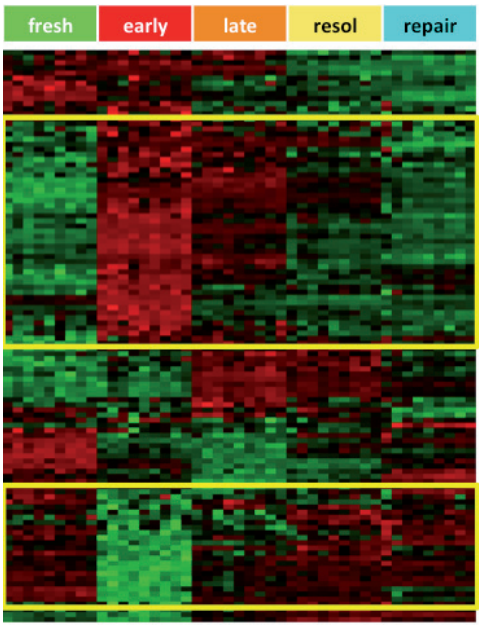

FIGURE 2: Gene signatures of the development of the innate immune response in vitro

Likewise, NP targeting macrophages and antigen-presenting cells can be designed as a therapeutic tool for the targeted therapy of immunological diseases based on excessive activation. As a simple approach, nanomedicines loaded with anti-inflammatory drugs may achieve effective suppression of the pathological reaction without inducing systemic side-effects. More complex approaches can be devised for selective immunostimulation, re-establishment of tolerance, and re-polarisation of altered responses in a series of immune-related pathologies.

CONCLUSIONS

To summarise, the interaction of nanomedicines with the immune system represents a key issue in the design and targeting of the nanodrug. Indeed, such interaction can be modulated so as to avoid toxic and pathology-inducing effects, to preserve the integrity of the nanomedicine and its reaching the target organs, but also exploited for specific approaches to the modulation of immune responses, starting from more effective vaccination strategies to the therapy of chronic inflammatory and autoimmune diseases.

MULTIFUNCTIONAL IRON OXIDE NANOPARTICLES FOR MACROPHAGE LABELLING AND MANIPULATION

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Multifunctional iron oxide (FeOX) magnetic nanoparticles (MNPs) represent a cutting edge tool for biomedical application. FeOX-MNPs offer some advantages in comparison to other MNPs due to their good biocompatibility and long term biodegradability. Furthermore they can be simultaneously functionalized and guided by a magnetic field, so they are studied as a new theranostic agent for cancer treatment [1].

In the present work we report the use of fluorescent FeOX-MNPs for label macrophage cells and their sorting and manipulation by external magnetic field. Magneto-fluorescent macrophage cells are an interesting device in nanomedicine, thanks to their possible use as carrier of MNPs into deep lesions, allowing multimodal magnetic resonance fluorescence imaging and magnetic field induced hyperthermia (fig. 1).

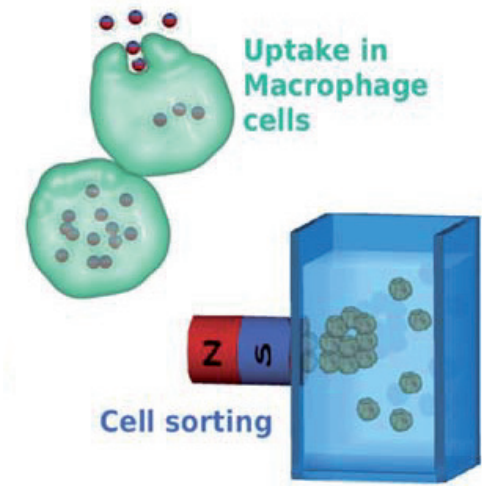

Figura 1: Macrophages sorting

The efficacy of nanoparticles uptaken was performed in PMA differentiated U937 cells (human non adherent monocyte like). Fluorescein conjugated FeOX-MNPs were first incubated with macrophage cells for 4 hours at 37°C and then assayed by flow cytometry and confocal microscopy. We evaluate the possibility of macrophage manipulation in a cell sorting experiment applying an external magnetic field after incubation as reported above.(fig 2).

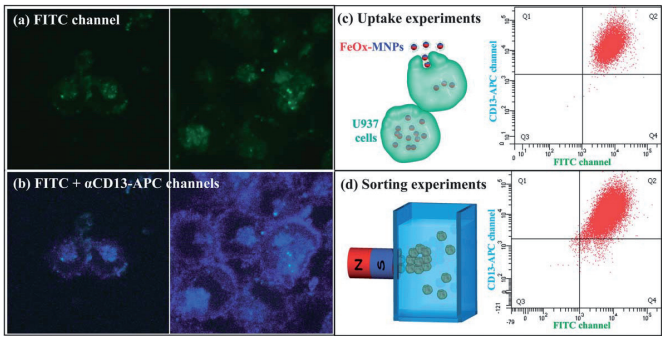

Figura 2: (a)(b) Confocal images of macrophages after incubation with MNPs and staining with anti CD13-APC. (c)(d) Cytofluorimetric analysis of macrophages cell uptake and sorting.

Moreover to analyze the MNPs effects on cell apoptosis and viability we performed the Annexin V Cytometry assay and XTT test. No sign of apoptosis and cell death was detected.

We found that these fluorescent loaded MNPs can be effectively used for cell sorting and manipulation by a magnetic field without cytotoxic effects. The results indicated that FeOX-MNPs can be a optimal new theragnostic tool for cancer management.

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CONTRAST ENHANCED OPTOACOUSTIC MICROSCOPY

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Optoacoustic imaging is a non-invasive modality under preclinical development that has been applied to several biomedical applications for obtaining anatomic and functional information. It combines the high resolution of ultrasound with the high contrast of optical methods.

In addition optoacoustic contrast agents can provide a higher contrast when the intrinsic optical contrast is insufficient. In this study, we prepared different types of nanoparticles as optoacoustic contrast agents. After verifying biocompatibility, biodegradability as well as the level of specific cellular binding, their suitability as optoacoustic contrast was evaluated with an optoacoustic microscope.

INTRODUCTION

Optoacoustic imaging is based on detecting acoustic signals generated from biological structures by local optical absorption. Short pulsed laser illumination is used to generate thermoelastic expansion resulting in acoustic pressure waves. An ultrasonic transducer detects the acoustic signal and the reconstructed image reflects the distribution of optical absorption. The technique combines the high spatial resolution and penetration of ultrasound with the high contrast related to the optical properties of tissue. The contrast potential of optoacoustic imaging can be significantly enhanced by auxiliary entities with strong optical absorption properties in the near infrared spectral range. Nanoparticles with superior physical and surface properties enable longterm stability and molecular specific binding to the cells. Before using the nanoparticles as an optoacoustic contrast agent *in vivo*, their biocompatibility and targeting efficiency have to be characterized. The optoacoustic detection of the particles attached on living cells has been carried out using our in-house developed optoacoustic microscope.

MATERIALS AND METHODS

1) Hardware platform – Our optoacoustic microscopy system (SASAM OPTO) consists of an inverted optical microscope (Olympus IX 81, Tokyo, Japan), a short pulsed laser source, a single element transducer and an appropriate readout electronics system2. In combination with reconstruction algorithms for optoacoustic imaging the recorded signals can be converted into a spatial representation of the absorbed light energy. The suitability of the synthesized nanoparticles as an optoacoustic contrast agent in terms of their ability to generate detectable acoustic signals was investigated with this optoacoustic microscopy platform.

2) Nanoparticle Synthesis – Gold nanorods were synthesized according to a method described in Pierrat et al.3. In a two-step process small gold colloids with an average size of 2-4 nm were synthesized by the reduction of HAuCl4 in a CTAB solution mixed with Natriumborhydrate. In a second step gold nanorods were produced by anisotropic growth of gold colloid. The synthesis of the magnetite nanoparticles is based on a method described in Osaka et al. by a controlled hydrolysis of an aqueous ferric chloride solution and the polyamine spermine4. Spermine was added dropwise to a FeCl2 solution. After 4 h of stirring, several nanoparticle fractions of different size ranges were obtained by centrifugation. The absorption spectra of the used nanoparticle solutions were measured using a spectrophotometer (Lambda950, PerkinElmer). Since the absorption of particles ranges in the near infrared, these particles can be used as contrast agents in the following experiments.

3) Antibody coupling – Specific targeting of tumor cells for sensitive imaging of early stage tumors is the major goal in cancer imaging and therapy. Coupling of tumor specific ligands on the surface of the developed nanoparticulate systems will result in active drug targeting. Using the example of gold nanoparticles the surface of the nanoparticles was modified by covalent attachment of trastuzumab (Herceptin®). Herceptin is a humanized monoclonal antibody with a specific binding to the HER2 receptor.

4) Cytotoxicity Assays – For assessing the suitability of the synthesized nanoparticles as an optoacoustic contrast agent, the biocompatibility of the prepared nanoparticles was investigated. For the in vitro toxicology study of the nanostructures two different cell culture systems were used: SK-Br3 cells (human breast cancer cells, DSMZ, Braunschweig, Germany) and A549 cells (human carcinoma epithelial cells, DSMZ). SK-Br3 cells were maintained in McCoy's 5A, A549 cells in RPMI 1640. Both media were supplemented with 10% FCS, 100 U/ml penicillin and 100 µg/ml streptomycin. The cells were cultured at 37 °C, 5% CO2 and 95% saturated atmospheric humidity. All experiments were conducted in 96-well plates at a cellular density of 1x104 cells per well. Standardized in vitro experiments were performed to characterize the cytotoxicity of nanoparticles using WST-1 assay, LDH assay and BrdU assay as described in literature5. For the experiments the cells were cultured on class slides and treated with the nanoparticles for 24 h. Afterwards the cells were fixed and embedded in VectaShield HardSet mounting medium and analyzed via CLSM.

RESULTS

1) Biocompatibility - According to ISO 10993 cell viability was determined as function of the exposure time and the incubated concentration using two different in vitro systems (SK-Br3 and A549). The results of the cytotoxicity studies indicate that all nanoparticulate systems are non-cytotoxic.

2) Targeting - After incubation of the particles for 24 h, the bioconjugation of the gold nanoparticles was verified by marking the antibodies by a secondary human IgG specific fluorescent antibody and subsequent confocal laser scanning microscopy (CLSM). HER2 over-expressing breast cancer cells SK-Br3 were used for cellular binding and uptake studies. SK-Br3 cells showed a specific cellular binding of the trastuzumab-functionalized gold nanoparticles (Figure 1).

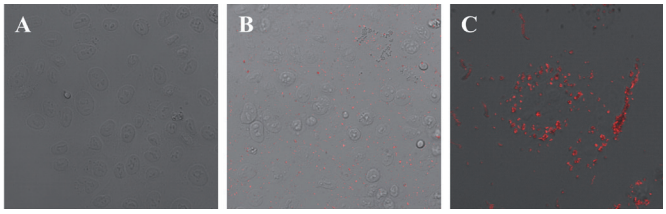

Figure 1: Specific and non-specific cellular binding of Herceptin-modified gold nanoparticles in HER2 over-expressing SK-BR3 cells studied by confocal laser scanning microscopy (CLSM). (A) Untreated control. (B) Non-specific binding. (C) Specific binding.

3) Optoacoustic suitability - After physicochemical and biological characterization of the particles the conversion efficiency of light into sound was investigated. The gold and magnetite particles were embedded in an alginate matrix and placed as small spots on top of a cover glass. The signal to noise ration (SNR) of the generated signal was detected as a function of the particle concentration (Figure 2A).

The targeted delivery of nanoparticles to the cells was investigated optoacoustically. A549 cells incubated with magnetite particles were imaged optoacoustically after the exposure time of 24 h (Figure 2 B and C). The optoacoustic data of the A549 suggest that this cell culture system does not fully internalize the particles but binds them at the outer part of the cell.

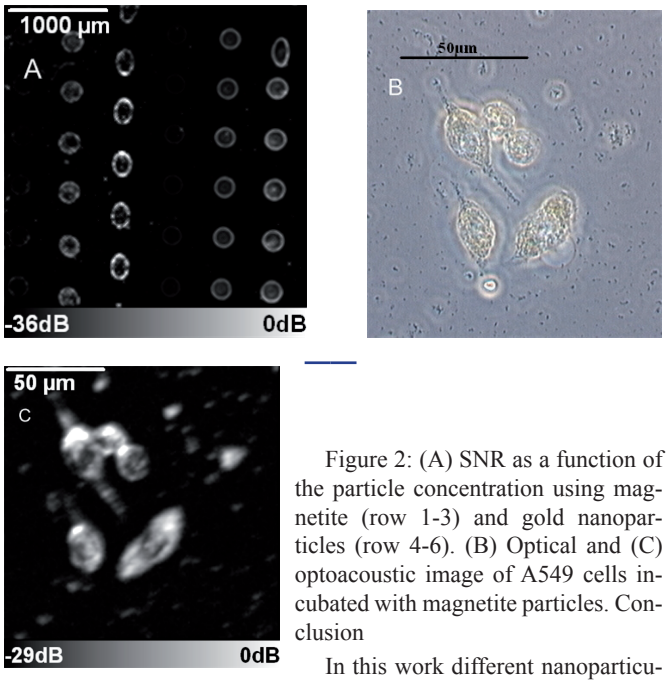

Figure 2: (A) SNR as a function of the particle concentration using magnetite (row 1-3) and gold nanoparticles (row 4-6). (B) Optical and (C) optoacoustic image of A549 cells incubated with magnetite particles. Conclusion

In this work different nanoparticulate systems were prepared and investigated concerning biocompatibility, their efficiency as an optoacoustic contrast agent and their specific targeting to tumor cell lines. The results of the biocompatibility studies verified suitability of the particles as basis for optoacoustic contrast agents. Using our optoacoustic microscope we investigated the ability to detect nanoparticular contrast agents. The contrast could be enhanced by 30dB comparable to the untreated cells. Finally, Herceptin functionalization of the synthesized gold nanorods leads to a specific targeting of HER2 over-expressing breast cancer cells. This was verified via CLSM. The nanoparticle-combined optoacoustic microscopy is under preclinical development and could be utilized for several biomedical applications *in vivo*.

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INFRASTRUCTURAL SETUP OF A ROBOTIC NANODRUG SCREENING PLATFORM

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Targeted gene inactivation via short interfering RNAs (siRNAs) represents a promising option for designing nanodrugs for personalized cancer nanomedicine. Yet, the identification of siRNAs with the desired activity profile against cancer cells represents a challenging task. Ideally, this includes systematic scanning of genome-wide siR-

NA libraries enabling the knock down gene by gene of each of the about 20,000 human genes in pairs of cell lines, where one cell line resembles normal cells, while the other cell line resembles cancer cells. Because this requires several tens of thousands of cell experiments, massive automation is required. Here we report on the layout and infrastructural setup of an HTS robotic platform and the required server resources.

The drug screening unit of the Lundbeckfonden Center of Excellence in Nanomedicine has the physical dimensions of 35m^3, which comprises the core unit. It is designed to process a set of 1,000 microtiter plates in one batch, starting from seeding of defined cell numbers up to genome-wide siRNA library transfection, cell incubation, and final readout of the assays. Multiple readouts are ascertained via a flexible interchangeable cassette-utilizing device. The overall capacity of the robotic unit is 120,000 individual experiments within 10-14 days, which corresponds to one differential genome-wide siRNA screen in triplicate in a 96-well format. Higher throughput can be achieved by using smaller well formats. Beyond that, the unit is designed to perform downstream processing of the cells, such as protein preparation to perform further readouts. A 60 TB computer server for data storage and processing as well as a biochip robot for expanding the readout options are coupled to the core unit. The entire process can be tracked down to the single well via a barcode system. The system represents one of the largest platforms in Northern Europe and has open and interchangeable architecture for expanding its options. At present, assay adaptation to the robotic workstation is performed, so that first screening activities are expected to start within 2011.

DESIGN, MODELING, EXPRESSION AND CHEMO-SELECTIVE PEGYLATION OF A NEW NANO-SIZE CYSTEINE ANALOGUE OF ERYTHROPOIETIN

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BACKGROUND AND PROPOSE

The recombinant human erythropoietin (rhEPO) is considered as one of the most pivotal pharmaceutical drugs in market because of its clinical application in the treatment of anemia associated disorders worldwide. However, like other therapeutic proteins, it has not suitable pharmacokinetic properties whereas it must be administrated at least 2-3 times per week. Chemo-selective cysteine PEGylation, employing molecular dynamics and graphics *in silico* (Figure 1) studies can be considered to overcome such problem.

MATERIALS AND METHODS

A special kind of EPO analogue was elicited based on literature review, homology modeling, molecular dynamics simulation and factors affect the PEGylation reaction. Then, cDNA of selected analogue was generated by site-directed mutagenesis and subsequently cloned into the expression vector. The construct was transfected to CHO/dhfr$^-$ cells and highly expressed clones were selected via methotrexate amplification. An ion immobilized affinity and size exclusion (SE) chromatography techniques were used to purify the expressed analogue. Thereafter, chemo-selective PEGylation was performed and a nano-size PEGylated EPO was obtained through dialysis. The *in vitro* biological assay and *in vivo* pharmacokinetic parameters were studied. Finally, E31C analogue FTIR, analytical SE-HPLC, zeta potential and size before and after PEGylation were characterized.

RESULTS

Findings indicate that a novel nano-size EPO31-PEG has five-fold longer terminal half-life with the same biological activity as compared to unmodified rhEPO. The results also show that EPO31-PEG size and charge versus un-modified protein was increased in a nano-spectrum and this may be one criteria of EPO biological potency enhancement (Figures 2-3). **Discussion**: This kind of novel engineered nano-size PEGylated EPO showed better remarkable advantages than rhEPO.

Key Words: Nano-PEGylated EPO, Cysteine PEGylation, Pharmacokinetic Property

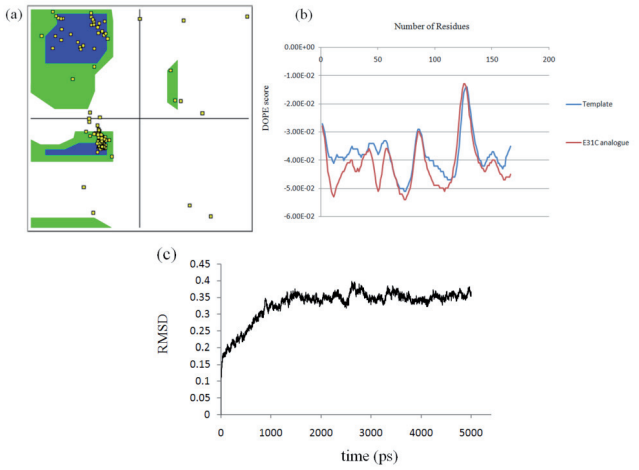


Figure 1: Quality assessment of the modeled 3D-structure of R31C analogue as a sample: (a) >98% of amino acids are located in allowed areas in the Ramachandran plot. (b) DOPE score profile of modeled E31C analogue and template. (c) RMSD plot for modeled E31C analogue during MD simulation. The fluctuations of modeled structure were reached to plateau after ~1500 ps of simulation.

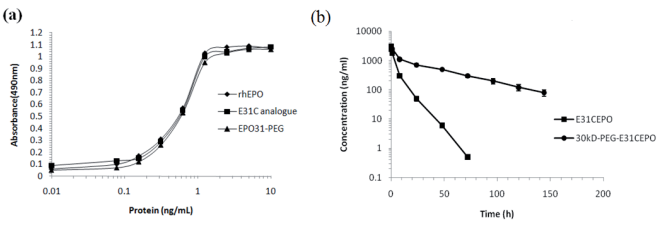
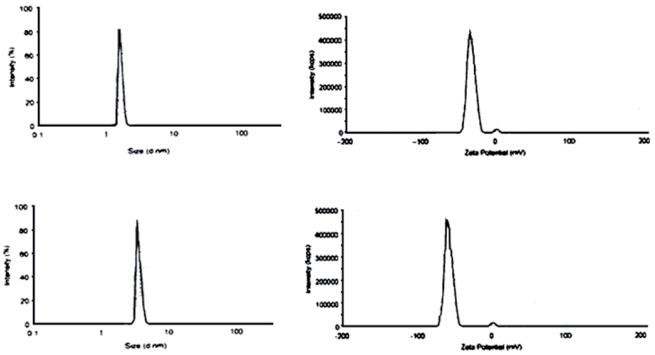


Figure 2: (a) In vitro biological assay based on proliferation of UT-7 cells. Both E31C analogue and EPO31-PEG had same biological activity similar to rhEPO. (b) Single i.v. injection of EPO31-PEG and E31C analogue in rats.



(a) (b)

Figure 3: changes in (a) Size and (b) zeta potential of E31C analogue before (up) and after (down) attaching to the PEG-30kDa. As depicted, the size and negative charge of molecule increased due to attaching of polymer.

IMPROVED TRANSDERMAL DELIVERY SYSTEMS BASED ON H_{II} MESOPHASE AND CELL-PENETRATING PEPTIDES.

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This research utilizes the unique properties of the GMO-based reverse hexagonal (H_{II}) mesophase with those of the selected cell penetrating peptides (CPP), for the development of improved transdermal drug delivery vehicles. The gel-like H_{II} mesophase serves as an excellent matrix due to its extremely high interfacial area which enables high solubilization capacities, and the CPPs provide tuning the skin permeation rate for a controlled release system.

Sodium diclofenac (Na-DFC) was incorporated within the H_{II} mesophase, in addition to a CPP. The effect of the guest molecules on the mesophase dimensions was determined by polarized light microscopy and SAXS. The location of the guest molecules within the mesophase was probed by DSC and ATR-FTIR techniques. Na-DFC migration kinetics out of the mesophase and through the skin were examined by Franz diffusion cells.

Na-DFC seems to populate mainly the interfacial region. The specific locations of two chosen CPPs, "A" and "B", were examined, and their effect on the mesophase nanostructure was determined. Both peptides increase Na-DFC permeation through skin, yet there were differences in the penetration profile of Na-DFC with the presence of each CPP, resulting probably from the different chemical natures and solubilization sites of the different peptides.

The different CPPs might serve as tuning agents for controlling the drug permeation rate through the skin, as needed. The effect of the CPPs on the skin will be further investigated, as well as their specific interactions with Na-DFC, and their effect on the drug migration from other mesophases with different characteristics.

FACTORS AFFECTING THE SIZE OF PRODUCED CHITOSAN NANOPARTICLES WITH SONICATION METHOD- USE OF ARTIFICIAL NEURAL NETWORK

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Concurrently evaluating and modeling the effective of namely parameters, chitosan concentration, buffer pH, amplitude and time of sonication on the diameter size of chitosan nanoparticles prepared with ultrasonicatin method carried out by artificial neural networks (ANNs).

INTRODUCTION

It has already been shown that efficacy and pharmacokinetics of nanoparticles in body mainly depends on their size, polydispersity and surface charge [1]. For instance, a study about in vivo antitumor activity of chitosan nanoparticles against sarcoma-180 and mouse hepatoma H22 denoted that the particle size makes a great effect on their antitumor efficacy [2]. Therefore, precise control of the size of chitosan nanoparticles is a critical parameter when developing chitosan based carriers for delivery of drugs, peptides or genes. Among several methods to fabricate chitosan nanoparticles, ultrasonication method is a simple, fast, green and popular method for producing these nanoparticles [3]. The aim of this study is to concurrently evaluate and model the effective parameters, namely, chitosan concentration, buffer pH, amplitude and time of sonication on the size of chitosan nanoparticles prepared with ultrasonicatin method using ANNs.

MATERIALS AND METHOD

Different concentrations of chitosan solutions (0.1-2 mg/ml) with sodium acetate buffer (0.1 M) in different pH values (3.0-5.3) were sonicated at ambient condition under continuous mode of sonicator at different amplitudes (10-100) over different duration times (20-600s). The particles hydrodynamic diameter was measured immediately after preparation of the samples by photon correlation spectroscopy (PCS) using a zetasizer Nano zs® (Malvern, UK) at 25°C. Then ANNs studies were performed by INForm v4.0 (Intelligensys, UK to model the relations between inputs and output. The response surfaces between the variables, illustrated as 3D graphs of two inputs vs. the single output, were employed to obtain the rules describing the relationships between inputs while considering the interactions between input parameters instead of "one-factor-at-a-time" approach.

RESULTS

After modeling the data by ANNs, the best predictive model gave R^2 values of 0.87, 0.98 and 0.82 for the training, test and validation data, respectively, indicating a satisfactory trained model with predictive capability. Training data set was used to train the software and characterize the relationships between the inputs and output using the training parameters listed in Table1. The 3D graphs illustrate the relationships between input variables and their effect on the size of chitosan nanoparticles (i.e. Output). In figure 1, details show that in general, to obtain the smallest particle size, chitosan concentration needs to be kept at minimum. At high values of time and amplitude (Figure 1a), the raise of chitosan concentration has no significant effect on nanoparticles diameter. Additionally the pH of buffer shows important effect on chitosan nanoparticles size produced with this method- increasing the pH value of buffer tends to decrease the size of nanoparticles. In other pictures was shown that amplitude does not have a linear effect on the size. In most cases, increase in amplitude leads to smaller size and increase in pH of buffer causes substantial

reduction in nanoparticles diameter.

Figure1. 3D plot of particle size (nm) predicted by the ANNs model at low, mid-range and high values of amplitude and time of sonication.

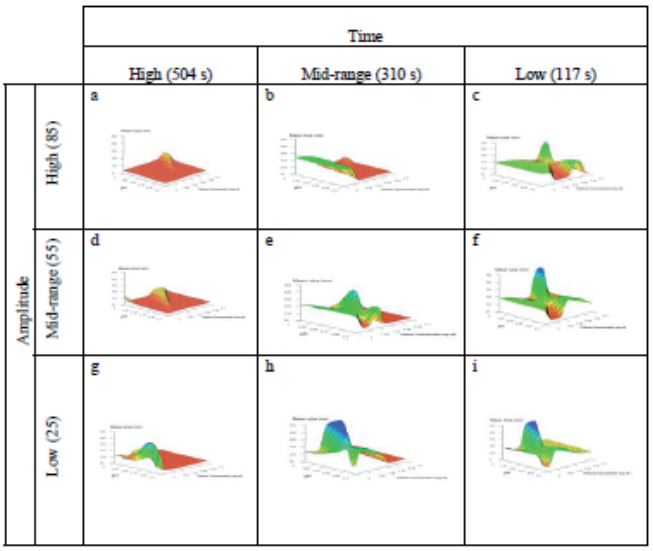

Table1. The training parameters set with INForm v4.0

Network structure	No. of hidden layers	1
	No. of nodes in hidden layer	5
Backpropagat type		Quick Prop
Back propagation parameters	Momentum factor	0.8
	Learning rate	0.7
Targets	Maximum iterations	1000
	MS error	0.0001
	Random seed	10000
Smart stop	Minimum iterations	20
	Test error weighting	0.1
	Iteration overshoot	200
	Auto weight	On
	Smart stop enabled	On
Transfer function	Output	Tanh
	Hidden layer	Asymmetric Sigmoid

CONCLUSION

From the 3D plots developed from the model, it was shown that all four input parameters studied have some effect on prepared nanoparticles. Concentration of chitosan was found to have direct effect on the particle size but the effect of this parameter on particles diameter may be substantially covered by other parameters. A reverse and dramatic effect between the pH of buffer and the particle size was shown for the first time in this study. To obtain the minimum size, it was indicated that the amplitude and duration of sonication need to be more than specific values. The time of sonication in comparison with other parameters was probably the major factor leading to smaller nanoparticles diameter, with some dependency on other parameters. From the model, the optimum conditions to obtain the smallest particles was ~4.9 and ~500 s as values of pH and time, respectively with amplitude not less than medium values (~55).

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MODULATION OF METHYLENE BLUE DYE ACCUMULATION BY THE MULTIDRUG TRANSPORTER P-GLYCOPROTEIN: IMPLICATIONS FOR ITS USE IN PHOTO-DYNAMIC THERAPY

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Photodynamic therapy (PDT) is an established approach for cancer treatment based on irradiation of a tumor-accumulating photosensitizer. Recently, photo-sensitizers which localize in mitochondria have been recognized as promising candidates in PDT because they can interact with pre-apoptotic factors, and trigger apoptosis in cancerous cells. The cationic dye methylene blue (MB) widely fulfils these requirements as it readily accumulates in the mitochondrial compartment and efficiently generates singlet oxygen ($^1O_2$) upon irradiation at an appropriate wavelength. However, prior to utilization of MB for PDT, factors affecting the pharmacokinetics (PK) of MB should be taken into account to estimate the concentration of dye available at the irradiation site. We predicted from the chemical structure of MB (with a cationic charge and dimethylphenothizinium scaffold) that it may be transported by the human multidrug resistance transporter P-glycoprotein (Pgp; MDR1), a protein known to play an important role in modulating the PK profile of drugs. We investigated this hypothesis by real-time monitoring of changes to the extracellular acidification rate (ECAR, %) and respiration rate (OCR, %) of living cells using the Bionas 2500 microphysiometer instrument. By treating wild type (wt) and MDR1-transfected mouse fibroblast cells (NIH-3T3 and NIH-3T3-MDR1-G185, respectively) with increasing concentrations of MB, we found that the ECARs and OCRs were increased at low MB concentrations, but inhibited (decreased) at high concentrations in both cell lines. However, the overall magnitude of this response was dramatically higher in wt cells compared to MDR1-transfected cells. These data suggest that I) intracellular accumulation of MB induces metabolic activation in mouse fibroblast cells, II) MB accumulates in mitochondria, enhancing the activity of the electron transport chain, leading to an increase in OCR, and III) the presence of Pgp reduces the intracellular accumulation of MB, as shown by the reduction in the magnitude of this metabolic effect in *MDR1*-transfected cells.

CROSSING IMPERMEABLE BORDERS: THE UNCHARTED FRONTIER OF LAW AND SCIENCE IN NANOTECHNOLOGY

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I. Crossing Impermeable Borders: The Uncharted Frontier of Nanotechnology

The sound of freedom that resonates from civil and political rights rings hollow to a newborn who has low birth weight, because the baby's mother had no access to a clean and safe workplace, good nutrition or adequate prenatal care. And, what good are political and civil rights to a different baby, who has lost a parent due to an occupational accident, or whose parents are debilitated by an occupational disease such as lung cancer, or to the baby who may suffer personal injury due to the effects of a parent's workplace exposure to mutagens or unchecked but foreseeable harms caused by unregulated applications of nanotechnology, at home or in their parents' workplace?

The issues raised by nanotechnology are new, but not as novel as they seem. International Laws governing the human right to health provide important precedent. These laws underscore the fundamental character of occupational health protections as human rights that are universally necessary as a condition to protecting health and promoting the future development of civilized society. The universal need for such rules of law promotes and ensures the work, health and survival all people: without health there can be no work; without work there can be no civilization. The international consensus regarding the value of occupational health is reflected in the WHO Constitution, ILO Constitution and conventions, EU directives, the International Covenant on Economic, Cultural and Social Rights, the Universal Declaration of Human Rights, the World Trade Organization (WTO) treaties national laws. Therefore, this paper examines existing international treaties signed by governments, to examine the question whether law is needed, and if so, what approach should be taken for regulating nanotechnology on the frontiers of science where there is no law.

«The protection and promotion of the health and welfare of its citizens is considered to be one of the most important functions of the modern state». Consistent with this ancient responsibility implicit in maintaining social order for governance, many types of government have begun projects to develop laws that regulate the use of nanotechnology: nations, international organizations, municipalities and local governments, regional constoria of governments and even multinational employers alone or acting through non-profit organizations, have started to post guidelines and safety procedures on their websites.

Nanotechnology is already here, bombarding consumers with applications of nanotechnology in paint coatings, refrigerator linings, sun tan lotion and even a car called the "nano". For example, the chain store "migros" in Switzerland gives out "nano mania"; a collectable set of toys marketed nationally in their stores. The state of the art of scientific scrutiny regarding acute health hazards and long-term health effects, however, lags far behind the innovative uses of nanotechnology. Although scientific uncertainty cannot stop the wheels of commerce, international scientific consensus nonetheless calls for precaution regarding major risks to public health and the health and well-being from the use of nanotechnology.

The Royal Commission on Environmental Pollution of the United Kingdom informed Her Majesty, in 2008: paragraph 1.37 "As we have noted, history is replete with instances where such assumptions were shown to be flawed too late to avoid serious consequences." Consistent with such concerns, the Swiss National Science Foundation warns, "Physically confining materials at the nanoscale alters the behaviour (sic) of electrons within them, which in turn can change the way they conduct electricity and heat, and interact with electromagnetic radiation. Moreover, materials engineered at the nanoscale can enter into places that are inaccessible to larger materials, and can therefore be used in new ways. These behaviours (sic) also have potential consequences on the abilities of synthetic nanomaterials to cause harm in novel ways. Furthermore, claims that seven Chinese factory workers developed severe lung damage from inhaling nanoparticles appear in a paper published in the European Respiratory Journal . Therefore, it is not surprising that in 2011 the US government sought public comments, « Whether the hazard identification, risk estimation, and discussion of health effects for carbon nanotubes and nanofibers are a reasonable reflection of the current understanding of the evidence in the scientific literature » Previously, in November 2007, the OECD Working Party on Manufactured Nanomaterials established a NIOSH-led project to raise awareness about- and harmonize approaches for- exposure measurement and mitigation for nanomaterials.

When these stakeholders form a critical mass of political will, their collective societal efforts can succeed to get it right by promoting the growth of new industries without catastrophic harm and with remarkably low risk to the general population. Answering the questions of concern to such stakeholders therefore can be the linchpin that will make or break the insurability--- and economic feasibility--- of new projects. In this context, the international consensus of a loosely defined scientific comunity plays a crucial role, defining the parameters of unquantifiable but foreseeable risk and suggesting the precautionary measures that may effectively minimize the risk of avoidable harm. When such opinion leaders conclude that the state of the art of scientific research promoting safety and health in the workplace lags behind the implementation of new technologies in commerce, the limits on the present state of the art for quantifying risk sharpen the edge of the dilemma that regulators, industrial stakeholders and all civil society must courageously examine on the cutting edge of science. Governmental structures at all levels of society presently face a situation in which there is potential risk to public health, but insuffiencient data exists about actual risk in order to make key policy judgments. Consequently, the regulatory picture of the legal landscape presently looks like a scene from the wild, wild west of the USA from the 19th century: large gaps in the law where there is undisputed uncertainty about the magnitude of risk, and many different sources of law clustered around tangible established practices for any toxic or hazardous materials, such as medical surveillance through employer-based occupational health services and global sharing of chemical hazard information, using engineering controls.

II. LESSONS LEARNED FROM PRECEDENTS REGULATING TECHNOLOGY UNDER INTERNATIONAL LAW

The question of managing risk in order to protect public health despite the immature state of the art of nanotechnology therefore involves a subtle analysis of how to apply accepted engineering controls, rather than asking what framework, should be applied, if any. The vital question of « how?» sounds simple, but is not easily answered.

The law is beautiful; one of the most precious gifts in our society. Unfortunately, when people bump into the law for the first time, without legal education, typically the law is saying «No!». No you can't walk across the street at the red light when you want to, no you can't tell those people not to go to your school, no you can't run your business the way you wanted no you don't have the right to control your land and neighborhood the way you thought you could. No you can't drink as much alcohol as you want before driving; no you can't drive along open roads as fast as you want. Inevitably, someone else is standing there yelling about their rights under law; wanting money or to curb personal liberty to make up for some invisible harm. To a citizen who has lived years in a nation without studying the law, it must be a shocking introduction; easy to dislike the law. By contrast, Citizens who are aware of the law rarely wake up in the morning with goals of breaking the law-- deliberately running red lights, followed by deliberately making racist comments or deliberately hurting people. Instead, People who are aware of the law gladly comply with laws that ultimately help them.

Big risks have been successfully addressed by governments in the past to enable industry and commerce to flourish by promoting technology in face of risk. Fotering so- called «big science»: nuclear energy genetics, the agricultural revolution and astrophysics, in order to bring new benefits to humanity was a recurring phenomenon in the twentieth century. Then, scientists, lawyers, stakeholders and policymakers worked together to safely incubate new potentially dangerous industries around. Occupational health and safety management laws, using well-honed prevention strategies, are part of the fabric of this protective safety net for society. More than merely reducing costs of accidents and the overall burden of disease in society, the fiscal savings are merely the tip of the iceberg of hidden costs that are avoided by applying the best practices and well understood methods of reducing risks. Rightly prioritized, implementing such laws saves money and therefore is a lifeline that keeps marginal employers afloat in turbulent economic times. Historically, complying with occupational health risk management laws has saved the life of a company from the brink of bankruptcy by avoiding loss as well as preventing liability. Nanotechnolgy is no exception to this basic human need for social order driven and protected by the rule of law. Clear thinking and gaps analysis in current policies, followed by education of the people who will purchase nanotechnology as intellectual property, adequate training of impacted workers and ultimately, discussing the laws with the general public at large can prevent some problems.

III CONCLUSION: THERE OUGHTTA BE A FLEXIBLE LAW

In conclusion, international laws discussed in this paper establish the principle that there is no dearth of international laws to provide a legal basis for implementing sound industrial hygiene practices and occupational health protections worldwide, no need for a popular hue and cry that there "ought to be a law" to protect people while working . Rather, there is a universal need for health and for implementing laws that already exist, in a model that is classless, candidly internalizing the hazards faced by policymakers and occupational health practitioners, and embracing the basic human need for health and work that preserves civilization. Such efforts must be sustained by refreshing data and training under the auspices of on-going flexible compliance programs. New approaches to developing stakeholder views and presenting them in various media are coming to the fore, but, along with consensus, there must be clear language in order to draft precise and useful law. But, it is not easy to translate seemingly logical and clear scientific consensus into applicable law. Such definitions seem to be easy to write because «everybody» knows them, or at least «everybody in the scientific community» does—despite the reality that crafting such definitions is very difficult. Taking into account changes in political will and new discoveries that will reshape the policy response to these issues requires a flexible regulatory framework, reflecting the lessons learned from existing technology-based international legal frameworks discussed in this paper. The new nanotechnology framework cannot simply be a one-shot firecracker approach that looks at a situation, but then ceases to monitor the situation for long term effects. Regulatory efforts must be reviewed periodically to refresh the program, daring to ask, What is the question that we are not asking ourselves, and when we finally ask it, are we confronting it properly?

BIOANALYTICAL PLATFORM FOR THE INVESTIGATION OF THE BIODISTRIBUTION OF FLUORESCENT MAGNETIC NANOPROBES TARGETING BREAST CANCER CELLS

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Diagnosis of axillary localization of breast cancer metastases is currently based on invasive detection methods, which imply the sentinel lymph node biopsy. In this context, one of the most significant challenge is represented by the identification of new target-specific contrast agents able to optimize breast cancer diagnosis and metastatic localization by Magnetic Resonance Imaging (MRI).[1,2]

Recently, we have synthesized a versatile nanoprobe functionalized for site-specific immobilization of antibodies. This magnetite nanocrystal (MNC) has been conjugated with a recombinant monodomain of protein A, with a strong affinity for human and rabbit IgG molecules. In particular, we designed a magnetic nanocrystal conjugated to the anti-HER-2 trastuzumab (TMNC). TMNC were demonstrated to be effective in selectively recognizing HER-2 receptor in MCF7 breast cancer cells in vitro.3 Here, we report the in vivo targeting of these nanocomplexes to MCF7 tumors and their biodistribution in Balb/c nude mice, by combining highly sensitive epi-fluorescence tracking and tissue analyses.

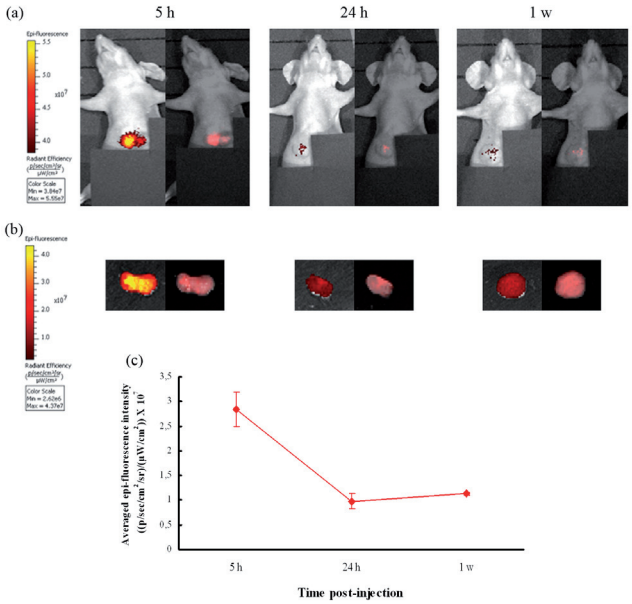

Figure 1: IVIS Lumina II images of mice with MCF7 xenografts (a) and of the isolated tumors (b) at 5 h, 24 h and 1 week post-injection of AF660-TMNC (5 µg/g body weight). Epi-fluorescence intensity images and spectrally unmixed fluorescence images are reported on left and right, respectively. (c) Averaged epi-fluorescence intensity of the isolated tumors. Mean ± s.e. of 3 different samples for each experimental time.

AlexaFluor 660-TMNC (AF660-TMNC) were injected in the tail vein of mice and their localization was observed at 5 h, 24 h and 1 week post-injection by the sensitive CCD camera IVIS Lumina II (Calipers Life Sciences, UK). Epi-fluorescence (Epf) images of supine mice (Figure 1a) indicate that TMNC accumulated in tumor at 5 h from the injection and, after 24 h, the epi-fluorescence intensity decreased. Ex vivo analysis of the isolated tumors confirmed the maximal Epf intensity at the shorter time of exposure (Figure 1b), as also indicated by the averaged Epf intensity, reported in Figure 1c as the mean value of three different tumors for each experimental condition. The surface-weighted properties of epi-fluorescence imaging could explain the significant decrease of Epf over time: this investigative approach, although characterized by an optimal detection sensitivity, is strongly limited by the penetration depth of photons.4 It is reasonable that the high Epf intensity observed at 5 h reflects the rapid displacement of AF660-TMNC in the peripheral vasculature of tumor, while a decrease of fluorescence signal occurs when nanocrystals distribute within the tissue. On the other hand, the drop of fluorescence observed in CCD images could be the effect of AF660-TMNC degradation, subsequent to their receptor-mediated internalization. The metabolization and/or excretion of the fluorochrome, associated to the saturation of membrane HER-2 receptors, could result in fluorescence decrease.

In order to verify this latter hypothesis, we analyzed spectrofluorimetrically the lysates obtained from the same tumors observed in CCD camera. We found that the fluorescence intensity, normalized to the protein content, was maximal at 5 h and progressively decreased over time (Figure 2). It is therefore feasible that AF660-TMNC are internalized by tumor cells and then subjected to degradation. Preliminary results obtained by relaxation measurement of the lysates showed an increase of the amount of iron over time, confirming that TMNC follow a lysosomal pathway in tumor cells.

The biodistribution of AF660-TMNC was evaluated by analyzing in CCD camera liver, kidneys, spleen and lungs, and the Epf of these organs was determined 5 h, 24 h and 1 week after the injection. Figure 3a, reporting the averaged Epf intensity of each imaged organ expressed as the mean value of three different replicates for each experimental time, indicates a significant distribution of nanocrystals in liver and kidneys, with a certain decreasing tendency over time.

A more accurate evaluation of TMNC amount in each organ was obtained by measuring the fluorescence intensity of the lysates, and a normalization of the fluorescence values to the total protein con-

tent allowed a comparison between the different samples. Figure 3b confirms a preferential distribution of TMNC in liver and kidneys at 5h, which gradually decreases over time, and an appreciable fluorescence was also observed in lungs at the shorter time of exposure.

Relaxation analysis of lysates seems to confirm the localization of TMNC in liver, kidneys and lungs after 5 h from the injection, and indicates a time-dependant accumulation of iron in liver, in line with the detoxification role of this organ.5

In conclusion, with this study we developed a multifaceted investigation approach aimed to determine the in vivo distribution of fluorescent magnetic nanoprobes targeting breast cancer cells. An highly functionalized magnetic nanoprobe has been identified as a good candidate for the diagnosis of this tumor.

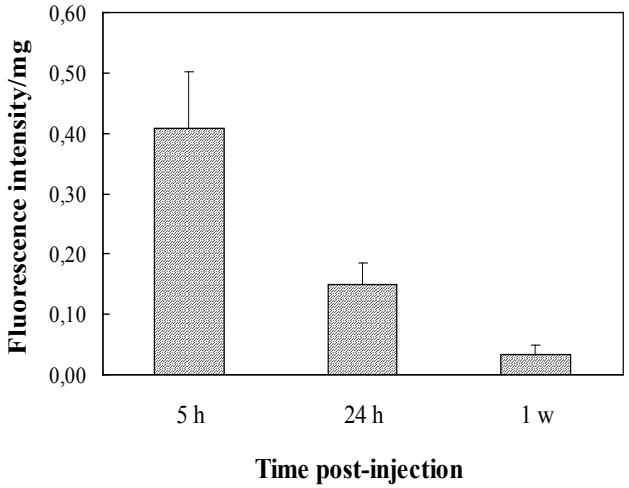

Figure 2: Fluorescence intensity of MCF7 lysates, measured 5 h, 24 h and 1 week after the injection of AF660-TMNC. Mean ± s.e. of 3 different replicates.

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ASSESSMENT OF BACTERICIDAL AND CYTOTOXIC EFFECTS OF SILVER NANOPARTICLES

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By the present time the problem of Mycobacterium tuberculosis strain drug resistance has taken global significance and determines largely spread, clinical course of TB and prognosis of its outcome. In this connection of special importance is the design of anti-TB drugs effective against Mycobacterium tuberculosis drug resistant strains.

Colloidal silver systems known since XIX century due to a wide range of antimicrobial effect of silver, lack of resistance to it in most pathogenic microorganisms, low toxicity and lack of data about the allergenic properties of silver in the literature as well as good tolerability of patients have contributed to increased interest to them. Unlike antibiotics, nanoparticles of silver do not cause resistance in microorganisms. This fact enables silver nanoparticles to be widely used in medical practice.

The objective of the proposed work is to evaluate cytotoxicity of silver nanoparticles in eukaryotic cells, to determine bactericidal activity of them against M. tuberculosis using chronic TB mouse models.

RESEARCH METHODS

The following nanosilver drugs were used for research: -colloidal silver, an aqueous solution of particles of a diameter of 10 nm; -spherical nanospheres of a size of 50 nm; -needle-like nanorods of a size of 150 nm.

Cytotoxicity of silver nanoparticles was determined using MTT test and ATP-measurement in cell lines such as murine macrophage-like cells J774.1A and murine fibroblasts L929; and in primary cell cultures such as mouse peritoneal macrophages and neutrophils of human blood.

Bactericidal effect of silver nanoparticles was determined in vitro by assessing CFU of M. tuberculosis H37 Rv grown on selective nutrient medium 7N11 after incubation for 1 hour in physiological solution without silver nanoparticles and with 10 ug / ml of silver nanoparticles added.

C57Bl mice infected with M. tuberculosis H37 Rv were used as a chronic TB model. The mice were treated with a single dose of suspension containing 0.8 mg of silver nanoparticles within 20 minutes in a dynamic mode at the calculated concentration of nanoparticles 8x10-4 ug / liter of air in Glass-Coll aerosol chamber. After 24 hours, the experimental and control mice (treated with physiological solution) were euthanized. The content of M. tuberculosis H37 Rv in lung homogenates of experimental and control mice was determined by seeding the microorganisms on selective medium 7N11 and counting the number of colony forming units (CFU) 21 days after seeding.

RESULTS.

Fig. 1 displays the percentage of viable cell lines J774.1A and L923 after exposure to silver nanoparticles for 18-24 h.

As is evident from the graphs, colloidal silver drug had no cytotoxic effect on cell cultures. Nanospheres and nanorods added in concentrations greater than 10 micrograms / ml induced the death of J774.1A and L923 cell lines. Similar results were obtained using peritoneal macrophages of mice and human blood neutrophils. MTT-test and ATP- measurement showed identical results. A correlation between the data obtained on cell lines and primary tissue cultures was observed. This suggests that cell lines J774.1A and L923 can be used as models to determine the toxicity of silver nanoparticles.

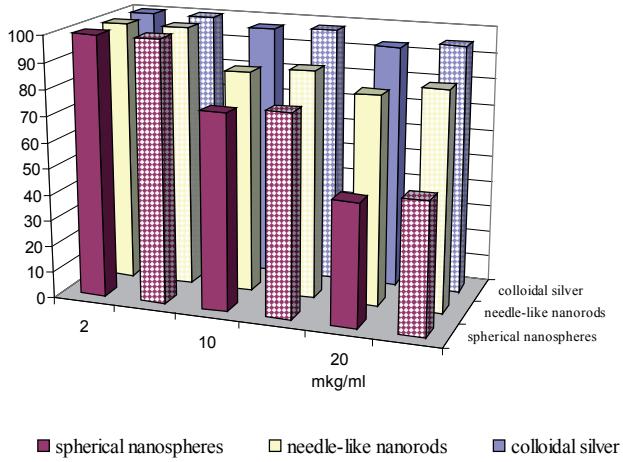

Fig. 1. Percentage of viable cell lines J774.1A and L923 (polka dot bars) after exposure to silver nanoparticles for 18-24 h

Since colloidal silver nanoparticles exhibited low toxicity in eukaryotic cells, this drug was used in subsequent experiments.

The study of bactericidal activity in vitro showed that as early as 1 hour after the addition into the cultivation medium colloidal silver nanoparticles reduced CFU of M. tuberculosis H37 Rv by 30%.

Single aerosol treatment of TB chronic mice with colloidal silver nanoparticles resulted in the decrease of M. tuberculosis H37 Rv CFU lung homogenates.

Thus, colloidal silver nanoparticles do not cause cytotoxic effect in eukaryotic cells and exhibits a pronounced bactericidal effect on Mycobacterium tuberculosis, namely M. tuberculosis H37 Rv. Further studies are required to develop treatment schemes and assess the efficacy of colloidal silver nanoparticles used either as a separate product or in combination of other drugs.

DEVELOPMENT OF GLUTATHIONE PEGYLATED LIPOSOMAL DOXORUBICIN (2B3-101) FOR THE TREATMENT OF BRAIN METASTASES FROM BREAST CANCER

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INTRODUCTION

A new treatment option for patients with brain metastases from breast cancer is being developed by initiating a phase I/IIa clinical study with glutathione pegylated liposomal doxorubicin (2B3-101) this year. In this report, the translational research with regard to the large-scale GMP production method, extended GLP preclinical safety studies, efficacy studies, and pharmacokinetics and biodistribution, will be summarized.

BRAIN CANCER – AN UNMET MEDICAL NEED:

Each year up to 200,000 persons in the US are affected by brain cancer. Brain metastases are by far the most common tumor in the brain and a major complication in metastatic breast cancer. Incidence is 10-16% for patients with late stage breast cancer, with the only main available treatment option being local therapies like whole-brain radiotherapy (WBRT), which results in a median survival after relapse of only 3-6 months (largely depending on the specific tumor cell subtype). Triple negative breast cancers are particularly aggressive and treatment-resistant. Patients with HER2+ breast cancer are usually systemically well controlled by anti-HER2 based treatment, but have a high risk of developing brain metastases.

MECHANISM OF 2B3-101

The drug substance of 2B3-101 is the powerful and well-established cytotoxic agent doxorubicin. 2B3-101 is a brain-targeted version of the marketed product Doxil®/Caelyx® (pegylated liposomal doxorubicin). Glutathione (GSH) coupled to the pegylated liposomal formulation safely enhances the brain delivery of doxorubicin through interaction with concentrating glutathione transporters at the blood-brain barrier. The pegylated liposomal packaging further still ensures a long circulation time in plasma and significantly reduces the risk of doxorubicin-induced cardiotoxicity.

MANUFACTURING (GMP-GRADE) OF LIPOSOMES

2B3-101 for use in nonclinical and early clinical studies is manufactured by TTY Biopharm (Taiwan). TTY Biopharm is highly experienced in producing pharmaceutical products with complex formulations like liposomes. For example, they also currently manufacture Lipo-Dox (a generic form of Doxil®/Caelyx®) for the Taiwanese market.

2B3-101 was prepared using the pre-insertion method, in which GSH-PEG micelles, produced by conjugation of GSH to DSPE-PEG-maleimide, were added to the lipids (HSPC and cholesterol) before extrusion and active loading of doxorubicin. Three batches (each 2L) have been prepared for nonclinical studies and tested for their stability at 5, 25 and 40 °C. 2B3-101 was stable up to 12 months when stored at 5°C, while storage at higher temperatures results in an anticipated decrease in doxorubicin loading. Another three batches of 8L each for the clinical studies are scheduled for production in March 2011.

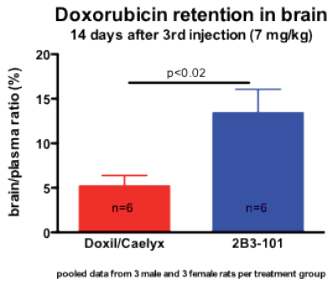
GLP TOXICOKINETIC, TOXICOLOGY AND NEUROBEHAVIOR SAFETY STUDIES

NOTOX, an independent CRO, has performed the GLP studies for 2B3-101. The formulation was administered by intravenous bolus injections on days 1, 15 and 29 to Wistar rats at dose levels of 0 (vehicle), 1.75, 3.5 and 7 mg/kg. As a reference substance, Doxil/Caelyx was dosed at 7 mg/kg to an additional group of rats.

Also, a placebo group (empty GSH-PEG-liposomes) was included in this study. In total 6 groups were used, each consisting of 10 animals/sex/group. An extra 5 animals/sex/group were allowed 4 weeks of recovery. Additional satellite animals were used for toxicokinetic blood sampling (n=3 per group). The dose levels were selected following a dose range finding study in both mice and rats using 3 injections of 4.5, 9 and 18 mg/kg of 2B3-101 and 9 mg/kg Doxil/Caelyx.

After 1st and 3rd administration, slightly higher exposures were obtained in the Doxil/Caelyx group when compared to the 2B3-101 treated group at the same dosage of 7 mg/kg, with AUC values 10-44% higher, while C0 values were considered to be similar for both drug substances. Apparent half-lives for Doxil/Caelyx were 36.1 and 32.6 hours in males and females after the 3rd administration respectively. For 2B3-101 these values were slightly shorter at 30.0 and 28.8 hours in males and females, respectively.

Tissue samples were analyzed for their doxorubicin content by a HPLC analysis with fluorescence detection at the NKI. At 24 hours after the 1st administration, the tissue levels of 2B3-101 were dose dependent and mainly driven by the plasma concentration, with highest levels in liver and spleen. The results obtained 2 weeks after the 3rd administration show a slightly higher doxorubicin concentration in the brain after administration of 2B3-101 compared to Doxil/Caelyx. This difference was even more pronounced in spleen. In other tissues, higher doxorubicin concentrations were found after administration with Doxil/Caelyx, which was in line with the higher plasma and AUC values. The doxorubicin brain/plasma ratio showed retention of both Doxil/Caelyx and 2B3-101 after repeated doses. 2B3-101 administration resulted in a significantly higher doxorubicin retention in the brain (Figure 1). Figure 1: Doxorubicin brain/plasma ratio in male rats



From observations during the in-life part of the study, as well as after histopathological investigations of a full range of tissues, it was concluded that no major differences were noted between 2B3-101 and Doxil/Caelyx when dosed at 7 mg/kg every two weeks. Most prominent and expected findings were dose-dependent inflammatory lesions of the skin, body weight gain depression, myelosuppression, and changes in clinical biochemistry parameters including cholesterol, phospholipids (both also constituents of the liposomes) and electrolytes. Important to note that there were no treatment-related changes in the brain neither after repeated 2B3-101 exposure of rats up to 7 mg/kg, nor after placebo (empty GSH-PEG liposomes) treatment. In addition, heart tissue did not show doxorubicin related toxicity in any of the 2B3-101 treatment groups.

An extensive modified Irwin test in rats was performed in male Wistar rats (n=8 per group) to assess any potential effect on neurobehavior and body temperature following a single intravenous bolus injection of 1.75 mg/kg, 3.5 mg/kg and 7 mg/kg 2B3-101. No neurobehavioral effects and no effects of 2B3-101 on body temperature were noted. Also, placebo formulations (empty GSH-PEG liposomes) and Doxil/Caelyx up to a dose of 7 mg/kg did not result in neurobehavioral effects and effects on body temperature.

EFFICACY OF 2B3-101 IN EXPERIMENTAL BRAIN TUMORS

Initial experiments with research-grade 2B3-101 already indicated a positive treatment effect of 2B3-101 compared to saline, Doxil/Caelyx, and free doxorubicin treatments on glioblastoma in mice. To confirm the efficacy of the GMP grade 2B3-101, produced by TTY Biopharm, two regimens at maximum tolerated doses (MTD) were tested in a mouse glioblastoma model (n=10 per group); 10 mg/kg q4dx4 and 18 mg/kg q8dx2 showed a survival benefit of 57% and 60% respectively when compared to saline, where 18 mg/kg q8dx2 seemed superior (Figure 2).

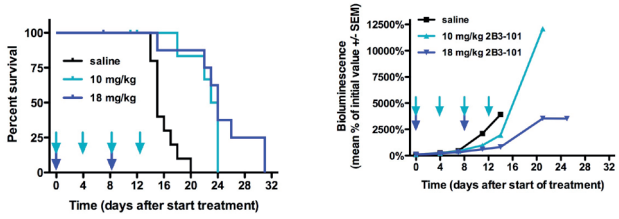


Figure 2: Significant glioma growth inhibition (left) and significant survival benefit (right) for 2B3-101 at 10 mg/kg q4dx4 and 18 mg/kg q8dx2 compared to saline (n=10 per group).

EFFICACY OF 2B3-101 AGAINST HUMAN BREAST CANCER XENOGRAFTS: Charles River Laboratories used a mouse model of systemic breast cancer in which it was shown that treatment with 2B3-101 and Doxil/Caelyx at 10 mg/kg q4dx3 both produced significant anti-tumor activity, were well tolerated, and gave similar weight loss profiles against subcutaneous MDA-MB-231 human breast carcinoma xenografts.

FUTURE PERSPECTIVES

The therapeutic benefit and predictable safety profile of 2B3-101 has been successfully demonstrated in pre-clinical studies and the product is ready to enter clinical trials. The first study in patients is scheduled mid 2011. The purpose of this study is to determine the safety, tolerability, and pharmacokinetics (PK) of 2B3-101 in patients with solid tumors with or without brain metastases. In addition the preliminary efficacy of 2B3-101 will be explored at the maximum tolerated dose level in patients with brain metastases of breast cancer. If successful, 2B3-101 development will continue in patients with progressive brain metastases of breast cancer. Market authorization for this initial indication could be obtained in 2017.

CONSTRUCTION OF NEW CANCER CELL MODELS FOR NANODRUG DISCOVERY AND TESTING

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Cancer stem cells (CSCs) represent a rare population of only one to few percent of the cells within a given tumor. CSCs can re-create a full-blown tumor from one to few cells, have a high metastatic potential and are particularly resistant against conventional drugs and radiotherapy. Accordingly, there is common consent that the next generation of anti-cancer drugs should aim at eliminating the CSCs. Yet, it is problematic to obtain sufficient amounts of CSCs for systematic approaches aiming at the discovery or testing of nanodrugs, because soon after isolation asymmetric division results in a rapid decline of the CSCs.

Recent reports indicated that the introduction of genes like SNAI1 and SLUG into breast cancer cells results in a shift towards more mesenchymal cells, which are in certain regards comparable to CSCs. Thus, the overexpression of these genes resulted in breast cancer cell lines enriched for CSC-like cells. Within this project, we selected a set of 42 candidate genes and miRNAs that could play a potential role in the cancer stem cell phenotype. The goal is to introduce these genes into MCF7 breast cancer cells via a new technique to create stable cell lines with inducible overexpression of the candidate set. Consecutively, we will systematically explore, which genes/miRNAs have the greatest potential to induce a shift to a CSC-like phenotype via mammosphere assays and the determination of CSC-markers. Here, we report on the state of this recently started project.

DEVELOPMENT OF NOVEL NANOTECHNOLOGY BASED DIAGNOSTIC SYSTEMS FOR RHEUMATOID ARTHRITIS AND OSTEOARTHRITIS NANODIARA

M. Hofmann-Amtenbrink and A. Hool

Scientific Coordination NanoDiaRA, MatSearch Consulting Hofmann, Pully (CH)

Inflammatory joint disorders like Rheumatoid Arthritis (RA) together cause the destruction of articular cartilages in about 2-3 % of the population. These disorders are accompanied by significant pain, morbidity and mortality, leading to a reduction in the capacity to work. Osteoarthritis (OA), a degenerative arthritis, is the leading cause of disability among the elderly population affecting over 50% of those over 60 years of age. Effective disease modifying drugs called biologics are now available to treat RA. But in OA drugs only reduce pain but not slow the disease. This often finally results in joint replacement in OA, or treatment may cause side effects in RA. Existing methods often do not permit early diagnosis of arthritis nor its progression and early responses to therapies if available.

Based on such clinical unmet needs the recent research in safe nanoparticles and in biomarkers of RA and OA disease onset and activity offer the new invaluable technology that will address these as yet unmet needs. The main objective of the project is therefore to develop nanotechnology-based novel diagnostic tools for easy and early detection of arthritic disease and its activity using modified superparamagnetic iron oxide nanoparticles (SPION) for ex-vivo application of sensitive biomarker micro-immunoassays for use with body fluids and in-vivo detection using functionalized nanoparticles targeted to detect by MRI molecular events in joints involved in arthritis onset and activity long before structural damage occurs that is presently detected by available imaging technology.

Superparamagnetic nanoparticles whose surfaces can be derivatised and coated with special targeting molecules used in the NanoDiaRA project exhibit their special magnetic properties only in the nano-range between 5 and about 20 nm. To become highly specific and capable of being targeted to specific cells, and even more specifically to specific cell surfaces or cell compartments or to specific biomolecules present in biofluids, it is necessary to functionalize the surface of the particle such that it is able to “recognize” and bind to specific joint tissues and components thereof. This “targeted tailoring” of nanoparticles will be made possible using specific coatings and functionalization steps and thereby attach targeted molecules already developed or under development in the NanoDiaRA project. For this to happen expert collaborations must be formed for pre-clinical and clinical applications by involving the multidisciplinary expertise of researchers at universities and research institutes.

POLYMER/SILICA HYBRID HOLLOW NANOSPHERES WITH PH-SENSITIVE DRUG RELEASE IN PHYSIOLOGICAL AND INTRACELLULAR ENVIRONMENTS

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Recently, ordered mesoporous silica nanoparticles have been investigated as efficacious drug carriers due to their large surface area, tunable pore size and volume, bio-inert and biocompatible properties.[1-4] These silica based drug delivery system showed a sustained drug release manner at mimic physiological condition. However,

"zero drug release" before reaching the targeting site and "fast release" in the select site, such as intracellular environment, are highly desirable in practical clinics. Herein, we report a preparation strategy of hybrid hollow silica nanospheres which have a controllable drug release function in physiological and intracellular environments, which shows improved antitumor effect in vivo. These hybrid hollow silica nanospheres were obtained in complete aqueous solution, through the deposition of silica on the surface of soft template: chitosan-polyacrylic acid nanoparticles (CS-PAA NPs), which are biodegradable and biocompatible. These hybrid nanoparticles possess a porous silica outer shell, offering good mechanical strength and high permeability for anticancer drugs, while the inner shell, consisting of polyelectrolyte layer, provides pH-sensitive switch in the physiological and intracellular environments for the hybrid spheres to release the guest materials. Such hybrid hollow nanospheres show "little release" at physiological condition but "fast release" in intracellular environments for an antitumor agent.

Hollow CS-PAA nanospheres were first prepared through core-template-free strategy according to our previous work.[10] Silica was deposited on the surface of these CS-PAA nanospheres in alkali aqueous solution by hydrolyzing tetraethyl orthosilicate (TEOS) through the interaction between the chitosan and silicate oligomers as shown ih figure 1. Finally, with further hydrolysis, hollow CS-Silica Nps were obtained in solution without the need of template removing step. During this procedure, the PAA molecules were fully deprotonated and released from the nanospheres into the solution, leaving only CS shell inside the hybrid spheres. The continuing release of PAA results in the formation of small pores on the silica shell of the hybrid nanospheres, partly offer the channels for drug to be released out.

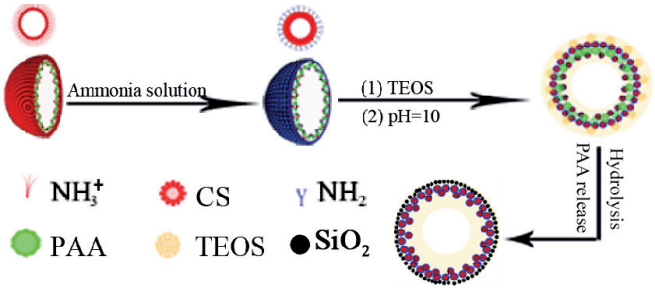


Fig. 1 Schematic depiction of the fabrication of hollow CS-Silica nanospheres with CS-PAA hollow nanospheres as soft template.

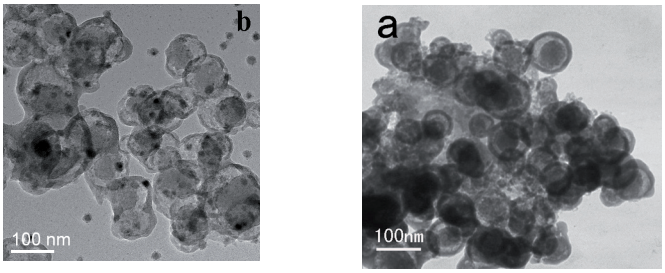
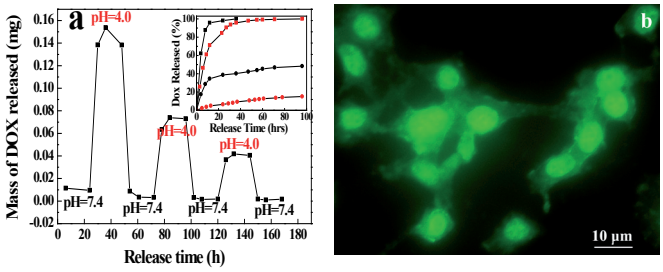


Fig. 2 TEM images of CS-Silica nanospheres before (a) and after calcination at 550°C for 4 hrs (b)

The transmission electron microscope (TEM) image of CS-Silica Nps is shown in Fig. 2a. A hollow spherical structure with a dark shell is clearly visualized. The mean thickness of the shell is about 15 nm, and the average diameter of the hollow interior is 70 nm. After calcination of CS-Silica Nps at 550 oC for 4 hrs to remove CS, the hollow pure silica nanospheres with a shell thickness about 10 nm were obtained.

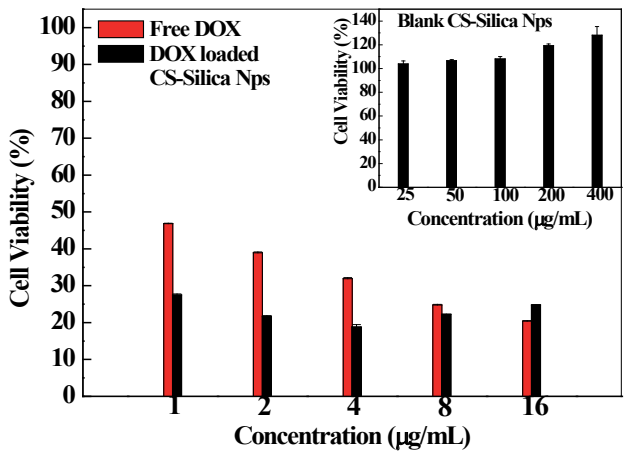
To evaluate the effect of CS inside the CS-Silica Nps on gust molecules loading and their pH-sensitive release, antitumor agent doxorubicin (DOX) containing an amino group (pKa = 8.6) was loaded into CS-Silica and Silica Nps, respectively. It was found that the drug loading content for CS-Silica and Silica Nps was 8.9% and 4.2%, and the loading efficiency was 97.5% and 43.5%, respectively. Compared to the Silica Nps, due to the larger surface area and more significant porosity of CS-Silica Nps, more DOX molecules were diffused and encapsulated inside the interior of CS shell of CS-Silica Nps, resulting in the higher drug payload and loading efficiency. Fig. 3a shows the DOX release from DOX-loaded CS-Silica Nps at 37 °C with the release medium being alternately changed between pH = 7.4 and pH = 4 PBS.



FFig. 3 In vitro release profile of doxorubicin loaded CS-Silica nanospheres at 37°C with the release medium alternately changed between pH = 7.4 and pH = 4.0 PBS (a). Inset shows the release behavior of CS-Silica (red) and Silica (black) Nps at pH = 7.4 (●) and pH = 4.0 (■); fluorescence image of DOX loaded CS-Silica nanospheres incubated with LoVo cells for 4 hrs (b).

It can be seen that the release profile exhibits a pulse appearance and the drug release is switched on and off by alternatively changing the medium pH value. When DOX loaded CS-Silica Nps were in an acidic condition (pH = 4.0), a rapid drug release is observed (80% within 20 hrs), while they were in physiological condition (pH = 7.4), only 15.1% of DOX was released within 96 hours (Inset in Fig. 3a). In contrast, the DOX release from DOX-loaded pure Silica Nps in pH 7.4 medium is much more and rapider than that from CS-Silica Nps, and the difference in DOX release magnitude between pH 4.0 and pH 7.4 medium also much smaller than that of CS-Silica Nps. This indicates that CS plays a key role in releasing DOX molecules with pH stimulation, and the enhanced pH-controlled release can be achieved in CS-Silica Nps.

The pH-sensitive release behaviour of DOX loaded CS-Silica Nps is attractive. Targeting delivery not only requires to deliver drugs to certain cell types or tumor organ, moreover, some drugs even have to be targeted to a specific cell organelle and can be directly released to specific subcellular organelles, such as endo- and lysosomes and nucleus. It has been found that the tumor microenvironment is acidic and significant pH distinction between the intracellular and extracellular compartment.[5] Thus, When DOX loaded CS-Silica Nps are at the physiological condition, such as blood circulation (pH = 7.4), the DOX will be entrapped inside the carriers with little release. After they are in the tumour site or uptake by the cells, due to the low pH values (pH = 5.5-6 in endosomes, pH = 4-5 in lysosomes.[6]), DOX will be released from the CS-Silica Nps rapidly, which provides a new generation of site-selective, controlled-release delivery system. Fig. 3b shows the fluorescence image of DOX loaded CS-Silica nanospheres incubated with LoVo cells for 4 hrs obtained in the green channel, which clearly shows that most of the DOX is inside the cells even in the nucleus. Compared to DOX loaded CS-Silica nanospheres, much weaker green color was observed inside the cells for free DOX, indicating fewer DOX in the cells or nucleus.



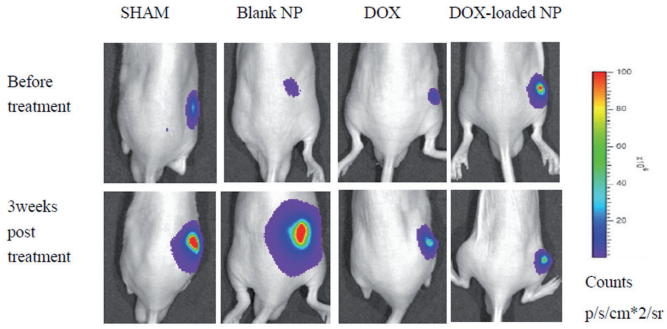


Fig. 4 In vitro cytotoxicity of free DOX and DOX loaded CS-Silica Nps against LoVo cells. Inset is the cytotoxicity of empty CS-Silica Nps against LoVo cells.

Fig 5. BLI images of representative subcutaneous U87 MG-luc2 xenografted mice with or without treatment. Using Xenogen IVIS Living Image Software, luciferase expressing tumor cells are displayed in the right flank of mice with a red–blue color bar, red color indicating the highest BLI signal intensity.

The cytotoxicity results of the free DOX and DOX loaded CS-Silica Nps against LoVo cells are presented in Fig. 4. The DOX loaded CS-Silica Nps show higher cytotoxicity than free DOX at same drug dose, which is similar to the other drug loaded nanoparticles.[7,8] It is pointed that the intracellular action site of DOX is within the nucleus, and only effective intracellular delivery of DOX into cancer cells and accumulation in nucleus can improve its anti-tumour activity.[9] In our case, more DOX inside the nucleus should significantly improve DOX anti-tumour activity. As a control, the blank CS-Silica Nps have no cytotoxicity even at high concentration (400 μg/mL) (inset of Fig. 4).

Fig 6. In vivo subcutaneous U87 MG-luc2 tumour BLI signal profiles of mice treated with different groups for 21 days. Animals were sacrificed after 21 days. The symbol *,# denotes a significant difference (P< 0.05) compared with sham, blank NP and Dox groups. The symbol *** denotes a significant difference (P< 0.001) compared with blank NP group.

The in vivo antitumor performance of DOX loaded CS-Silica NPs was investigated by using subcutaneous U87 MG glioma tumor-bearing mice as the model animals through local drug delivery system. Sham-operated group (no drug and no NPs implanted), blank CS-Silica NPs (blank NPs) and free DOX with concentration of 1 mg/mL were used as control. For animals in the blank NP and DOX-loaded NP groups, the drug and NPs were implanted around the tumor and the wound was closed using subcutaneous suturing. Animals in the DOX group were administered with 0.1 mL DOX solution directly into the tumor mass. Fig 5 shows the BLI signal intensity on 0 day and 21 days of treatment for the various groups. The sham and blank NP groups show marked increase in BLI signal intensity, and the area of tumor is largely extended. This reveals that the blank NPs show no toxicity to the tumor cells. For the free DOX group, there is no significant difference observed before and after treatment, indicating that free drug can inhibit the growth of tumor to a certain extent. While in the DOX loaded CS-Silica NPs group, the area of tumor after 21 days treatment presents obvious reduction than the untreated one, displaying optimal antitumor performance among the four groups.

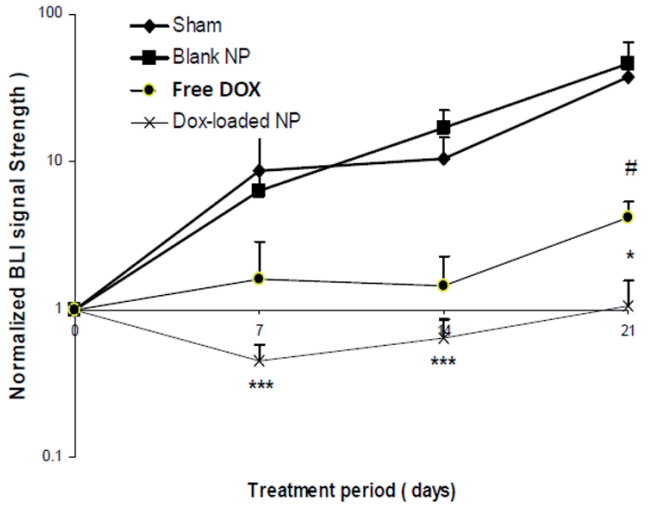


Fig 6. shows normalized BLI signal intensity over time for each group. The sham and blank NP groups display similar exponential amplification of glioma cells during the entire period of study. In comparison to the sham, animals in the DOX group have less BLI signal, but the difference is only on day 21 ($p > 0.05$), not significant on day 7 and 14 ($p<0.05$). For DOX-loaded CS-Silica NPs treated animals, the BLI signals significantly decrease in comparison to those treated with the blank NP ($p < 0.001$ on day 7 and 14; $p < 0.05$ on day 21) for the entire treatment period. For the DOX group, the BLI signal is significantly higher than the DOX-loaded NP treated mice under the same treatment period, especially on day 21 ($p<0.05$), which suggests that CS-Silica NPs can provide stronger anti-tumoral effect than the free doxorubicin for long-term treatment. In conclusion, the drug loaded CS-silica NPs exhibit excellent tumor inhibition performance. These results are reasonable that nanoparticles may facilitate enhanced intracellular delivery as they can carry a large "payload". For the CS-Silica NPs, due to their pH-dependent drug release property, DOX will be rapidly released out within the acidic tumor site or inside the tumor cells, which results in a higher antitumor efficiency in vivo.

We successfully prepared a novel kind of hybrid hollow CS-Silica nanospheres by constructing the internal surface of hollow silica nanocarriers with pH-sensitive polyelectrolyte materials. DOX can be efficaciously incorporated inside the hybrid nanospheres and released little at physiological condition but released much in intracellular environments, and they exhibited superior antitumor effect than the free drug in vivo.

This work is supported by the National Natural Science Foundation of China (No. 50603008, No. 50625311, 5057301),

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SELECTIVE IN VITRO TARGETING OF LIVER CANCER BY MULTI-WALLED CARBON NANOTUBES FUNCTIONALIZED WITH BOVINE SERUM ALBUMIN DELIVERY

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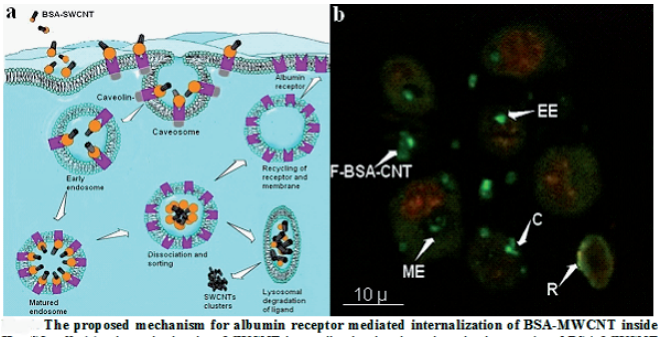
We present a method of selective targeting of HepG2 using carbon nanotube bound to bovine serum albumin (BSA).

Hep G2 cells were treated with MWCNT-BSA (non-covalently functionalized) at various concentrations and various incubation times. Confocal microscopy and immunochemical staining were used to demonstrate the selective internalization of BSA-MWCNTs via Gp60 receptors and caveolin-mediated endocytosis followed by lisosomal degradation inside the HepG2 cells.

First, we investigated the possibility that a 60 kDa glycoprotein, gp60, which is known to function in albumin transcytosis in malign cells, was involved in the selective uptake of albumin bound to carbon nanotubes. To accomplish this, we allowed the cells treated with 5mg/L BSA-MWCNTs for one hour to incorporate cy3–anti-gp60 antibody for 30 minutes at 37°C. To that end, we obtained fluorescent images demonstrating the internalized cy3 fluorescence.

Alternatively, we showed that HepG2 cells internalized with albumin-bound MWCNTs (fluorescently labeled with FITC) were distributed into the punctate structure inside the cells. 4'-6-Diamidino-2-phenylindole (DAPI), which is known to form fluorescent complexes with natural double-stranded DNA, was used for nuclei staining. To that end, nearly complete co-localization of the FITC fluorescence (green) and cy3 fluorescence (red) was evident by yellow in the merged image. This finding suggests that albumin bound to MWCNTs was incorporated into plasmalemmal vesicles containing gp60 as a membrane protein, further validating BSA-MWCNTs specificity for gp60 receptors. Based on these data, we showed that BSA-MWCNTs can act as specific and sensitive site-targeted nanosystems against gp60 receptor located on the liver cancer cells' membrane.

Literature suggested that caveolae-mediated endocytosis in cells is stimulated by the binding of albumin to gp60, a receptor located in the caveolae. Next, we reasoned that the mechanism of BSA-MWCNTs internalization in HepG2 cells was similar. To test this hypothesis, we immunostained the HepG2 cells with Cy3-anti-caveolin-1 Ab. Confocal imaging revealed that the majority of FITC-BSA-MWCNTs containing plasmalemmal vesicles stained for caveolin-1 used this fluorescent anti-caveolin-1 monoclonal antibody. Taken together, all these data demonstrate that BSA-MWCNTs selectively internalize in human hepatocellular cancer cells via caveolae-mediated endocytosis, by the binding of the albumin carrier to gp60, a specific albumin-binding protein.



The proposed mechanism for albumin receptor mediated internalization of BSA-MWCNT inside HepG2 cells.(a) schematic drawing: MWCNT internalization involves the selective uptake of BSA-MWCNT with the aid of albumin (GP 60) receptors. This usually begins with the formation of caveolar invaginations on the plasma membrane surface. These pits are called caveosomes. The vesicles then transform into early endosomes. Matured (late) endosomes receive internalized material en route to lysosomes, usually from early endosomes in the endocytic pathway and most of the associated receptors circulate back to the cell membrane. Late endosomes mediate a final set of sorting events prior to delivery of material to lysosomes. Lysosomes are the last compartment of the endocytic pathway. The MWCNTs are released inside the cytoplasm forming clusters and BSA is digested by lysosomes.(b) Hep G2 cells were treated with MWCNT-BSA-FITC 5 mg/L for 60 minute; Confocal fluorescence image showing the internalization and accumulation of fluorescent nanobioconjugate inside the cytoplasm (various phases of endocytosis mechanism are demonstrated: R-selective binding to albumin receptors;C-caveosomes; EE-early endosomes; ME-matured endosomes

Our findings could be of significance for the specific targeting of liver cancer cells by using BSA-MWCNT and their destruction by laser photothermolysis. Our presented results clearly show that BSA-MWCNTs selectively attach on the albondin (aka gp60) receptor located on Hep G2 membrane followed by uptake through a caveolin dependant endocytosis process. These results may represent a major step in liver cancer treatment using nanolocalized treatment.

CELLULAR DELIVERY AND INTRACELLULAR TRANSPORT OF NANOPARTICLES: REQUIREMENTS FOR CLINICAL USE OF NANOPARTICLES

Tore Skotland[1,2], Tore Geir Iversen[1,2], Kirsten Sandvig[1,2,3]

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The main focus within the field of nanomedicine is in the development of nanoparticles for targeted drug delivery and diagnostic in vivo imaging of tumours and other diseases. Carefully designed experiments both in vitro and in vivo with different types of NPs are required to fully explore the potential of this technology. In poster 1, we present the main issues that need to be solved in order to bring medical imaging with new metal-based NPs into common clinical use. These include increased knowledge about biodistribution, metabolism, excretion and toxicity of the NPs. Rapid clearance of imaging agents from blood is essential in order to obtain low background signals and good images. The surface charge and hydrodynamic diameter of the NPs in the presence of plasma proteins are important for their biodistribution, excretion and rapid clearance from blood. Remaining challenges regarding safety and metabolism issues with NPs are discussed. Measurements and optimization of the critical parameters will shorten the time needed for such particles to be accepted for widespread medical use. Sheet 1 is a summary of our recent review article in Nanomedicine 6 (2010) 730-737.

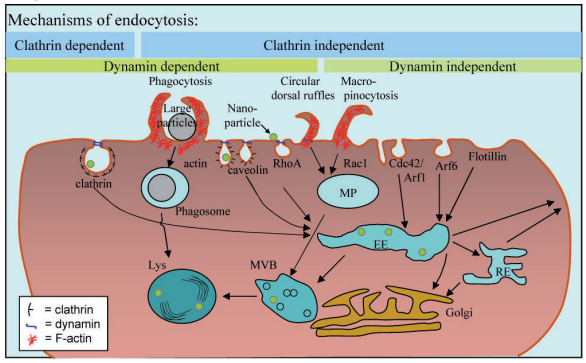

Figure 1 Overview of mechanisms of endocytosis and intracellular transport identified by the specific regulatory and structural proteins involved. NP (green dots) taken up by endocytosis are enclosed within early endosomes (EE), phagosomes or macropinosomes (MP). They may mature into multivesicular bodies (MVB) and lysosomes (Lys), or be transported back to the cell surface through recycling endosomes (RE).

Successful use of nanoparticles for in vitro studies and for delivery of drugs and contrast agents in animals and humans is also depending on a detailed knowledge about how the nanoparticles are taken up and transported within cells. In poster 2, we summarize our discussion of the possibilities and challenges of studying endocytic pathways (see Fig.1) involved in cellular uptake of nanoparticles (accepted for publication in Nano Today2).

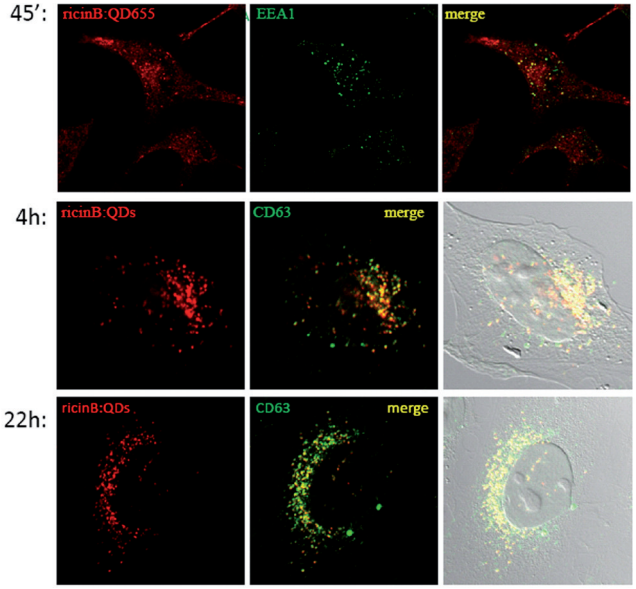

Figure 2 Confocal immunofluorescence microscopy images showing uptake (for 40 min/4h/22h) and colocalization of ricinB:QD bioconjugates with the early endosomal marker EEA1 and the lysosomal marker CD63 in HeLa cells.

Our research aims at gaining a deeper understanding of the mechanisms of endocytosis and intracellular transport of nanoparticles. Furthermore, the use of nanoparticles has also provided us with a new tool to obtain insight into cell biological questions of basic character. The extent to which nanoparticles are able to distort the normal intracellular trafficking was studied in three different cell lines by measuring the uptake and intracellular transport of quantum dot (QD) nanoparticles coupled to three different proteins that bind to different cell receptors. The proteins studied were transferrin (Tf), the plant toxin ricin and Shiga toxin. Tf:QDs were endocytosed by clathrin-mediated endocytosis, but in contrast to transferrin did not recycle out to the plasma membrane. RicinB:QDs were efficiently endocytosed and accumulated within late endosomes of the cells, displaying good colocalization with lysosomal markers such as CD63 (Fig. 2 and Sheet 2). Moreover, a significant fraction of ricinB:QDs and Tf:QDs was also routed into non-lysosomal vesicles of HeLa, HEp-2, and SW480 cells3,4. Shiga:QDs were endocytosed less efficiently in Hep-2 cells compared to HeLa cells. However, in contrast to ricin and Shiga toxin itself, neither the ricin:QDs or Shiga:QDs were not transported to the Golgi apparatus. Clearly, ligands coupled to particles do not behave as normal and the particles can affect intracellular trafficking in general3,4,5.

Importantly, we found that uptake and endosomal accumulation of Tf:QDs and ricin:QDs nano-conjugates perturbed the intracellular transport of ligands between endosomes and the Golgi apparatus (see sheet 2), demonstrating that nanoparticles may have significant effects on cell function. Thus, safety assessment of nanoparticles should include studies addressing their cellular uptake in various cells and their ability to perturb cellular processes.

In our further studies we will investigate whether a reduction in size and changes in composition and surface chemistry will give particles with less cellular side-effects and a low extent of cellular retention.

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SYSTEMATIC IDENTIFICATION OF NOVEL BREAST CANCER DRUG TARGETS

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Nucleic acid-based nanodrugs that utilize short interfering RNAs (siRNAs) and cause synthetic lethality to cancer cells are a promising option for personalized cancer nanomedicine. Synthetic lethality means that inactivation of a certain gene by RNA interference only causes death to cells with a particular cancer-specific mutation in another gene. The advantage of this strategy is that a patient`s tumor can by typed for the presence of the mutation to identify prospective responders. Furthermore, nanodrugs causing synthetic lethality are proposed to exert minimized toxic side effects.

Recent next generation sequencing approaches recovered large numbers of genes with mutations in cancer, representing a favorable source for the discovery of novel drug targets. However, for more than 90% of these genes either the functions or the consequences of the mutations are still unknown.

To bridge this gap, we selected a set of 140 genes from these new profiles with the goal to recover novel potential drug targets via a systematic functional genomics approach. Here we report on cloning and functional studies of a first subset of this gene set, which already at this early stage identified one novel gene reducing cancer growth (see Figure 1). Downstream studies to evaluate its suitability as drug target and continuation of the analyses are in progress.

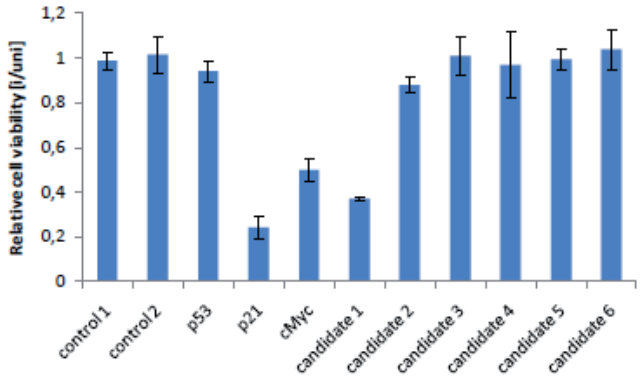

Figure 1. Discovery of novel breast cancer genes. First data from the systematic identification of novel breast cancer genes. Candidate gene 1 is a strong candidate for a new breast cancer gene, because it decreased cancer growth to a similar strong degree as the known cancer genes P21 and cMyc, i.e. by more than 60%.

THE PATHWAY OF CARBOXYL-COATED QUANTUM DOTS ACCUMULATION IN THE EMBRYONIC FIBROBLAST NIH3T3 CELLS

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Quantum dots (QD) are fluorescent semiconductor nanoparticles that possess ideal physicochemical properties for biological imaging and therapeutic applications. The mechanism of non- targeted (non-functionalized) QD uptake by living cells is largely unknown, although, few studies show that receptor-mediated endocytosis is the most likely scenario. Our study shows that carboxyl-coated CdSe/ZnS QD accumulate in the NIH3T3 cells by lipid raft/caveolin-dependent endocytosis pathway. QD uptake mechanism could be divided into several steps: a) adsorption to the cell surface during 5-30 min. of incubation with QD; b) early stage of endocytosis where QD follow lipid raft/caveolin-dependent endocytic route c) late stage of endocytosis where QD and other vesicles fuse into multivesicular body, which does not proceed subsequent fusion with lysosomal compartment and therefore does not lead to QD intracellular degradation.

FROM SINGLE MOLECULES TO THE LIVING ORGANISM: NANOSCIENCE AT SEMMELWEIS UNIVERSITY

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Nanosciences carry the promise of specialized methodologies for understanding nature at the nanoscale and of devising novel diagnostic and therapeutic tools in medicine. At Semmelweis University, a recently-formed Nanoscience Network coordinates research, development and innovation related to nanoscale science by nurturing communication and facilitating intellectual exchange between the various thematic units. The Nanochemistry Group aims at developing intelligent biocompatible ad biodegradable polymer systems for use in various biomedical applications ranging from tissue-engineering to controlled drug-release. The Nanomedicine Group participates in devising, synthesizing and testing liposome-based drug delivery systems and in addressing a set of nanotoxicology problems. The Nanoscience Education Unit facilitates the development of nanoscience courses, the assembly of educational materials and the streamlining of knowledge from the laboratory bench towards the students and laypeople. Finally, the Nanobiotechnology and In Vivo Imaging Center houses a novel instrumentation base that enables the exploration of the nanoscale world ranging from the mechanical manipulation of individual biomolecules all the way to imaging nanoparticles administered into in vivo organismal systems. Aided by atomic force microscopes and optical tweezer work-stations, the structure and elastic behavior of filamentous biomolecular systems such as cytoskeletal and extracellular proteins, DNA, chromatin, chromosomes and RNA are investigated. By using novel microscopies and imaging devices such as confocal, TIRF and multi-photon microscopes and a nanoSPECT/CT instrument the nanoscale mechanisms of in vivo processes are addressed. Thus, by launching the Nanoscience Network, Semmelweis University had set the stage for reaching landmarks along the contortous path of understanding and controlling biomedical systems on the nanoscale.

EDUCATIONAL NANO-BIOSAFETY CENTER (PUSHCHINO STATE UNIVERSITY) BASED ON THE STATE RESEARCH CENTER OF APPLIED MICROBIOLOGY & BIOTECHNOLOGY

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A nanotechnology is currently recognized a high priority trend in the advancement of science and technology of the 21st century. This is a reason why great attention now focuses on the exploration of this advanced technology that enters steadily different spheres of modern society (medicine, economy, ecology, engineering, etc). Like any novel technology, the nanotechnology is not only indubitably advantageous, but is also potentially hazardous for ecosystems and human health. Data reported on impact of nanomaterials on laboratory animals highlight their potential risk to people handling nanomaterials. Nanomaterials may provoke fibrosis and some other lung diseases in people after short-time exposure to carbon nano-sized tubes, stimulate translocation of nanoparticles to brain via the olfactory system and to blood, and activate thrombocytes accountable for blood vessel thrombosis, etc.

It is obvious that for tackling problems of people health and environmental protection because of production and wide spread of nanomaterials interdisciplinary investigations are needed to study molecular and nanoparticle-induced processes taking place in humans and animals, and in the environment. It is necessary to elucidate mechanisms of interaction of organic and inorganic structures during specific small-scale processes. Knowledge of the dynamics of processes specific for nanostructures would not only illuminate mechanisms underlying their action on people and ecosystems, and routes of their transfer and transformation by biotic or abiotic factors, but would also identify beneficial applications of nanotechnologies in the sake of human health and to improve biological and ecological safety standards.

Owing to unique physicochemical and biological properties, engineered nano - particles and – materials are used in nano-medicine to diagnose and to prevent many diseases. Main areas of their application are:

- medical nano-equipment-based diagnostics;
- medical nanorobots;
- biocompatible nanomaterials;
- nano-therapeutics.

Nanotechnologies open fascinating prospects not only for medicine, but also for other areas of science and technology. However, potential risks of engineered nanomaterials and nanoparticles for people and animals, and for the environment have been evaluated in part only. Sophisticated methods and equipment are needed to evaluate all potential, actual and delayed risks of nanomaterials for people health. Experienced and trained specialists are also required.

In this context on the basis of the State Research Center for Applied Microbiology and Biotechnology (SRCAM&B) a faculty (Educational Center for Biological & Environmental Safety) structured into the Pushchino State University was set up in 2006. The Center was then changed for the Educational Nanosafety Center, whose objective was to train relevant specialists.

The State Research Center for Applied Microbiology & Biotechnology has a unique science and technology base and highly qualified specialists to carry out complex research both on biosafety and nanotechnology safety. There are conditions for experimentation to assess impact of nano-particles and – materials on various living organisms. There is a certified test center that meets all requirements in compliance with GOST ISO/IEC 17025-2000. In the center there are internationally certified animal facilities. There is a staff of experienced scientists who are capable to determine influence of potentially hazardous compounds on living organisms. All this allows extensive testing of toxicity of various agents for laboratory animals and primary and resuscitated tissue cultures in vitro, evaluation of their potential mutagenic, carcinogenic and teratogenic effects, and the study of histological and histochemical and some other characteristics of the vital activity of animals.

A curriculum available for training master degree holders assures the basic knowledge to have highly proficient specialists in the field of nanosafety, as well as obligatory disciplines on biological and ecological nanosafety. It covers the following key topics: «Nanotechnology Safety», «Biosecurity in the Modern World», «Environmental Toxicology of Nanomaterials», «Principles of Nanomaterial Toxicology», «Nanomaterial Immunotoxicity», «Principles of Nanobioriskology», «Laboratory Animals & Nanobiosafety Research», «Nanobiosafety of Aerosols», «Nanomaterial Genotoxicity».

Since 2008 works to assess toxicity of different nanomaterials have been fulfilled. Three theses for obtaining a master degree were defended in the Center in 2010. They were:

1. Boutyrkina A.S. «In vitro assessment of nanomaterial toxicity for cell cultures».
2. Polezhaev O.V. «Influence of carbon nanoparticles on the function of murine neutrophils».
3. Timoshinova E.V. «Assessment of integral toxicity of xenobiotics and nanomaterials by growth assays».

Two theses are planned to be reviewed in 2011.

1. Voropaev A.A. «Development of methodical approaches to the assessment of genotoxicity and mutagenicity of some nanomaterials».
2. Rakitsky Yu.N. «Assessing integral toxicity of xenobiotics and nanomaterials in biotests.

MODIFICATION OF SURFACE ENERGIES OF DRUG DELIVERY SYSTEMS TO REDUCE COMPLEMENT REACTIONS IN CANCER THERAPY

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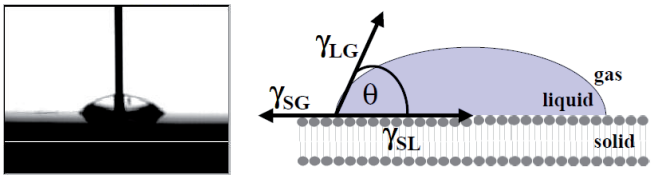
To improve cancer therapy, efficient delivery systems like liposomes, nanocapsules and nanoparticles are increasingly employed (Farokhzad and Langer 2009; Shi, Votruba et al. 2010). A challenging issue is to avoid their recognition by the complement system, which leads to a clearance of liposomes or nanoparticles from the blood system (Nilsson, Nilsson Ekdahl et al. 2007; Ricklin and Lambris 2007; Ricklin, Hajishengallis et al. 2010). Blood clearance may be initiated by two crucial processes: Firstly, opsonization, i.e., binding of complement proteins to foreign substances followed by phagocytosis. Secondly, lysis of the liposome bilayer by the membrane attack complex (MAC) formed by complement proteins. A well established strategy is to cover liposome surfaces with suitable substances, such as poly(ethylene glycol) (PEG) to protect them from blood clearance (Vermette and Meagher 2003; Mora-Huertas, Fessi et al. 2010). PEGylated liposomes show a prolonged circulation lifetime. However, a second dose of injected PEGylated liposomes demonstrates a significant immune response, leading to the phenomenon of accelerated blood clearance (ABC). A current hypothesis assumes that antibodies against PEG are formed (Laverman, Brouwers et al. 2000; Kim, Kim et al. 2009; Pham, Mitchell et al. 2011). The key open question is: Which surface properties are exposed by the PEGylated liposomes to prolong blood circulation time by avoiding any unspecific immune response like, e.g., opsonization and insertion of MAC? If these properties and their underlying mechanisms could be described quantitatively, it should be possible to cover liposomes with substances other than PEG which offer similar protection against unspecific immune reactions, but do not activate specific immune reactions.

Supported lipid bilayers (SLBs) are used as a model for biological membranes and can be formed in different ways (E.Kalb 1992; R.Richter 2005). However, the challenging issue is to ensure their stability in air. We achieved this by using a silicon chip with grafted PEG and tethered cholesteryl anchors as a support (Deng, Wang et al. 2008). SLBs were fixed to the surface by the cholesteryl anchors. Investigations about surface energies with respect to biological functionalities like adsorption of serum proteins were established. Using measurements of advancing contact angles we characterized these surfaces consisting of different phospholipid bilayers and their modification by introducing covalently bound poly(ethylene glycol) (PEG). A schematic representation of the measurement is given in figure 1. The measurements were taken at physiological temperature and isotonic buffer systems.



a) b)

Figure 1: a) Measurement of the advancing contact angle of an expanding drop. b) Schematic presentation of the equilibrium of interfacial forces characterized by Young's equation.

There are different methods to calculate energy properties of lipid bilayers from contact angle measurements (Zenkiewicz 2007; Deshmukh and Shetty 2008). Here, we consider a method reported by van Oss (Oss 1993), which implies contact angle measurements of different liquids on a dry lipid bilayer surface. The total surface energy gS is the sum of the polar and nonpolar components of the examined surface, gSAB and gSLW, respectively. According to van Oss, the polar term is divided into proton acceptor and donor component:

$$\gamma_S^B = 2\sqrt{\gamma_S^- \gamma_S^+}$$

,with g + being the proton donor and g – the proton acceptor component. The proton acceptor term is of main interest to compare the characteristics of phospholipid and lipid-PEG bilayers. AFM measurements prove the existence of homogeneous lipid bilayers and low surface roughness with root mean square values well below 1 nm.

The results in figure 2 show that g – is higher for all SLBs compared to the subsurface consisting of PEG with tethered cholesteryl

anchors. For the saturated phospholipid dipalmitoyl phosphatidylcholine (DPPC) g – is lower than for the unsaturated lipids dioleoyl phosphatidylcholine (DOPC) and palmitoyl oleoyl phosphatidylcholine (POPC). The insertion of only 1 mol% PEG (covalently bound to distearoyl phosphatidylcholine, DSPE) to POPC leads to an almost twofold magnitude of g –, which reaches already 25 % of the magnitude for a surface with 100 % PEG (data not shown). However, an increase of the PEG component to 5 mol% of the SLB does not further increase g –. Instead, g – is decreased with increasing the PEG content from 1 to 5 mol% by about 13 %. A higher degree of entanglements which results in less amenable OH-groups is interpreted to be the cause for this reduction in g –. For comparison, SLBs of POPC with 1 and 5 mol% of the lipid anchor DSPE (without PEG) do not produce a significant increase in g – compared to pure POPC.

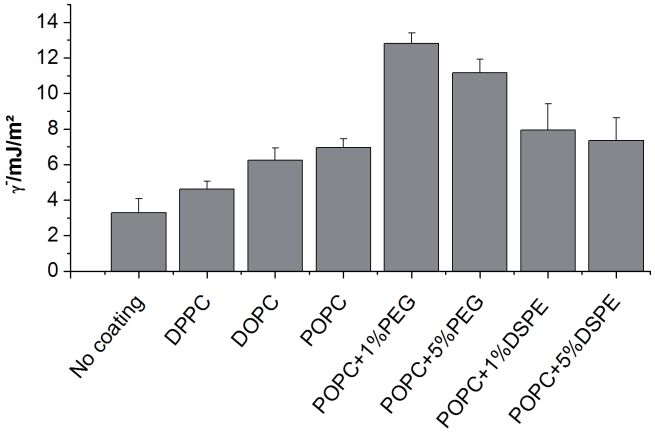


Figure 2: Proton acceptor component g – for different SLBs. Layers containing 1 or 5 mol% PEG demonstrate a higher proton acceptor component.

This means that DSPE does not contribute to the increase of g –, only the PEG chain (molar weight 2 kDa, 45 ethylene oxide units) which is covalently bound to DSPE.

The measurements presented contribute to elucidate the mechanisms which lead to a reduction in phagocytosis, destruction and elimination of liposomes by coating them with PEG. Based on the developed method, we are able to investigate bilayers with adsorbed blood proteins as a next step and learn more about the adsorption mechanisms. Other lipid mixtures can be tested for surface energetic properties, their influence on the adsorption of blood proteins and their potential activation of complement proteins. The ultimate goal is to improve drug delivery for long circulation and targeting of solid tumors and metastases without activating the formation of antibodies against the coating polymer, which seems to be the case for PEG as indicated by the ABC phenomenon.

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BIODEGRADABLE NANOPARICLES LOADED WITH TEMOPORFIN AS PROMISING DELIVERY SYSTEM FOR PHOTODYNAMIC THERAPY

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INTRODUCTION

Photodynamic therapy (PDT) has emerged as one of the important therapeutic options in management of cancer and other diseases. It is based on the combined use of photosensitizers and visible light, which induce selective photochemical damage of tumour cells with minimal effect on the surrounding normal tissues (Chatterjee et al. 2008). Most photosensitizers are highly hydrophobic and can agregate easily in aqueous media therefore, reduction in biological activity is often observed (Bechet et al. 2008). Incorporation of photosenzitizers in biodegradable nanosized particles is a promising technological approach (Kristl et al 1996), since nanoparticles can accumulate at tumor site, internalize tumor cells and enable prolonged intracellular release of photosenzitizer. The aim of the current research was formulation and in vitro evaluation of non-PEGylated and PEGylated poly-(D,L-lactide-co-glycolide) (PLGA) nanoparticles loaded with highly potent photosenzitizer temoporfin (3,3',3",3'"-(2,3-dihydroporphyrin-5,10,15,20-tetrayl)tetraphenol).

METHODS

Preparation of temoporfin loaded PLGA and PEGylated PLGA nanoparticles: PLGA and PEGylated-PLGA NPs were prepared by modified nanoprecipitation method (Kocbek et al. 2010). Briefly, 45 mg of PLGA (Resomer RG 503H, Boehringer, Ingelheim, Germany) and 5 mg of temoporfin (Biolitec AG, Jena, Germany) were dissolved in 1 ml acetone and the solution was slowly injected into 50 ml of 0.25 % (w/v) aqueous poloxamer 188 solution with moderate magnetic stirring. The resulting nanoparticle dispersion was stirred for 15 min at room temperature and then centrifuged at 15.000 rpm

for 15 min to separate nanoparticles from non-incorporated temoporfin and the excess of the stabilizer. The nanoparticles were further washed with 20 ml of distilled water, centrifuged at 15.000 rpm for 15 min, redispersed in 10 ml of 5% (w/v) aqueous trehalose solution, and freeze-dried at −57 °C and 0.090 mbar for 24 h (Christ Beta 1-8 K, Germany). PEGylated nanoparticles were prepared by the same procedure, the only difference being the polymer composition. The weight ratio of PLGA and PEG-PLGA (Resomer RGP d 50155, Boehringer, Ingelheim, Germany) used for preparation of PEGylated nanoparticles was 1 to 1.

Characterization of nanoparticles: Nanoparticle size was determined by photon correlation spectroscopy (PCS) using a Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK). PCS yields the mean particle diameter and the width of the particle size distribution (polydispersity index). The particle charge was quantified as zeta potential by laser Doppler anemometry using a Zetasizer Nano ZS. Freeze-dried NPs were dispersed in 10% FBS solution in PBS prior to measurement. All measurements were made in triplicate.

The total amount of temoporfin entrapped in nanoparticles was measured after complete dissolution of the nanoparticles in DMSO. The temoporfin fluorescence (λexcitation 423 nm, λemission 652 nm) was recorded using microplate reader Tecan Safire2 and the concentration was determined from the corresponding standard curve.

For release studies PEGylated and non-PEGylated freeze-dried PLGA nanoparticles loaded with temoporfin were dispersed in PBS containing 10% FBS. The samples were incubated at 37 ºC for 1 to 24 h. Then the samples were ultracentrifuged for 20 min at 50.000 x g using Thermo Scientific Sorvall® WX100 ultracentrifuge equipped with rotor F50L-24x1.5ml. After ultracentrifugation, the supernatants were discarded and the sediments were dissolved in DMSO. The concentration of temoporfin in solution was determined by measuring the solution fluorescence as described above and the amount of temoporfin released was determined indirectly from the obtained data.

Cellular uptake: MCF 10A neoT cells (1x105) were seeded in 24-well plates and grown to confluence. U937 (4x105 cells/ml) were differentiated using 50 nM phorbol 12-myristate 13-acetate (PMA) (Sigma, St. Louis, MO, USA) for 24 h to achieve macrophage properties and attached to polystyrene plate (Welgus 1986). Cells were washed with PBS and incubated for 4 h and 24 h with 0.5 or 1 μM temoporfin in nanoparticles dispersed in cell growth medium supplemented with 10% FBS. Cells were then washed twice with PBS to remove uninternalized nanoparticles, detached from growing surface and immediately analyzed by flow cytometry. The mean fluorescence intensity (MFI) of a population of 1x104 cells was determined on a FACSCalibur (Becton Dickinson, San Jose, USA). An argon ion laser at 488 nm was used for excitation, and fluorescence emission was measured at 670 nm (FL-3 detector).

RESULTS AND DISCUSSION

Poor aqueous solubility of temoporfin demands specific formulation approach to acchieve assocciation of photosensitizer with polimeric matrix and its delivery to the tumor site. The result of the modified nanoprecipitation method were sferical polymeric nanoparticles loaded with temoporfin. Average particle size of PLGA nanoparticles was around 179,0 ± 0,3 nm and polydispersity index (PI) 0.27. Combination of PLGA polymer with PEG-PLGA caused decrease in mean particle size (144,7 ± 2,0 nm) and polidispersity (PI 0.13) as well. The particle size of both types of nanoparticles was small enough to enable their accumulation at the tumor site in vivo due to the enhanced permeability and retention effect, which is followed by nanoparticle internalization into tumor cells as reported by Maeda et al. (2000).

The surface charge of both nanoparticle formulations was negative, however, it was more negative for PLGA nanoparticles (- 5.5 ± 0,4 mV) compared to PEG-PLGA nanoparticles (-2.5 ± 1,1 mV). The results indicate the presence of higher number of the free carboxylic end groups on the PLGA nanoparticle surface compared to PEG-PLGA nanoparticles, which have on the surface additionally covered with uncharged PEG chains. Low surface charge may be the reason for limited nanoparticle stability, aggregation and sedimentation after contact with cell culture medium containing FBS. However, it is known that PEGylation prolongs the retention time in the blood stream, since the nanoparticle surface becomes unrecognizable for the mononuclear phagocyte system, resulting in higher accumulation of stealth nanosystem at tumor site (Owens and Peppas 2006).

Temoporfin loading was comparable for both types of nanoparticles (7%, w/w), therefore, it can be concluded that polymer composition did not affect association of drug with polymeric matrix. Temoporfin was non-covalently entrapped inside the polymeric matrix and partially bound to the nanoparticle surface as well. The results presented in Fig. 1 indicate distinctive initial burst release for PEG-PLGA nanoparticles, followed by sustained temoporfin release. The burst release was more pronounced in case of PEG-PLGA nanoparticles, which were smaller and had therefore bigger surface area per mass unit available for surface adsorption of temoporfin. Temoporfin entrapped in PLGA matrix was suggested to form aggregates, which dissociate into monomeric form after release from nanoparticles and transfer to plasma proteins (Konan-Kouakou et al. 2005). Monomeric form is necessary for efficient PDT as published previously (Bezdetnaya et al. 1996). Therefore, it is expect that when delivered to the site of action, temoporfin is monomerized and thus present in a form, which is preferable for effective PDT.

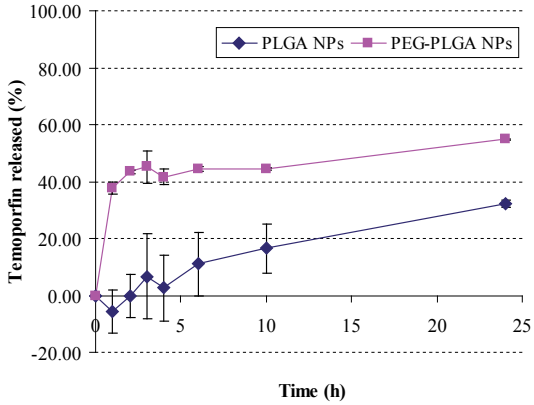

Figure 1: Release of temoporfin from PEGylated and non-PEGylated PLGA nanoparticles in aqueous medium supplemented with 10% FBS.

The amount of internalized nanoparticles correlated well with duration of cell incubation with nanoparticles (Fig. 2). On the other hand, nanoparticle surface properties significantly affected the cellular uptake of temoporfin-loaded nanoparticles. The ratio between the mean fluorescence intensity for PLGA and PEG-PLGA nanoparticles clearly showed a reduced internalization of PEG-PLGA compared to the PLGA nanoparticles in both tested cell lines after 4 and 24 h incubation, confirming stealthy properties of PEGylated nanoparticles.

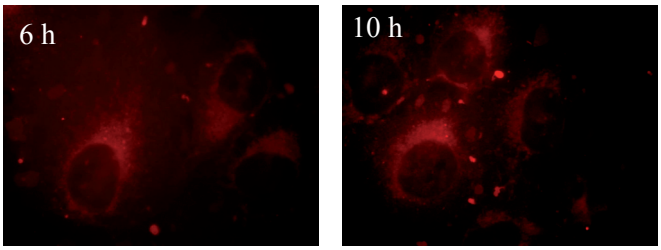

Figure 2: MCF 10A neoT cells with internalized temoporfin loaded PEG-PLGA nanoparticles after 6 and 10 h incubation.

CONCLUSION

In this research biodegradable PLGA nanoparticles loaded with photosenzitizer temoporfin were formulated. In vitro studies revealed that the PEGylated nanosystem poses promising properties for application in PDT (particle size, surface properties, temoporfin release profile), however, further in vitro as well as in vivo studies are needed to confirm its efficacy in PDT.

AKNOWLEDGEMENTS

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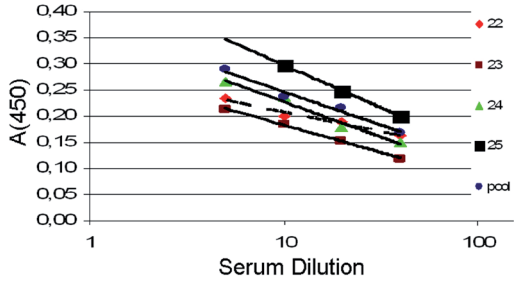
ANTI DRUG ANTIBODIES (ADAS) AS PREDICTIVE MARKERS OF COMPLEMENT ACTIVATION-RELATED PSEUDOALLERGY

Gergely Tibor Kozma

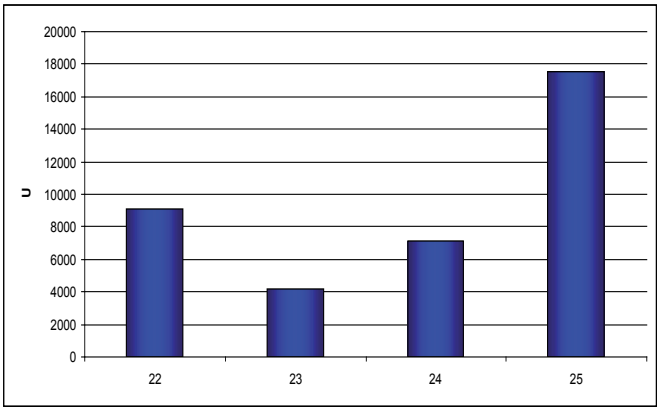
Colloidical structures used for delivery and/or targeting drugs often cause hypersensitivity reactions (HSRs) in man accompanying by the activation of the complement system. The frequency and the severity of HSRs is different, depend on size, charge and molecular content of the particle; but there are no methods, which could provide an effective individual prediction. Nowadays the only way to prevent fatal reactions is the pre-medication (with steroids) or medical alert during the first administration. Complement activation is initiated by pattern recognizing proteins and/or antigen specific antibodies (IgM and IgG) leading to instant systemic hyperreactivity caused by toxic protein products such as anaphylatoxins and membrane attacking complex (C5b-9). In our study we aimed to investigate whether drug specific antibodies (ADAs) in the serum against the whole colloidical structure could be suitable for individual prediction of HSR. As the first step we started to investigate IgG molecules produced by the organ against micellar Paclitaxel.

To determine the concentration of IgG specific for Paclitaxel in the serum, Enzyme Linked Immunoassay (ELISA) was used. Briefly, Paclitaxel was attached on the surface of an ELISA plate; after blocking the nonspecific binding sites and washing the unbound materials, serially diluted sera were added; during incubation, ADAs were bind to the attached Paíclitaxel layer; removing the non-reacting part of the serum, bound IgG molecules were labeled with Peroxidase (HRP) conjugated anti human IgG antibodies; after the unbound conjugate was washed, color reaction was developed by Tetramethyl benzidine (TMB); the reaction was stopped with sulfuric acid and the absorbance was identified by reading at 450 nm for each test well. A pool of the investigated sera was used for quantification. Sera were obtained from patients, before Paclitaxel treatment. During medication the status of the patients were observed by a doctor. To monitor the level of S-protein (vitronectin) associated terminal complement complex (SC5b-9), a marker of the complement activation, blood was drawn several times during Paclitaxel infusion and concentration of SC5b-9 was determined by commercial ELISA (Quidel Corporation).

Plotting the absorbance values (A(450)) against the dilution linear regression could be applied with similar slope, excepted patient ID22. This observation could be explained by the similar antibody affinity in the blood of patient ID23-25 to Paclitaxel, but a decrease affinity in patient ID22. A450 values of the pooled serum were between the points originated from the individual sera.



In the (concentrated) serum pool, ADA concentration was defined as 10 000 U. Using this pool, the relative concentration of Paclitexel ADA was also calculated in every sera at the highest dilution (40-fold), where the matrix effect could be the smaller:



The relatively highest ADA concentration was detected in the serum of patient ID25, while serum from ID23 rendered with the smallest one. Comparing the calculated relative ADA concentration with the observed clinical symptoms and the SC5b-9 elevation on the peak reaction, a strong correlation could be found:

Patient ID	Clinical symptom	Fold elevation of SC5b-9 concentration in the serum
22	Increased blood pressure from 30 to 60 minutes of the infusion.	1,0
23	Blood pressure is increased during the whole period of the infusion.	1,1
24	Dinus from 30 to 140 minutes of the infusion, patient feel warm. Drop number was decreased.	1,3
25	Increased blood pressure from 30 to 95 minutes of the infusion. Patient feel sick and has headache.	2,9

The highest elevation in the SC5b-9 during the treatment was observed in the serum of patient ID25 with relatively sever clinical symptoms. Patients ID22-23 showed hardly anything sign of HSR.

Our results suggest that the individual identification of relative ADA concentration could be a useful tool to predict HSR. To increase the power of our observations, further studies are needed involving increased number of patients and different type of colloidical materials.

INTRA- AND INTERCELLULAR TRAFFICKING OF SOLID LIPID NANOPARTICLES

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There are still many open questions concerning the intracellular fate of nanosized drug delivery systems. In our approach we have used a biodegradable and biocompatible nanosized system – solid lipid nanoparticles (SLN) – fluorescently labeled with a newly synthesized probe, SPP-189. We observed the dynamic intracellular movement of SLN and their final positioning. Detailed evaluation of their location showed that a few SLN could be found in mitochondria and actin fibers but none could be found in the nuclei or lysosomes. Interestingly, for the first time, transport of NPs between cells through tunneling nanotubes (TNT) has been observed. Moreover, cells treated with SLN exhibit many more TNT, demonstrated by greater roughness of treated than of control cells.

These results of the detailed intracellular location of SLN and the proof of SLN inter-cell transfer over TNT are important for clinical therapy, where the main goal is to control the cellular movement of NPs and to achieve specific intracellular location. The newly discovered intercellular trafficking of SLN over TNT can be particularly important for treatment procedures. Once SLN are internalized, their

acceptance into neighboring cells is already guaranteed, which would enhance NP efficiency or toxic effect up to nanoparticles fate.

INTRODUCTION

The use of nanoparticle-based drug delivery systems is a rapidly developing area within nanotechnology. The advantages are high stability, high carrier capacity, low to non-existent carrier toxicity, incorporation of both lipophilic and hydrophilic drugs, increased stability of loaded drug, possibility of controlled drug release and drug targeting, feasibility of variable routes of administration, etc. (1). Solid lipid nanoparticles (SLN) constitute a very promising part of nanocarriers. While much is known about the preparation and sophisticated structure of SLNs', we know less about their internalization, trafficking and final position in the cells (2). For this reason we investigated the fate of SLN in the cellular environment.

FLUORESCENTLY LABELED SOLID LIPID NANOPARTICLES (SLN). Using the melt-emulsification process (3) we prepared SLN with a lipid core of behenate and phospholipid, and poloxamer acted as a surface active polymer. A newly synthesized fluorescent probe, SPP-189, is used to visualize SLN by fluorescence microscopy. It has a lipophilic anchor and was therefore proposed to be well integrated into SLN (Fig. 1). They are seen as distinct, bright blue dots without high background (4).

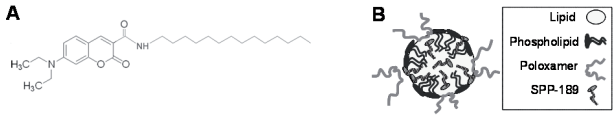

Figure 1: (A) The chemical structure of the coumarin-derived fluorescent dye, SPP-189. (B) Schematic presentation of SLN and integration of SPP-189 into the NP structure.

Atomic force microscopy (AFM), as a high-resolution microscopy technique, confirmed the NPs' size of approx. 120 nm, determined by photon correlation spectroscopy. NPs' dispersion included mostly spherical SLN of various sizes (Fig. 2) that were repulsed due to the high zeta potential.

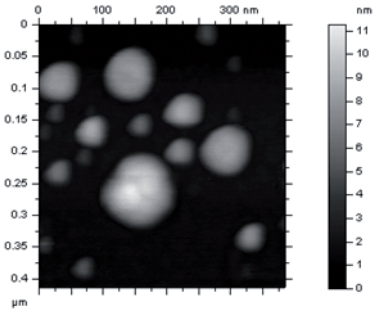

Figure 2: AFM topography picture of SLN showing their size, heterogeneity and shape.

INTRACELLULAR LOCALIZATION OF SLN. Live cell imaging on human keratinocytes NCTC2544 (ICLC, University of Genova, Italy) shows rapid internalization of SLN that were then trafficked rapidly to distinct compartments within the cell. The majority of SLN move from the cell periphery towards the nucleus (data not shown).

The intracellular distribution of SLN was studied qualitatively using a co-localization technique, and quantitatively using fluorescence intensity profiles (Fig. 3). In co-localization we were looking for pink fluorescence in the over-lay of the blue fluorescence (SLN) with red emitting dyes, which were used for staining particular organelles (nuclei, actin, mitochondria and lysosomes) that play important roles in cell functionality. Fluorescence intensity profiles were prepared from fluorescence pictures, enabling the positioning of NPs to be determined precisely relative to specific organelles.

Majority of SLN were located perinuclearly (Fig. 3). Next, some NPs were integrated among actin filaments, indicating transfer of NPs intracellularly or extracellularly. A few SLN were captured also in mitochondria. Finally, SLN appeared not to find their way to lysosomes.

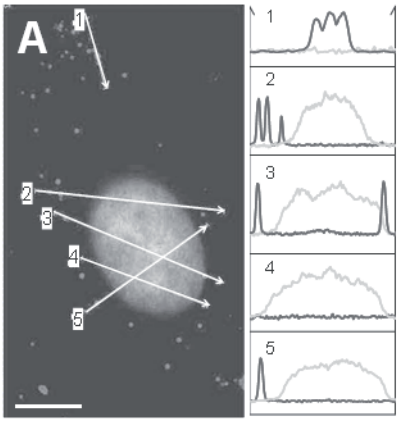

Figure 3: Localization of SLN, seen as (blue) dots, in the cell, stained for (red) nuclei. Graphs represent relative fluorescence intensity of NPs (dark (blue) curve) and nuclei (bright (red) curve) regarding the arrows marked on the fluorescence picture to the left. Profiles 2-5 are showing perinuclearly localization of SLN. Bar is 10 µm.

INTERCELLULAR TRANSFERRING OF SLN OVER TUNNELING NANOTUBES. SLN were for the first time found in tunneling nanotubes (TNT) (Fig. 4). TNT are formed de novo between cells and appear to be the general mode of cell-cell communication. They can mediate the intercell transfer of endosome related organelles, lipid anchored proteins, calcium flux, surface receptors, mitochondria, soluble proteins, lysosomes, etc. (5). Thus TNT provide a very sophisticated way of SLN transfer between cells. In an in vivo system (e.g. for tissue), neighboring cells could easily be accessed by moving SLN over TNT, which would enhance the efficiency of a particular colloidal system.

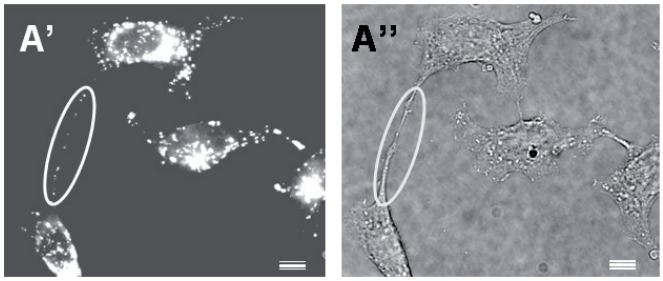

Figure 4: Transport of SLN (blue dots) between cells via tunneling nanotubes. (A') Cells were stained for lysosomes (red fluorescence). (A'') the corresponding trans-mission picture, showing the border of the cells. The yellow circle marks a tunneling nanotube with SLN inside. Bar is 10 µm.

Using AFM, more TNT were seen between cells treated with SLN, than between control cells (Fig. 5). This was confirmed by roughness analysis, which showed that treated cells are – due to their multiple outgrowths – rougher (the average roughness (Ra) is 19.3 and the root mean square roughness (Rq) is 24.7) than control cells (Ra = 14.5 and Rq = 17.8). The presence of SLN appears to trigger increase of TNT, thus enabling SLN to be transported between cells.

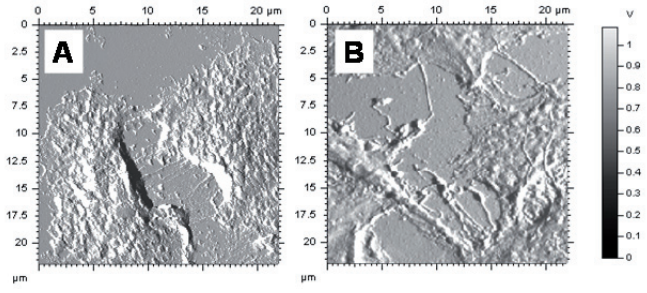

Figure 5: AFM imaging of cells untreated (A) or treated with SLN (B). The latter show more tunneling nanotubes than the control.

CONCLUSION

The novel fate of SLN in cells has been elucidated in vitro, casting new light on mechanisms of drug intracellular delivery by NPs. This should help customize treatment procedures with respect to the routes of SLN intra- and intercellular trafficking.

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INVESTIGATION OF QUANTUM DOTS DISTRIBUTION PATHWAYS IN MICE

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Nanoparticles (NP) penetration through the biological barriers of the tissues is an important issue of nanotoxicology as the field of NP applications is expanding and the number of commercial products in the market is increasing. On the other hand, comprehensive knowledge on NP distribution in organism is required for the development of NP based drug delivery systems. Quantum dots (QDs) are semiconductor nanoparticles, emerging as alternative bioprobes. The exceptional physicochemical properties represent QDs as a perfect model for the NP distribution in vivo investigations.

The aim of our study was to investigate tissue localization and migration patterns of subcutaneously injected QDs by the means of fluorescence spectroscopy, histological analysis and fluorescence microscopy.

The whole body imaging indicates that QDs were drained with the interstitial fluid via the lymph vessels into regional lymph nodes (LN). The findings show that NP are not fully retained in the regional LN and may be passed by the drainage system to the further LN and later on, possibly, up to the superior vena cava entering the blood circulation system.The tissue analysis revealed that QDs are able to penetrate locally through dermis and connective tissue between the muscle fibers. However, the NPs were prevented from passing to the skin structures, which are outlined by the epidermis basement membrane.

This study shows that three processes determine in vivo distribution of subcutaneously injected QDs a): local QDs penetration through intracellular space in tissues, b) QDs migration via lymphatic system, and c) systemic distribution with blood circulation.

DESIRED CHARACTERISTICS OF SILICA-COATED QUANTUM DOT SQUARE (QD2) FOR IN VIVO IMAGING APPLICATION

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The usability of newly-developed optical nanomaterials for in vivo imaging application depend upon their biodistribution characteristics including urinary excretion and/or possible blood absorption of emission spectra of these optical nanomaterials. In this work, we report that our 120 nm-sized highly-sensitive quantum dots embedded silica nanoparticles (QD^2s) are excreted via kidneys by in vivo microPET study using 68Ga-labeled QD^2 and report the absorption phenomena of fluorescence-emitting QD^2 by hemoglobin in blood due to the overlapped wavelength between emission spectrum of QD^2 and absorption spectrum of the hemoglobin.

Though easy renal excretion of small-sized particle (ca 3-6 nm) was proven in various in vivo biodistribution studies using different sized quantum dots (QDs) as well as in MRI studies using different sized magnetic contrast agents (1,2), the cylindrical and fibrilous shape of single-walled carbon nanotubes having average length of 300-1000 nm showed effective urinary excretion within a few hours (3). Recently silica-doped QD (QD605) was also reported to be cleared via kidneys with lower liver uptake (4).

QD2 was produced to contain 500 single QDs doped with silica shell. Amine-functionalized QD2 was coupled with NOTA-NCS chelator and the resulting QD2-NOTA was labeled with 68GaCl3. On biodistribution study using microPET, 68Ga-labeled QD2 showed urinary excretion 2 minutes after intravenous injection of 68Ga-labeled QD2, with significant radioactivity in the liver, kidneys, and bladder. The urinary excretion of QD2 was proven by transmission electron microscopy (TEM) of urine specimen, and QD2 degradation products were not found in the urine.

QD2 in the mouse blood was found to be absorbed by red blood cells (RBC) but not by plasma upon the mixture study by individual blood components. RBC concentration was the major factor of photon absorption on further serial dilution study (Figure 1). Fluorescence of commercially available single QD was also similarly absorbed. QD2s were absorbed by RBC lysates by concentration-dependent manner.

In this investigation to test in vivo applicability of the newly-developed QD2, microPET/TEM images revealed that these relatively large-sized QD2 passed through kidneys, but that this fluorescent QD2 was absorbed proportionally to the concentration of RBC lysates. In vivo distribution characteristics including urinary excretion of these new 120 nm-sized QD2 endowed the advantage of making in vivo contrast suitable for in vivo imaging. However, the absorption characteristics of these nanoparticles by blood/hemoglobin compromised severely the sensitivity. This expected disadvantage of QD2 implied that we need to evaluate absorption characteristics of every new optical nanomaterial as well as the previously-reported optical nanomaterials implied for in vivo use. This is the case especially when the imaging target tissues contain fair amount of blood as blood was going to absorb the emitting light of those optical nanoparticles.

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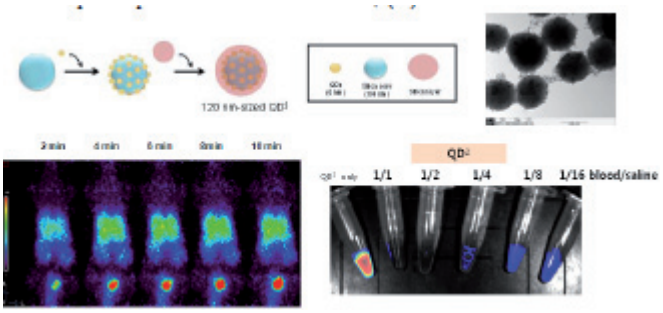

Figure 1. In vivo behavior of 68Ga-labeled QD2, and fluorescence absorption of QD2 in mouse blood. QD2 (120 nm) consists of numerous single QDs embedded on the surface of silica nanoparticle core, and the silica shell layer. TEM showed intact QD2 in the urine specimen. On microPET study, in vivo 68Ga radioactivity of 68Ga-labeled QD2 was detected in the kidneys and bladder 2 minutes after intravenous injection into mouse. The fluorescence of QD2 was significantly absorbed in diluted RBC/lysate solutions on concentration-dependent manner.

THE MECHANISM OF P-GLYCOPROTEIN INHIBITION - INSIGHTS GAINED WITH DETERGENTS

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Biozentrum of the University of Basel

The efflux transporter P-glycoprotein (ABCB1, MDR1) strongly contributes to multidrug resistance which is a major problem in several therapeutic areas and is moreover responsible for drug-drug interactions. Detergents have long been used as excipients in drug formulations however, their mode of interaction has been understood only recently. We demonstrated that many detergents directly bind to the efflux transporter P-glycoprotein. Binding of substrates to the transporter occurs in two steps a lipid-water partitioning step and a transporter binding step which takes place in the lipid membrane. Partitioning into the lipid membrane is based on hydrophobic interactions while binding to the transporter in the lipid membrane is due exclusively to hydrogen bond formation between the hydrogen bond acceptor groups of the detergents and the transmembrane domain of the transporter. We quantified the interaction of several detergents with P-glycoprotein and demonstrated that the affinity to the transporter increases with the number of hydrogen bond acceptors in the polar end of the detergent. Depending on their affinity to the transporter and the concentration applied detergents modulate or even inhibit the interaction of drugs with P-glycoprotein (1). We present the tools for a simple a priori estimate of the membrane and P-glycoprotein binding ability of non-charged detergents based on a modular binding approach (2).

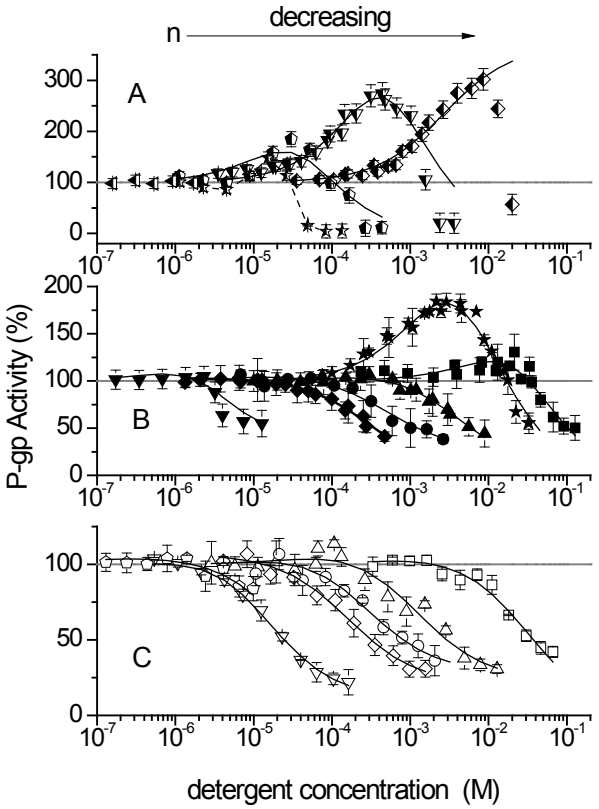

Fig.1A, B&C. P-gp ATPase avity measured as a function of concentration in plasma membrane vesicles of NIHMDR1- G185 cells for n-alkyl-trimethylammonium chloride (A), for n-alkyl-β-D-glucopyranoside (B) and for n-alkyl-β-D-maltoside (C) at pH 7.0 and T = 37 °C.

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MEMBRANE BINDING OF ßAP(25-35)

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Amyloid peptide aggregation leads to plaque formation1,2, characteristic of more than 15 neurodegenerative diseases. The smaller oligomers are known to be the most toxic species to neuron cells3,4 however their interaction with cellular membrane is poorly understood. Biophysical studies with model membranes can provide insight into the peptide-membrane interaction with consequences for both peptide and membrane structure. βAP(25-35) is often used as a model peptide for the full-length amyloid β peptide, βAP(1-40), because the neurotoxic and neurotrophic properties and also the aggregation behavior of these two peptides are similar. The main goal of our work was the description of the amyloid fragment βAP(25-35) binding to a model lipid membrane.

Peptide secondary structural changes upon binding to lipid can be followed with circular dichroism. βAP(25-35) random-coil to β-sheet structural transition at pH 4.0 was first reported in 1994 and is known to be concentration-dependent. The addition of lipid vesicles was shown to shift the structural equilibrium of a 50 μM βAP(25-35) solution towards β-sheet secondary structure[5], at a peptide-to-lipid ratio of ~ 1/30. However our studies showed that the lipid membrane plays an opposite role at lower peptide concentrations and a peptide-to-lipid ratio of ~ 1/120. At 10 μM, the random-coil content of βAP(25-35) in buffer is increased upon binding to lipid vesicles.

This seems to indicate the presence of a threshold in peptide aggregation and further fibrillization defined by the peptide-to-lipid ratio.

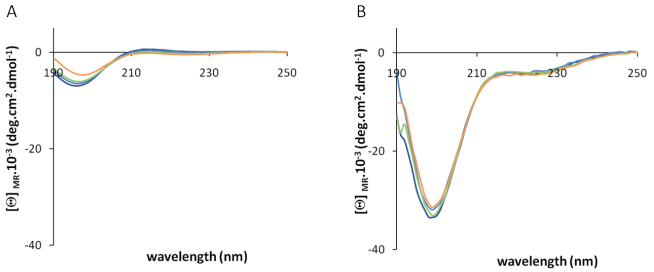

Figure 1: Influence of the temperature on the secondary structure of βAP(25-35) in solution or bound to the lipid membrane in 10 mM AcONa buffer at pH 4.0. HFIP treated βAP(25-35) (A) in solution and (B) bound to lipid. POPC/POPG SUVs (3:1, mol/mol)were added to a peptide solution (~ 20 µM) at a 1/120 ratio. The scans were made at 10 (___), 20 (___), 30 (___), and 40 °C (___).

The thermodynamic parameters such as enthalpy, entropy and free energy were measured at pH 4.0 with isothermal titration calorimetry, a direct high-sensitivity technique which does not require any labeling of the molecules. The intrinsic binding constants were rather low and vary between 10 M-1 at 30°C and 12 M-1 at 20°C. The free energy of binding ΔG0 is stable in the temperature range studied and has a value of ΔG0 = -3.7 kcal.mol-1.

Table 1: Thermodynamic parameters for the binding of βAP(25-35) to SUVs composed of POPC/POPG (3:1, mol/mol) at various temperatures and pH 4.0. The data result from the average of two experiments and the standard deviation is indicated for the enthalpy and binding constant. The number of effective charges, z, is also indicated.

	T (°C)	ΔH0 (kcal/mol)	K0 (M-1)	ΔG0 (kcal/mol)	TΔS0 (kcal/mol)	z /
βAP(25-35)	10	-1.42 ± 0.23	12 ± 0	-3.7	2.2	2
	20	-1.37 ± 0.13	11 ± 0	-3.7	2.4	2
	30	-1.36 ± 0.53	9.8 ± 0.4	-3.8	2.4	2

Eventually we studied the effect of the insertion of βAP(25-35) on the lipid membrane structure by means of solid-state NMR at high peptide-to-lipid ratio (1/50). We demonstrated that the insertion of βAP(25-35) into the lipid membrane leaves the bilayer structure intact. However we evidenced an increase in the fluidity of the lipid acyl chains that has not been observed in the past.

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TARGETED DELIVERY TO, AND IMAGING OF, WHITE ADIPOSE TISSUE USING PEPTIDE-FUNCTIONALIZED GOLD NANOPARTICLES AND QUANTUM DOTS

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BACKGROUND

Nanotechnology involves the use of small particles at atomic and molecular scale, and has found applications in medical diagnostics, drug delivery and molecular imaging. Gold nanoparticles (GNPs) and quantum dots (QDs) are extensively used as drug delivery, labelling and imaging tools in biomedical research. Obesity is a worldwide epidemic affecting millions of people, and its pharmacological management is hampered by drug toxicity and undesirable side effects. Therefore, a need still exists for the development of safe medication for the treatment of obesity. Nanotechnology offers a new potentially useful avenue for solving the problem of toxicity of anti-obesity drugs, through targeted drug delivery. The system involves designing nanoparticles that have a combination of a targeting molecule to deliver either a therapeutic payload to adipose tissue-associated cells, or fluorescent nanocrystals to image the adipose tissue.This study reports the use of peptide-functionalised GNPs and QDs as delivery and imaging agents to the white adipose tissue vasculature.

METHODOLOGY

GNPs and QDs were functionalized with an adipose tissue-specific peptide (CKGGRAKDC) and polyethylene glycol by streptavidin-biotin chemistry. The specificity of the ligand was evaluate in vitro using isolated microvascular endothelial cells and CHO or MCF7 cells, and in vivo using a rat model of diet-induced obesity. In vivo: Male Wistar rats were made obese by feeding a diet high in fat and sugar, and the control group was fed normal rat chow diet. Rats were housed under controlled temperature, humidity, ventilation and 12-hour photoperiod. Rats were injected iv with peptide functionalized-QDs (Chow-QDs, HF-QDs) or GNPs (chow-GNPs, HF-GNPs) and the other with saline (Chow-PBS, HF-PBS). Rats were terminated by exsanguination under anaesthesia. Organs were dissected out, weighed and analysed by Xenogen IVIS imaging system and inductively coupled plasma mass spectrometry.

RESULTS

Peptide-functionalized nanoparticles bound specifically to microvascular adipose tissue endothelial cells. The GNPs and QDs bound only to endothelial cells derived from inguinal and epididymal adipose tissue, and to prohibitin-expressing CHO/MCF7 cells, and not to other cells. Cellular uptake was confirmed by fluorescence microscopy and flow cytometry. The mechanism for the intracellular uptake was confirmed by competition of receptor binding sites by excess FITC-conjugated peptide, the blocking of receptors by an anti-prohibitin antibody and low temperature (4 °C) experiments. Binding studies suggested that receptor-mediated endocytosis largely contributed to the uptake of nanoparticles. In vivo studies indicated that peptide-functionalized nanoparticles localized specifically the adipose tissue. Non-functionalized nanoparticles accumulated in the spleen and liver (RES mediated uptake).

CONCLUSION

This study shows that the selective delivery of peptide-functionalized QDs and GNPs to endothelial cells in adipose tissue represents a potential approach for targeted drug delivery systems for targeting the vasculature in adipose tissue.

BIOENGINEERING A VERSATILE MAGNETIC NANOPROBE TO SITE-SPECIFIC LABELING OF ANTIBODIES FOR TARGETED DETECTION OF TUMOR CELLS

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Magnetic resonance imaging (MRI) is the gold-standard technique in noninvasive clinical imaging for prevention and treatment of malignant diseases. However, MRI potential for accurate detection and monitoring of malignancies at their early stages of development was dramatic affected by its low sensitivity. Therefore, great efforts have been made to develop efficient target-oriented contrast agents aimed at improving the signal difference between the target-area and the background. This drawback can be partially overcome by using magnetic nanoparticles based on iron oxide (MNPs), which have provided strong signal enhancement in T_2-weighted images.[1,2]

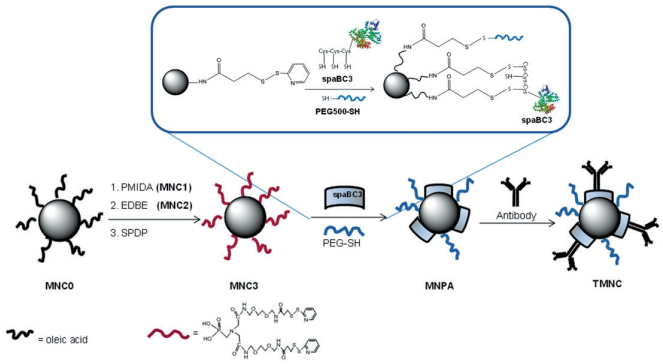
As magnetite nanoparticles (MNPs) display both magnetic properties and surface features suitable for direct conjugation of organic and biological molecules, they can be envisaged as a good candidate for use as contrast agent for MRI. In particular, many research groups have been involved in the development of reliable strategies for the conjugation of targeting biomolecules, especially monoclonal antibodies (IgGs), to MNPs.[3] These include passive/electrostatic physical adsorption of IgGs, tight immobilization exploiting the strong interaction of biological counterparts, such as biotin-streptavidin,[4] or the formation of covalent chemical connections, which is often considered the most practical choice.[3,5] Although these approaches have been successfully employed in several circumstances, they all share the same basic limitation, that is a non site-specific binding to IgG, which affects the targeting efficiency of the antibody. As a matter of fact, the actual conservation of the targeting bioactivity of immobilized IgGs remains a crucial issue, which must be addressed in designing a successful targeted nanoprobe.

We envisaged that the use of a natural peptide linker with high affinity for IgGs, such as protein A might be an interesting option. Protein A is a *Staphilococcus aureus* protein[6] consisting of a 42 kDa single polypeptide chain, folded into five highly homologous domains, named E, D, A, B and C.[7] The biotechnological interest for this molecule resides mainly in the stability of protein structure over a broad range of pH (2-12) and in presence of various detergents, reversible binding of a large variety of IgGs via their Fc fragment[7] and dissociation of IgG-protein A complex under controlled conditions (pH 3.5-4.5) without apparent loss of activity.[8] As protein A recognizes the Fc portion of IgGs, it is expected to mediate an orderly Fc site-specific antibody immobilization on MNPs resulting in a target-directed Fab presentation.[9]

Based on these advantageous features, we propose a multidisciplinary approach to the design and synthesis of a universal magnetic nanohybrid system. High-quality biocompatible magnetite nanocrystals (MNC) were obtained by phase transfer of oleate-coated iron oxides via successful ligand exchange with an iminodiacetic acid phosphonate exploiting the strong affinity of phosphonate for iron oxide. Such water-soluble nanocrystals exhibited a transverse relaxation rate of 255 mM-1 s-1, which is significantly higher than commercially available T2 contrast agents based on polymer-coated iron oxides.

A recombinant low-molecular-weight fragment of protein A modified to present a terminal cysteine tripod (spaBC3) was expressed in E. coli, purified and characterized in order to assess its IgG binding activity and the possibility of recycling after Na-citrate treatment.

Subsequently, spaBC3 was conjugated to MNPs via disulphide bridges formation through an appropriate bifunctional linker affording a molecular nanohybrid (MNPA) suitable for site-specific immobilization of antibodies (Scheme 1).

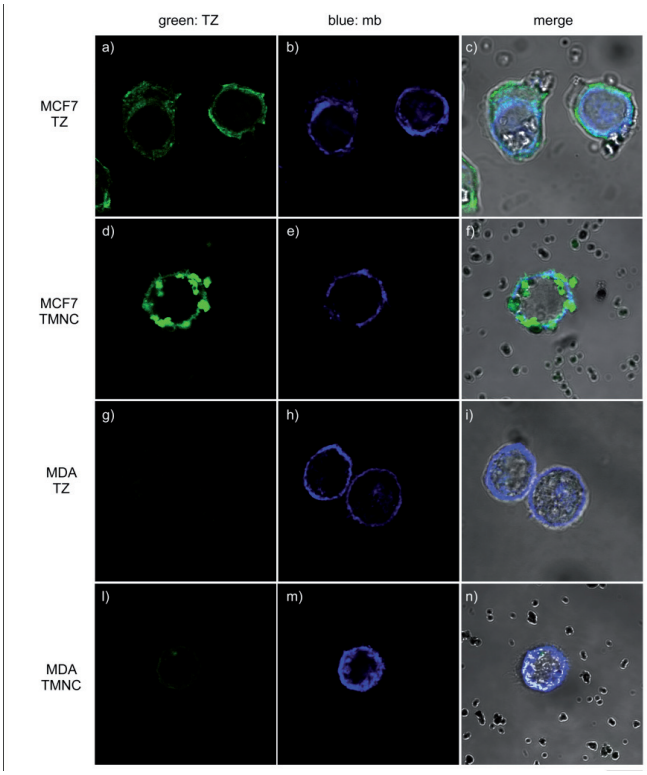


Then, MNPA were characterized in order to prove that spaBC3 was bound to MNC exploiting the tripod tail selectively via disulfide bridge avoiding nonspecific physical adsorption. Eventually, the antibody capture capacity and the recycling possibility of our hybrid nanoparticles were successfully tested with trastuzumab and rabbit IgGs.

As a case study for the development of our tumor-targeting nanoprobes, we focused on the humanized monoclonal antibody trastuzumab, which is commonly employed in clinical therapy because it can bind the HER-2 receptor, a membrane tumor marker overexpressed in several metastasizing breast cancer cells.10 In particular, trastuzumab-conjugated MNC were effective in immunoprecipitate HER-2 receptor expressed in MCF-7 breast cancer cells in a highly specific manner, as antibodies recognizing dynamin-related protein (Drp1) and Akt, two soluble cytosolic proteins, or calnexin (Clnx), an integral protein of the endoplasmic reticulum, which were tested as negative controls, displayed a detectable signal only in unbound sample. These results suggest that the interaction between TMNC and HER-2 is specific and related to antibody conjugation.

To validate the immunoprecipitation data, the specificity of binding between TMNC and HER-2 was observed by confocal laser scanning microscopy. Indeed, as HER-2 is a trans-membrane receptor, TMNC were expected to accumulate in correspondence of the external surface of HER-2-overexpressing cells. HER-2 positive MCF-7 cells11 (Figure 1a-c) and, as a negative control, HER-2 negative MDA cells (Figure 1g-i) were treated with tz in order to assess HER-2 expression and cellular surface distribution. As expected, after 15 min incubation, TMNC were observed in HER-2 positive MCF-7 cell surfaces (Figure 1d-f), but not in MDA negative control cells (Figure 1l-n), demonstrating that they localized selectively in correspondence of trans-membrane receptors owing to the presence of the specific targeting agent.

The model nanoparticle system (MNPA) presented here enables efficient and reversible labeling of a large variety of human monoclonal antibodies with highly conserved biological activity towards their natural receptors. The combination of all these properties renders our newly developed MNPA a promising versatile nanoscale probe for application in targeted diagnosis of tumor cells and tissues.



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EFFECTS OF MESENCHIMAL STEM CELLS EXPOSURE TO SINGLE WALL CARBON NANOTUBES INVOLVE OXIDATIVE STRESS MECHANISMS

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INTRODUCTION

Single wall carbon nanotubes(SWCNT) represent a material of high interest, recently, due to many biomedical application that have been imagined. Recent in vitro studies have demonstrated increased oxygen free radical production after cells exposure to carbon nanotubes. However, exposure effects on mesenchimal stem cells and the implication of oxidative stress mechanisms in SWCNT toxicity is still unclear.

MATERIAL AND METHOD

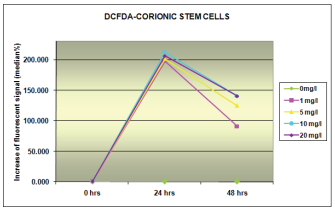
High purity carbon nanotubes were functionalized with single strand DNA through sonication(7 hrs). Estimation of concentration was performed (250 mg/l ss-DNA-SWCNT water solution).

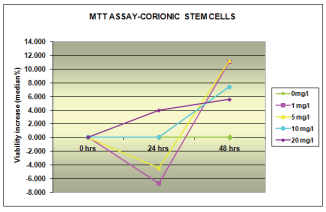
Addition of NaCl was performed up to the level of 0.9%. Consecutive dilutions were made to obtain 1, 5, 10 and 20 mg/l concentrated solutions. Separate samples of amniotic membrane stem cell line were exposed to the above mentioned solutions. For each concentration a control sample was assigned. MTT assay was performed to assess proliferation. For 10, and 20mg/l SWCNT concentration 5-(and-6)-carboxy-2',7'-dichlorofluorescein diacetate (DCFDA) assay was performed.

Evaluation was performed at baseline, 24 and 48 hrs interval. Baseline proliferation values were considered as 100%. Dynamic proliferation curves were constructed and differences between curves were tested using non-parametric methods.

RESULTS

Stimulation of proliferation was detected for low concentrations (1mg/L, 5mg/L), while high concentrations (10mg/l, 20mg/l) induced decrease of proliferation rate. All effects were transitory. Proliferation effects were dose- dependant, with significance obtained between 10mg/l and 20mg/l and low concentrations (1mg/l and 5mg/l), respectively ($p<0.05$). High concentrations of SWCNT induced high levels of fluorescent signals at 24 and 48 hours, respectively, with peak values at 24 hours ($p<0.05$).





CONCLUSIONS

Different concentrations of CNT induce different proliferation results. Inhibitory proliferation effects are induced by high dose of exposure and invove oxidative stress mechanisms. Antioxidant supplementation may be necessary for drug- vehicle applications of SWCNT.

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IRON BASED MAGNETIC PARTICLES FOR MEDICAL APPLICATIONS

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According to Roadmaps in Nanomedicine towards 2020, Joint European Commission /ETP Nanomedicine, Expert Report 2009) the following challenges are important among others: new contrast agent is a crucial requirement, activated nanoparticles and new nanostructures to optimise relaxation signals. Crystalline nanoparticles can be used for therapeutic purposes or for diagnostic applications in combination with external devices such as MRI, Laser, Radiotherapy, CT Scan, Ultrasound, HF. The nanoparticles should possess no toxicity and have to be metabolized by the human body. New types of carriers for contrast agents are required such as magnetic nanoparticles, empty viruses or magnetic bacteria.

In our laboratory we can synthesize and precisely characterize several types of biocompatible magnetic nanoparticles: nanocrystalline iron with small amount of structural promoters: iron – carbon nanocomposites (carbon nanotubes and nanocapsules), nanoparticulate iron oxides, nanoparticulate zinc ferrite (ZnFe2O4)

Nanocrystalline iron is obtained by fusion of magnetite followed by reduction under hydrogen. The obtained material can be next used as a catalyst to obtain carbon nanomaterials through decomposition of hydrocarbons (cabon nanotubes and nanocapsules are the products) or can be submitted to a procedure of oxidation-reduction-etching to obtain narrower crystal size distribution.

Iron oxide and zinc ferrite are synthesized using traditional wet methods of precipitation or sol-gel method or microwave assisted hydrothermal synthesis.

All samples are characterized concerning their chemical composition, surface composition, specific surface area, phase composition, morphology and magnetic properties. The examples of pbtained results are shown on the poster.

POLYMERSOME NANOPARTICLES FOR TARGETED PROTEIN DELIVERY TO NEURONS

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The treatment of neurodegenerative diseases is hampered by the current strategies used for drug delivery to neurons and the central nervous system. Neurodegenerative diseases are not easy to replicate through the use of in vitro models.. In this work we aim to use targeted nanoparticles to circumvent these obstacles and deliver protein directly to neurons to replicate aspects of pathology.

Alzheimer's, Parkinson's and Huntington's disease, have common cellular and molecular mechanisms including protein aggregation and inclusion body formation. Indicating that defects in protein processing may be linked to their development, progression and pathogenesis [1-2]. The identification of genetic mutations involved in neurodegenerative diseases has facilitated the establishment of in vivo and in vitro models of these disorders [3]. However the mechanisms linking cell dysfunction and death to excess protein aggregation and inclusion bodies remain unresolved, Thus misfolded and aggregated disease proteins may prove to be effective therapeutic targets. Engineered proteins delivered to neurons have been used as neuroprotectors and to block aberrant protein expression [4-5]. However, delivery of these proteins across the membrane of neurons is difficult. Traditional means of transfection, protein transduction and macromolecular delivery have proven to be difficult in neurons due to their terminally differentiated state [6]. Liposomes or charged lipid formulations have been used, but these have limited complex stability in serum and often result in toxicity [7]. Electroporation can yield high rates of transfection, but this is only effective when performed in young undifferentiated cells [8]. Viral vectors have shown limited efficacy in humans and animals. They also typically require invasive procedures such as direct injection into the brain to achieve targeted delivery[9]. We are interested in using polymersome nanoparticles as means to deliver different payloads to neurons.

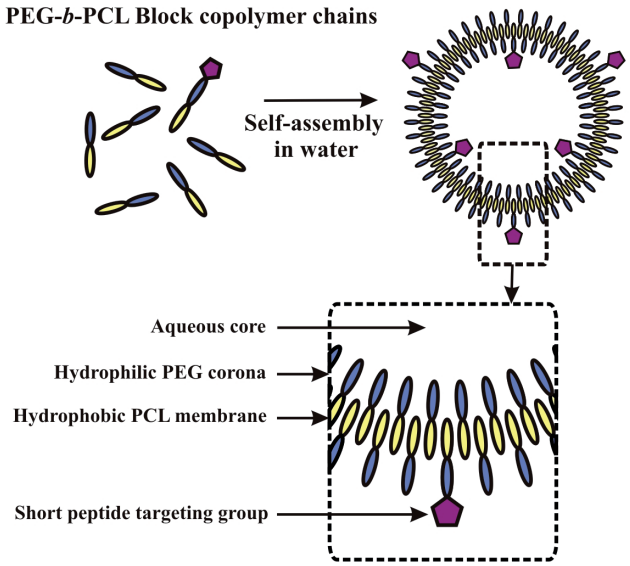

Figure 1. PEG-*b*-PCL block copolymers self assemble in an aqueous environment to form polymersomes.

Polymersomes are vesicle-like nanoparticles [10-11], that have the potential to be drug delivery vehicles.. They are produced from non-toxic amphiphilic block copolymers. Preparation is by slow addition of a block-copolymer solution in a water miscible organic solvent to water. Upon addition the polymer chains self-assemble, the hydrophobic units of the polymer form a spherical membrane encapsulating an aqueous core and the hydrophilic chains form a surrounding corona as well as line the aqueous interior cavity (Figure 1). Low molecular weight hydrophobic molecules can be incorporated into the membrane [12] or hydrophilic molecules encapsulated in the aqueous core [13]. Specific targeting to neurons can be achieved by functionalizing the polymersome surface with short peptide sequences that interact with cell surface receptors [14]. We have found that the polymersomes show excellent stability over several weeks. The polymersomes have been shown to be non-toxic in several in vitro and in-vivo studies.

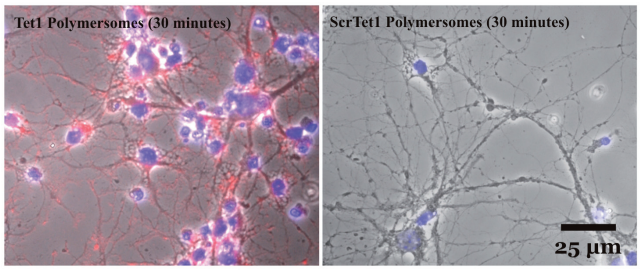

Figure 2. We observed rapid uptake of polymersome nanoparticles (red) functionalized with the Tet1 peptide in cultures of primary hippocampal neurons at time points as short as 30 minutes. Control polymersomes functionalized with a scrambled Tet1 sequence (ScrTet1) showed negligible uptake.

Recombinant tetanus toxin has been shown to be internalized by motor neurons and to undergo rapid retrograde transport, it binds to ganglioside receptor GT1b [15]. Liu et al [16] found a 13 amino acid peptide (Tet1) which competes with tetanus toxin for binding to the same receptor. We have attached Tet1 peptide to the surface of our polymersome nanoparticles, and have seen that at time points as short as 30 minutes after incubation it greatly increases the rate at which the NPs enter cultures of primary neurons. As a control a scrambled Tet1 peptide sequence was also attached, but negligible uptake was observed (Figure 2).

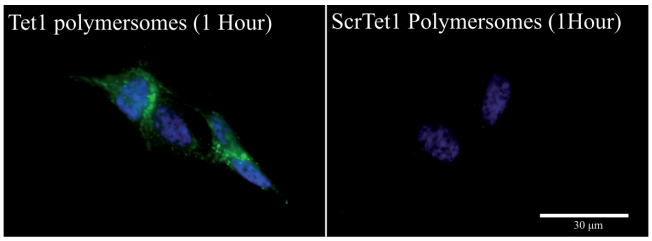

Figure 3. FITC-BSA (green) was transported into SH-SY5Y cells when loaded onto polymersomes functionalized with Tet1 peptide. Polymersomes functionalized with a scrambled Tet1 sequence (ScrTet1) did not transport the FITC-BSA.

SH-SY5Y human neuroblastoma cells treated with retinoic acid differentiate, and acquire neuronal markers and morphological, neurochemical and electrophysiological aspects of neurons [17-20]. We have shown a biological consequence of therapeutic agent delivered by polymersomes functionalized with the Tet1 peptide. In the future we are interested in using the polymersomes to deliver payloads of functional proteins such as enzymes. As a model protein we used bovine serum albumin (BSA) tagged with fluorescein isothiocyante (FITC). We loaded the FITC-BSA onto the polymersomes and observed that the Tet1 functionalized polymersomes were capable of transporting the protein into the SH-SY5Y cells. Fluorescence microscopy results and analysis of protein extracts by western blot revealed successful delivery of BSA-FITC using Tet1 functionalized nanoparticles. We observed cellular uptake of Tet1 functionalized nanoparticles in over a 24 hours period.

Negligible FITC-BSA was observed in cells incubated with free FITC-BSA, or FITC-BSA loaded onto control polymersomes functionalized with the ScrTet1 peptide (Figure 3).

Our results demonstrate that Tet1 functionalized nanoparticles can deliver a protein payload to neurons. These experiments indicate that block co-polymer nanoparticles may offer an effective strategy for protein delivery to neurons and could be used to deliver drugs necessary for the treatment of several neurodegenerative diseases.

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PEG-PCL NANOPARTICLE TARGETING OF THE HUMAN ENDOMETRIUM; A STEP CLOSER TO IMPROVED THERAPY IN REPRODUCTIVE HEALTH.

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INTRODUCTION

Endometriosis is an extremely common gynaecological disorder characterised by deposits of endometrial tissue within the pelvic cavity (figure 1). It affects around 20-30% of women with infertility (Fauconnier and Chapron 2005) Despite its prevalence, advances in diagnosis and management of this disorder remain limited. Imaging techniques including transvaginal ultrasonagrophy (Alcazar, Laparte et al. 1997) and magnetic resonance imaging (RCOG, 2006) have limited sensitivity and specificity in the detection of endometriotic foci.

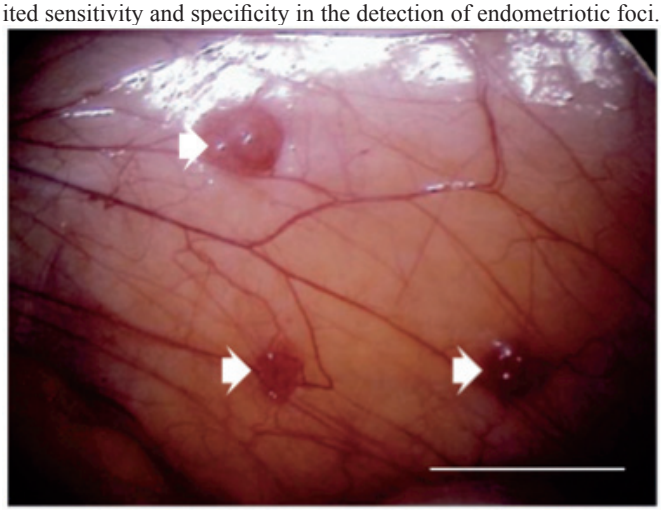

Figure 1. Endometriotic foci (arrows) in the pelvic peritoneum, laparoscopic image. (Scale bar 1cm).

Hormone therapy is the primary non-surgical intervention, this approach is associated with high disease recurrence once discontinued, and is contra-indicated for women considering future pregnancy. Laparoscopic excision of the lesion followed by histological confirmation remains the only reliable diagnostic and therapeutic option in a significant number of patients. However, because the early stages of endometriosis are often microscopic and hence not detected during surgery, the disease recurrence rate is high even after surgical therapy (Cheong, Tay et al. 2008) and there is ongoing concern about ovarian reserve in women who have laparoscopic excision (Wong, Gillman et al. 2004). The need for developing a non-surgical means of disease management and recurrence prevention is clear.

AIMS

The principle aim of this project was to ascertain the potential of polymeric nanoparticles (Johnston, Dalton et al. 2010), in enhancing the management of endometriosis. We aimed to investigate NP interaction with the endometrial surface epithelium/glandular epithelium characterised by Mucin-1 (MUC-1), a transmembrane protein expressed on most secretory epithelium. The effect of varying particle surface charge on levels of adherence and tissue selectivity was also explored.

METHODS

Endometrial tissue was obtained by pipelle biopsy from women of reproductive age (n=18). Patient characteristics and data regarding menstrual cycle were also obtained. NPs were PEG-b-PCL polymersomes labelled with fluorescein (FITC) and decorated with varying concentrations of TAT peptide (YGRKKRRQRRRA) to increase NP surface charge (0mg/mL, 0.5 mg/mL and 1.5 mg/mL). Freshly collected tissue was incubated in a NP suspension for 10 minutes. The tissue was then processed for histology. Consecutive cryostat sections were stained to reveal the gross morphology by light (Haematoxylin and Eosin) and fluorescence (4',6-diamidino-2-phenylindole-DAPI) microscopy. Double immunohistochemistry was used to determine the co-localisation of MUC-1 and the FITC labelled NPs.

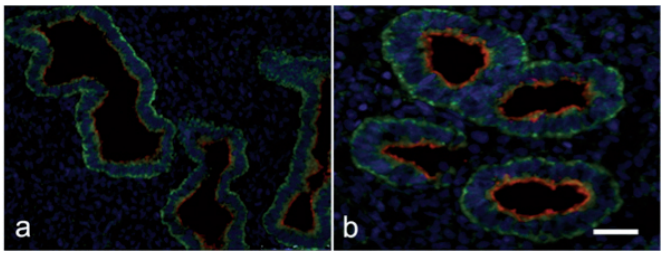

Figure 2a,b. MUC-1 expression on the luminal surface of endometrial glandular cells (red). NP uptake (green) into secretory glandular epithelium. MUC-1 and NPs co-localisation (orange). (Scale bar 50μμ, ΔΑΠΙ = βλυε)

RESULTS

MUC-1 was expressed on all secretory epithelial surfaces of the tissue. NPs lacking TAT were taken up into the single columnar epithelial cell layer surrounding the endometrial gland lumen only. MUC-1 and 0mg TAT NPs co-localised in some areas of this cell layer (figure 2). In contrast NPs carrying TAT were also visualised within the stromal tissue.

DISCUSSION

Medical treatment aimed at suppressing ovarian activity/menses and atrophy of endometriotic implants is often utilized, although the extent to which this is currently achievable is not clear (Hughes, Fedorkow et al. 2003). Current evidence that long-term hormonal treatment up to 24 months post-surgery results in an absence of improvement of symptoms indicates the need for new approaches to this management regime (Yap, Furness et al. 2010). Among post-surgical interventions that have been proposed in recent years, systemic hormone therapy with progesterone appears to be a potential method of reducing disease recurrence in endometriosis (Prentice, Deary et al. 2000). By specifically targeting glandular cells with a progestin-containing NP (Roy, Johnston et al. 2010) it may be possible that rates of recurrence could be decreased and treatment enhanced. NP driven progestin therapy may also be an effective approach to halting disease progression in stages I and II (mild forms with superficial disease) for young women with concerns about future fertility. This is the first study examining the interaction of nanoparticles with the endometrial glands. It highlights the potential of targeted nanoparticle therapy in endometriosis and other disorders where direct targeting of the endometrial glandular epithelium could be beneficial.

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A NEW TOOL FOR CONTROLLED SIRNA DELIVERY: POLYMER-PROTEIN CORE-SHELL HYBRIDS

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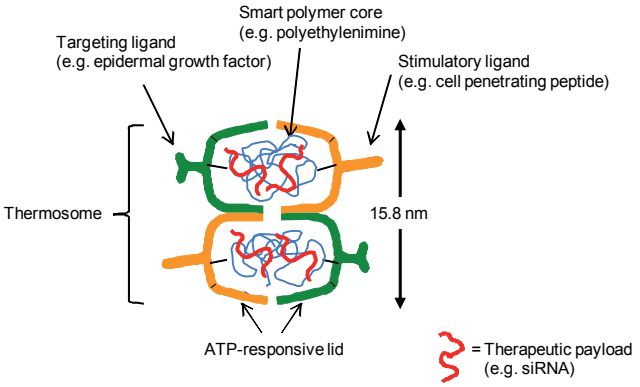
INTRODUCTION

Polymer nanoparticles and drug-polymer conjugates are highly important in modern drug delivery technology, as they can be tailored to fulfill several functions [1-3]. Polymers, particularly highly branched or dendrimeric polymers, are considered to be used as drug delivery vehicles. The advantages of such polymers are that they can bind or incorporate drugs, whereas their surface can be functionalized with targeting and/or reporting agents. Furthermore, the polymers can be designed to release their payload depending on the environmental conditions such as pH or temperature. Cationic polymers such as polyethyleneimine, poly-L-lysin and polyamidoamine (PAMAM) are able to form a complex with RNA or DNA [4]. PAMAM also can incorporate hydrophobic drugs, whereas the release is pH dependent [5]. A drawback of these cationic polymers is their toxicity to cells due to their positively charged surface. Several attempts to overcome this problem are based on the modification of their surface. But this reduces the ability of binding nucleic acids. Therefore, we incorporated PAMAM into the protein cage thermosome, a chaperonin from *Thermoplasma acidophilum*. The thermosome provides a closable cavity large enough to accommodate macromolecules such as PAMAM. Our concept is that PAMAM in the thermosome can bind small interfering RNA (siRNA), thus acting as an anchor to entrap oligonucleotides. The protein shell around a PAMAM-siRNA complex would protect encapsulated oligonucleotides against degradation and shields the positive charges of the polymer from the cells. The opening and closing of the thermosome cavity is ATP dependent, so it can be closed with ATP analoga.

We assume, that in a cell (high [ATP]) the cavity is opened and due to the low pH the payload is released. The surface of the thermosome could be modified with targeting and stimulatory ligands to create a controlled siRNA delivery tool (Figure 1).



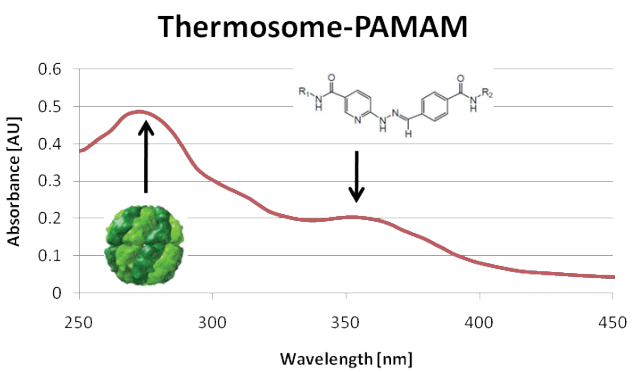
Concept of our polymer-protein core shell hybrid. The thermosome protects the polymer-therapeutic payload complex from degradation and shields the core from the surrounding media, which presumably will reduce toxicity of the drug-delivery system. Additionally, the modified thermosome directs the payload to its place of action.

RESULTS AND DISCUSSION

A bisarylhydrzone linker was used to obtain the polymer-protein core-shell hybrid using thermosome and PAMAM (generation 4). To this end, PAMAM was functionalized with a succinimidyl functionalized 6-hydrazinonicotinamide (S-HyNic) which reacts with primary amines of the PAMAM surface via succinimidyl chemistry. Thermosome was functionalized with maleimido trioxa-6-formyl benzamide (MTFB). An engineered thermosome was used that only possess accessible cysteins at the interior surface of the thermosome β-subunit [6], so that the MTFB attaches to the inner wall of the cavities. The purification of modified PAMAM and THS from excess linkers was carried out by extensive ultra-filtration. The molecular substitution ratio (MSR) of aromatic hydrazine per PAMAM was determiend to about 18 and the MSR of aromatic aldehyde per thermosome to about 3.4.

The aromatic hydrazine on the PAMAM surface and the aromatic aldehyde at the interior of the thermosome react under mild conditions to form a resonance stabilized Schiff's base. The conjugation reaction was done at pH 6.5 overnight at room temperature. Size exclusion chromatography was used to separate the polymer-protein core-shell hybrid from free PAMAM. Free PAMAM and the thermosome-PAMAM complex were baseline-separated.

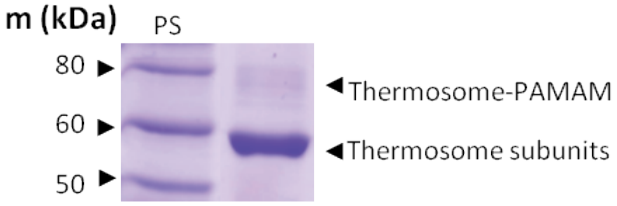
To verify the conjugation, gel electrophoresis and UV-spectroscopy was carried out. The formed bisarylhydrazone linker absorbs UV light at a specific wavelength (λ = 354 nm). The UV-Vis spectrum in Figure 2 reveals an overlay of three mean peaks. A peak at around 280 nm from the thermosome (ε_{280nm} = 210'880 M^{-1} cm^{-1}) and PAMAM (ε_{280nm} ≈ 1660 M^{-1} cm^{-1}), a peak at around 310 nm from not reacted aromatic hydrazine moieties on the PAMAM and a peak at 354 nm originating from the newly formed bisarylhydrazone bond (ε_{354nm} = 29'000 M^{-1} cm^{-1}). From this spectrum the ratio of PAMAM per thermosome was calculated to about three.



UV-Vis-absorbance spectrum of thermosome-PAMAM conjugate. It shows the absorbance of thermosome (ε_{280nm} = 210'880 M^{-1} cm^{-1}) and PAMAM (ε_{280nm} ≈ 1660 M^{-1} cm^{-1}) at 280 nm and the specific absorbance at 354 nm of the newly formed bisarylhydrazone bond (ε_{354nm} = 29'000 M^{-1} cm^{-1}).

An SDS-polyacrylamide electrophoresis (SDS-PAGE) gel is shown in Figure 3. Besides the band of the thermosome subunits (α- and β-subunits have quite the same molecular weight: 56'623 Da and 56'382 Da, respectively), a blurred band between 60 kDa and 80 kDa can be seen, which corresponds to thermosome-PAMAM. A blurred band is typical for PAMAM due to its molecular weight distribution [8]. Native PAGE data (not shown) confirm the integrity of the thermosome-PAMAM complex.

Experiments on the incorporation of siRNA into the polymer-protein core-shell hybrids, on the stabilization of siRNA due to the encapsulation, and release studies in vitro and in cells are in progress and will be reported soon.



Coomassie-stained SDS-PAGE of thermosome-PAMAM conjugate shows a blurred band at around 75 kDa corresponding to thermosome-PAMAM. (PS = protein standard)

Conclusions

We conclude a successful linking of PAMAM into the thermosome. The linking chemistry does not harm the protein and is therefore suitable for linking of polymer to protein. This polymer-protein core-shell hybrid should be able to take up drug payload and to hide the PAMAM so that, the cationic surface of PAMAM, which is needed to complex siRNA, is shielded from the cells. This should lower the toxicity of the PAMAM. Due to the ability of the thermosome to close its cavity depending on ATP, the payload might additionally be protected from degradation. Further aims are to modify the thermosome with a cell recognition moiety and a cell penetrating moiety. Such a polymer-protein core-shell hybrid could act as a specific targeted delivery tool for siRNA and other therapeutics.

ACKNOWLEDGEMENTS.

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SELECTIVE TARGETING OF LIVER CANCER VIA INSULIN GROWTH FACTOR BASED- GOLD NANOPARTICLES VECTOR

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Hepatocellular carcinoma (HC) represents a leading cause of cancer deaths worldwide. As chemotherapy and radiotherapy show modest results and surgery is possible in 10% to 30% of patients, new specific targeted therapies offer the hope for a better outcome in the near future for patients diagnosed with hepatocellular carcinoma.

For the identification of such therapies, gold nanoparticles (GNPs) platforms are particulary interest because of their surface plasmon resonance, non-cytotoxicity, chemical stability, high afinity to biomolecules and anti-angiogenic properties.

It has been observed that liver cancer cells overexpress the Insulin-like growth factor- receptor (IGF-R). IGF-R is a tyrosine kinase receptor (RTK) associated with caveolae, invaginations of the plasma membrane that regulate vesicular transport, endocytosis and intracellular signaling.

Here in, we report a selective therapy of HepG2 cells based on targeting the IGF-R with Insulin-like growth factor (IGF) functionalized gold nanoparticles.

Confocal microscopy along with Transmission electron Microscopy and combined with immunochemical staining was used to demonstrate the selective internalization of fluorescently labeled IGF-GNP via IGF-R.

The perfect overlapping of the IGF-R and IGF-GNP, showed in Figure1, lead as to conclude that the cellular targeting mechanism occurs via IGF-R targeting.

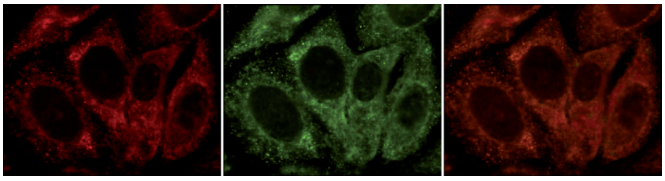

Figure 1. GNP-IGF(green), IGF-R co-localization(red), HepG2 cells were treated with IGF-GNP (15 mg/L) for 30 min, fixed in 4% formaldehyde, permeabilized with Methanol at —20°C labeled with a mouse anti-IGF-R antibody

We also showed that IGF-R internalization triggers Cav-1 and PTRF/Cavin translocation from plasma membrane to cytosol and support the critical role of caveolae in IGF-GNP intracellular traveling.

The presented results impose IGF as having a good potential for the development of GNPs- based targeting agents in human HC. This new therapy can aid in the development of a new generation of immunonanoconjugates for in vivo imaging and selective photothermal therapy of liver cancer.

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NONINVASIVE COLOR VISUALIZATION OF RED BLOOD CELLS

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Our understanding of biological organization of a live matter and its cellular process we can mainly get from microscopy. But visualization of a biological structures is based on interaction of electrons and photons with sample what leads to destruction of a sample. In some cases to get color contrast image of separate elements of biological sample need to use a variety of dyes and fluorescent substances but it leads to artificial staining of sample, destructive modification and loss very important structural information its native structure. For instance to get color image of morphological structure of blood smears are usually using staining of living blood smears by dyes. But this method leads to disruption of sample caused by the specimen preparation and viewing conditions [1]. Other ways of generating color image contrast is based on visualization of phase gradients within unstaining specimens, as realized by phase contrast [2] and differential interference contrast [3]. Usually in medical practice conventional bright-field microscopy let us see black and white image of separate morphological elements of blood smears only (Figure 1a).

In present paper we are offering the new nondestructive method of optical microscopy capable of examining the structures of living cells in their natural colors without staining them, using a specially designed substrate for deposition of biological sample and observing native structure in reflected light. This method based on physical phenomena of white light interference reflected from sample surface and special supporter on which this sample is deposited. As distinct from phase contrast [2] or differential interference contrast [3] microscopy there we have interference picture not for passed through sample and transparence object-plate two light rays but for two reflected light rays 1 and 2 on the sample surface and substrate respectively (Figure 2). It allows to occur at the image plane converting previously invisible gradients of refractive index within the specimen in to intensity gradients in the image. Color interference contrast image is achieved due to special condition of experiment is connected with chose of angle of incidental light, wave length of light of reflected ray, chemical composition of sample, thickness of sample, refractive index of sample, refractive index of substrate, chemical composition of substrate

The setup for color reflected interference microscopy was centered around ordinary optical microscope (Carl Zeiss, Germany) equipped for digital photo camera (Sony) and substrate which serve as object-plate for sample and as source of coherent light for scattering on morphological structures of sample. Light from a 100 watt xenon source was directed on to the specimen. Microscopic images were obtained with Zeiss lens and digital camera and recorded on a personal computer using commercially available software.

To demonstrate the potential usefulness of this method, we provide qualitative data describing color image of healthy and pathological damaged cells for alive and dry blood smears (Figure 1A-1E).

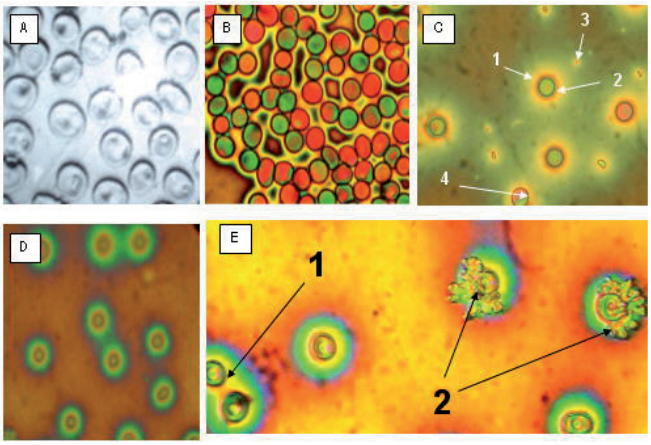

FIGURE1. A, Bright-field image of erythrocytes of healthy individuals. B, Color image same blood sample. C, Erythrocytes of healthy individuals, We can see multilayer structure of interference pattern (1), distinct line of cell shell (2), and platelets (3). Different colors can be seen in certain areas of an individual red blood cell (4). D, E, Living erythrocytes of a patient with diagnosed core rectal cancer. Images show a multi-layered pattern of interference picture. Differences between the coloration in left (D) and right image (E) caused by different experimental conditions. We can see double cells (1) and damaged cells (2), both are pathologic.

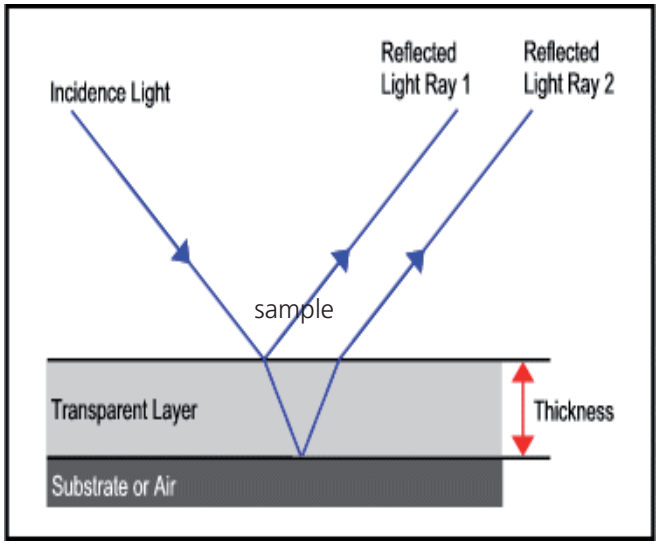

FIGURE 2. Schematic layout to get interference in reflected light rays 1 and 2 on the transparence sample deposited on the no transparence substrate.

Comparison Figure1A and Figure1B for same samples but obtained by conventional bright-field microscopy and by using new method correspondently showed distinguishing in color not only separate red blood cells but distinguishing difference parts in area separate erythrocytes too.

Usually for healthily individuals a albuminous aureole around the erythrocytes are mainly white-yellow (Figure 1C), but for cancer cells (core rectal cancer) the aureole color is quite different and reflects significant changes in chemical composition of both internal, and external contents of erythrocytes (Figure 1D, 1E).

Easy detection of organic shells around blood cells in our case is evident. Operations by fixing, smear coloring, prolonged processing, the availability for phase-contrast or interference microscope, special illuminators, radiating the exciting short-wave light beams are not required. Interferometric coloring of blood elements occurs on a surface of specially selected substrate. Corresponding colored images of blood elements are formed due to interference phenomena occurring under interaction of light beams reflected from front and back surfaces of blood elements, smeared on a substrate.

As it is seen from the given micro photos, the character of the colored image is the same, as though they were investigated with phase-contrast or interference universal microscopes.

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TITLE: ADHESIVE NANOSHEET FOR TOPIC DRUG RELEASE AND THERAPY IN THE GASTROINTESTINAL TRACT

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INTRODUCTION

The gastro intestinal apparatus (GI) is a complex and heterogeneous system with a total length of 9 metres1 extending from the oral cavity to the perineal region. This long organ consists of a complex system of layers which are vascularized and innervated by the enteric nervous system and by sensitive fibres2. Several diseases show their first symptoms in different GI tracts, altering functionality and healthy state of the mucosal barrier3. In particular these pathologies include chronic Inflammations of the Bowel (IBD), Crohn Syndrome (CS), Granulomatous Enteritis segmental and Ulcerative Rectocolitis (URC). Those pathologies are currently cured by systemic drugs administration, such as anti-inflammatory, corticosteroids, immunesuppressive and antibiotics agents4,5 with a final low efficiency of the drug for restoring the normal state of the mucosa. A definitive approach is represented by the surgical resection of the diseased portion of bowel in case of ulcerative phenomena. Unfortunately, in the particular case of CS, those lesions are characterized by chronic granulomatous transmural flogosis and segmentariety6,7, making resection hardly affordable.

An innovative technique would consists of the local release of anti-inflammatory drugs loaded on polymeric patches. These special patches must be biocompatible, resistant to mechanical stress, thin (at nano level), uniform, with negligible superficial roughness, resistant to the environment of the intestinal lumen and to the huge immune response.

In this framework, the fabrication of ultrathin films (so called "nanofilms") in poly lactic acid is presented together with the evaluation of their essential structural characteristics in terms of thickness and roughness, degradation time and adhesion characteristics on porcine mucosa in ex vivo conditions. Finally we discuss possible use of nanofilms as wound healing and tissue curing technique of the GI mucosa.

MATERIALS AND RESULTS

Nanofilms with area of 2 cm × 2 cm were prepared by spin coated assisted deposition8 by using Poly(L-lactide) (PLLA, Mw 80,000-100,000, Polysciences Inc. Warrington, PA) as first layer and Poly(vinyl alcohol, PVA, average Mw 13,000-23,000, Sigma-Aldrich, St. Louis, MO) as supporting layer. Samples with 3 concentrations of PLLA (5, 10, 20 mg\ml) have been tested, since the different concentration of the material influences the final thickness of the nanofilm.

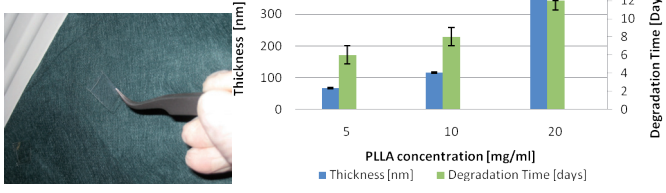


Fig. 1

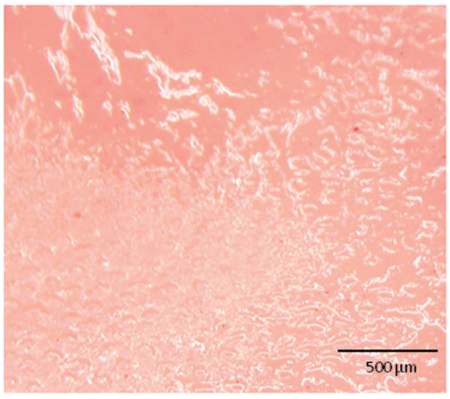
As reported in fig. 1.a, the nanofilm can be detached from the silicon wafer support employed for the fabrication and thus manipulated by traditional tweezers. As summarized in fig. 1.b the average thickness ranges from 67,9 nm for the lowest concentration up to 351,4 nm for PLLA 20 mg\ml, while the average roughness Ra is in the order of 2-6 nm (data obtained by sample analysis by atomic force microscopy).

The GI tract is characterized by values of pH that span from highly acidic values (1-3) in the stomach to 6-7,5 in duodenum, small intestine, ileum and colon, with a correlated reduction of thickness of protective mucus layer9. Aiming to design a curative nanopatch for protecting the mucosal surface and for healing wounds\ulcers that can appear along the entire GI length, the degradation time of the prepared nanofilms was tested in the worst case of highly acidic condition (pH = 1), immerging the nanofilm in hydrochloric acid filled glass container and counting the required days for dissolving the total structure. As reported in fig. 1.b, the synthetic nature of the nanofilm leads to a long lasting behavior (5 mg\ml PLLA nanofilm requires 6 days for complete dissolution) which could guarantee an even longer stability, and thus longer therapeutic effect, in more basic conditions, like the ileum and colon tracts where the first symptoms of CS are more frequent.

Freshly excised porcine small intestine and colon tissues were provided by a slaughterhouse 2 hours before the experiments and maintained at 4°C in order to preserve the inner mucus layer. Samples of tissues from the different tracts show the reduced amount of mucus in the colon compared to the small intestine10. The morphology of the mucosa, consisting of epithelial and mucus secreting cells aggregated in pits and villi, appears significantly irregular in small intestine with consistent deviation among organs extracted from different animals.

The 3 nanofilms samples were detached from the silicon support and deposited with stainless steel tweezers on the mucosa with the PLLA in contact with the tissue. By bringing the nanofilms into contact with the tissue, they spread out due to cohesive and capillary forces. The supporting PVA layer was removed after the deployment of the nanofilm adding water on the surface and aspirating the dissolved liquid solution from the nanofilm borders; a stable adhesion configuration was observed for PLLA 5 and PLLA 10 nanofilms, while the one of PLLA 20 was only weakly stable This phenomenon can be explained considering that, once the polymeric patch is detached from the wafer, the thickness of the PLLA 20 nanofilm coupled with the PVA supporting layer is on the order of tens of µm; this leads to a totally different behavior in terms of cohesive phenomena.

Fig. 2:



Experiments were firstly carried on mucus covered tissues, then by gently removing part of the protective layer with a spatula. As expected, the removal of mucus improved the adhesion of the nanofilm. This effect can be favorable in non-healthy conditions (CS or URC) where the mucus secretion is altered or reduced.

The film nanometric thickness appeared as a favoring factor on its adhesion on the wet surface. The thinner is the nanofilm, the higher is the effect of the surface tension force on the whole structure and of the cohesive force due to capillarity phenomena. As soon as the PVA layer is dissolved, this mechanism was even more evident, obtaining a fully distended sheet. Finally, thanks to the reduced presence of mucus and to the regular morphology of the tissue, the bond was higher on colonic than on small intestine tissues.

As shown in fig.2, the borders of the PLLA 5 nanopatch, which perfectly cover the mucosa following the tissue profile, could not be distinguished. This issue is regarded to as a positive aspect, since first symptoms of CS occur as inflammatory and fibrotic lesions exactly in colon and in distal ileum tracts, where mucus is less abundant and the bowel profile is smoother.

DISCUSSION AND CONCLUSIONS

The main properties of an adhesive film for application inside the body are the bonding capability on wet surface, the bio-absorbability, the cohesive and adhesive strength together with the flexibility and scaffolding properties of the structure and its reduced interference with the tissue and its activity. Since the PLLA nanofilm have been presented in literature as alternative suturing technique11 and innovative patch for topical drug release12, in this work a preliminary evaluation of nanofilm deployment and adhesion onto GI mucosa was presented. The provided results pave the way for the design of nanopatches for drug release directly onto lesions, thus improving therapeutic efficiency, enabling the concentration of the curative effects exclusively on the affected areas, thus avoiding side effects due to systemic administration. The drugs, once released by the patch, could penetrate in depth while the polymeric film protect the longitudinal extension of the ulcerative lesions; this aspect is being further investigated and will be documented through subsequent publications.

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A NEW DELIVERY SYSTEM OF CLOBETASOL PROPIONATE (LOADED LIPOSOMES 0.025 %) FOR TOPICAL APPLICATION.

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Clobetasol propionate is a potent corticosteroid especially useful for short treatment of recalcitrant dermatoses. However, their systemic side-effects, particularly inhibition of the hypothalamic-pituitary-adrenal axis, limit its use as maximum up to 14 days. In the present study Clobetasol propionate was encapsulated into liposomes for improving its dermal action.

METHODS

Liposomes composed of soy Phosphatitdylcoline, Cholesterol, Butylated hydroxytoluene and Clobetasol propionate (CP) were prepared using polyol dilution method and a factorial design approach was used. Amount of Cholesterol (Chol), homogenization speed and homogenization time were taken as variables at two different levels. Liposomes were characterized for vesicles size and encapsulation efficiency. Gel containing liposome dispersion (batch with higher liposome size) was prepared in Carbopol® 940 NF and was characterized for gel viscosity. Drug percutaneous absorption from liposomal gel and conventional formulations was evaluated in vitro through excised human skin using Franz diffusion cells and liquid scintillation to measure the drug content in samples. In vivo anti-inflammatory effect was tested on mice and rats. A phase II clinical trial was conducted to evaluate efficacy and safety of new liposomes loaded with 0.025 % of CP compared with a commonly used ointment formulation with 0.05 % of the same drug in the topical treatment of plaque-type psoriasis. One hundred twenty three patients were enrolled in such study, divided into two groups.

RESULTS

Polyol dilution method was found to produce multilamellar and homogeneous population of liposomes. Results of regression analysis revealed that vesicle size was dependant on the cholesterol concentration (positive correlation) and the homogenization speed (negative correlation) during preparation.

Table 1. Experimental design with coded levels of variables, their actual values and responses obtained for studied parameters. Values in parentheses indicates coded lev

Batch number	Variable X1 amount of Chol (mg)	Variable X2 homogenization speed (rpm)	Variable X3 homogenization time (min)	Particle size (μm ± SD), n=4	Encapsulation efficiency (EE% ± SD), n=3
1	250 (+1)	8000 (+1)	6 (+1)	3.359 ± 0.234	99.55 ± 0.014
2	250 (+1)	8000 (+1)	3 (-1)	3.366 ± 0.256	99.56 ± 0.014
3	250 (+1)	3000 (-1)	6 (+1)	3.957 ± 0.180	99.65 ± 0.071
4	250 (+1)	3000 (-1)	3 (-1)	4.047 ± 0.214	99.66 ± 0.028
5	125 (-1)	8000 (+1)	6 (+1)	2.498 ± 0.344	99.52 ± 0.035
6	125 (-1)	8000 (+1)	3 (-1)	2.674 ± 0.322	99.57 ± 0.028
7	125 (-1)	3000 (-1)	6 (+1)	3.523 ± 0.189	99.62 ± 0.180
8	125 (-1)	3000 (-1)	3 (-1)	2.855 ± 0.136	99.67 ± 0.085

Incorporation of liposomal suspension to 1 % Carbopol gel base at 1:3 ratio gave a suitable viscosity for apply to the skin. Percutaneous absorption study showed both better accumulation of CP into skin and lesser levels in receptor fluid than conventional formulations (figure 1). From these results one may expect a lower diffusion of the Clobetasol Propionate incorporated in liposomes towards the systemic circulation after in vivo application.

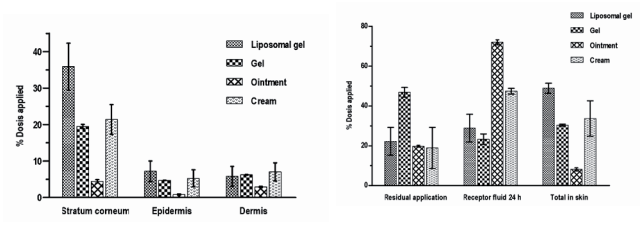

Figure 1. CP distribution from assayed formulations after 24 hours of application, a) into different skin strata, b) including receptor fluid and residual of application.

In vivo experiments demonstrated the same anti-inflammatory effect of CP liposomal formulation as the marketed cream and ointment, even when CP concentration in liposomal gel is a half of the commercial products (tables 2 and 3, figures 2 and 3).

Table 2. Assayed formulations for the croton oil-induced oedema in mice.

Groups	Dose (μg of CP/ear)
DECLOBAN® ointment (CP 0.05 %)	10
Clovate ® cream (CP 0.05 %)	10
Liposomal gel (CP 0.025 %)	5
Control	No treatment

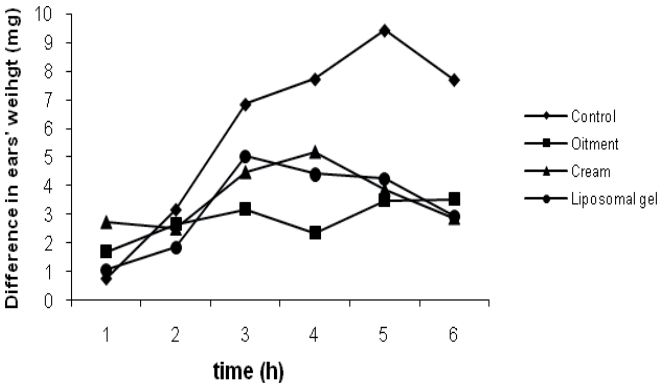

Figure 2. Inhibitory effect of different formulations on the oedema induced by croton oil.

Table 3. Assayed formulations for the cotton pellet-induced granuloma in rats.

Groups	Dose (μg of CP/pellet)
DECLOBAN® ointment (CP 0.05 %)	150
Clovate ® cream (CP 0.05 %)	150
Liposomal gel (CP 0.025 %)	75
Control	No treatment

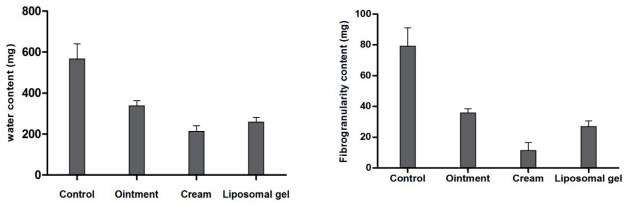

Figure 3. Inhibitory effect of different formulations on granuloma induced by cotton pellet implantation.

After a dropout of 17 patients a total of 106 patients with psoriasis were treated (53 with CP liposomal gel formulation and 53 with conventional CP ointment formulation) with the same dosage regime (figure 4). Both formulations were found similar for the reduction of Psoriasis Area and Severity Index (PASI) after three weeks of treatment. No significant differences in clinical responses (86.8 % and 94.3 % for treatment with CP liposomal gel and CP ointment, respectively) were found. Adverse events also were similar for both formulations followed two additional weeks after treatment (table 4)

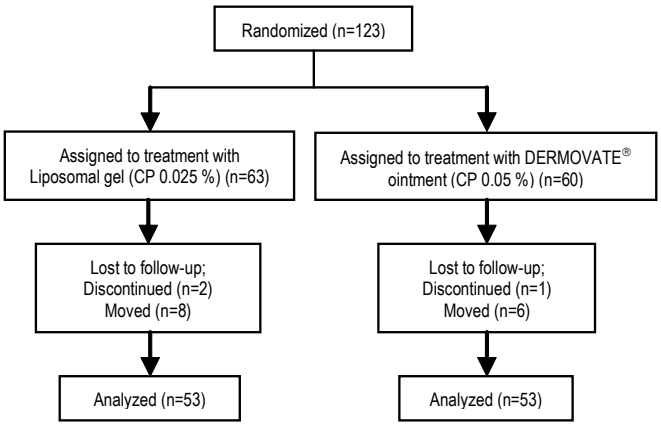

Figure 4. Flow diagram of the number of patients included in the statistical analysis.

Table 4. Clinical response at 3th week of treatment and occurrence of adverse events during the next two week after treatment.

	Group A Liposomal gel (CP 0.025 %)	Group B DERMOVATE® ointment (CP 0.05 %)	p
Clinical response (PASI reduction)			
Clean	62.3 % (33/53)	88.7 % (47/53)	
Responsive	24.5 % (13/53)	5.7 % (3/53)	0.006*
Not responsive	13.2 % (7/53)	5.7 % (3/53)	
Adverse events			
Itching	29.2 % (14/48)	8.2 % (4/49)	0.008*
Burning	8.3 % (4/48)	2.0 % (1/49)	0.161*
New psoriatic lesions	10.4 % (5//48)	10.2 % (5/49)	0.972*
Rebound effect	8.3 % (4/48)	8.2 % (4/49)	0.976*

Clean: ³ 65 % of PASI reduction. Responsive: 36 % £ PASI reduction < 65 %. Not responsive: PASI reduction £ 36 %. * Pearson's c2 test.

CONCLUSIONS

Our results suggest that a suitably developed liposomal formulation of Clobetasol propionate can be of actual value for enhancing its localization into the skin, thus improving its dermal action in topical therapy of dermatoses, particularly in psoriasis.

TISSUE ANALYSIS AND THERAPY MONITORING USING RAMAN SPECTROSCOPY AND SURFACE PLASMON RESONANCE OF THE NOBLE METAL NANOPARTICLES

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Ultrasensitive micro-Raman technique exploiting local optical fields of noble metal nanoparticles, namely surface-enhanced Raman scattering (SERS), was employed in characterization of biological tissue samples from mice models with photochemically induced skin cancer.

The tumor models required important and non invasive techniques to control the evolution of the pathology for early diagnosis and treatment surveillance. Early diagnostic in cancer still represents a challenge for the interdisciplinary field. In order to reduce or exclude the fatal pathologic evolutions, early diagnostic should concentrate on the subtle changes at molecular level. Raman spectroscopy has recently involved in an increasing number of cancer diagnostic applications [1-3] because of its unique ability to identify and differentiate molecular structures responsible in the tissue pathology. However, the spectral differences observed between normal and pathologic tissue are generally subtle when normal Raman scattering is measured. Moreover, different molecular components of the cells and tissue possess different Raman scattering cross section, resulting in overall Raman signal dominated by some molecular components in detriment of others, as shown in the Fig. 1. For example, the Raman spectra from skin tissue are dominated by the intense Raman signal of collagen. Generally, the proteins and lipids exhibit characteristic Raman fingerprint bands, whereas nucleic acids, responsible for cells proliferation along the malignancy, are less observable as distinct Raman bands in the tissue measurement. In spite of the large amount of recently reported Raman spectroscopic data on tissue pathology [1-3], for a reliable diagnostic additional statistical methods and important amount of recorded spectra are required. Therefore, the acquisition protocol optimization is essential. For clinical routine applications this technique has to be improved concerning the acquisition time, the signal-to-noise ratio and reproducibility. It has been recently demonstrated that surface enhanced Raman scattering (SERS) technique can be successfully applied in tissue measurements using Ag colloidal nanoparticles [1-2] inoculated into the tissue. The SERS signal from tissue is strong, sensitive and selective for the molecular tissue components from the close vicinity of the nanoparticles (Fig. 2).

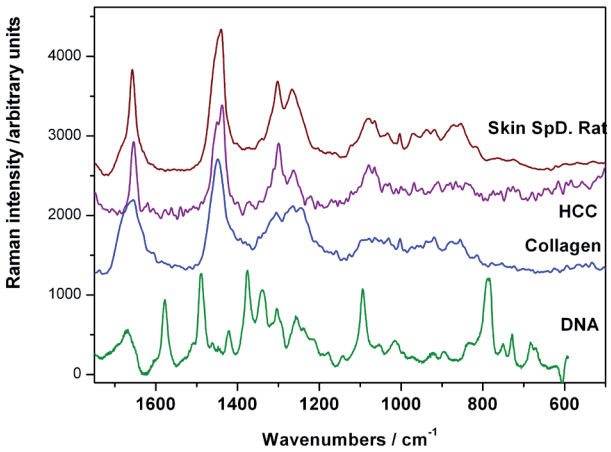

Fig. 1. Raman spectra of skin from ex-vivo Sprague Dawley rat, human colon carcinoma (HCC), Achilles tendon collagen and DNA, as indicated on each spectrum. The tissue spectra resemble well the proteins (collagen) pattern, whereas bands from nucleic acids are less representative. Laser excitation: 785 and 1064 nm.

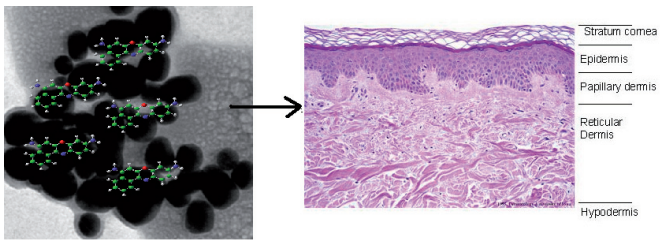

Fig. 2. Functionalised noble metal nanoparticles with a Raman tag (species with high Raman scattering cross section) could be easily embedded in tissue and Raman detected. Together with the signal from Raman tag it is obtained signal from the molecular components close enough to the functionalized nanoparticles.

Moreover, among the large molecular diversity, the nucleic acids bands are dominant, as shown in the Fig. 3.

Tumor and damaged skin were selected as target areas for FT-SERS measurements. from the NMRI and Sprague Dowley (SpD) mice specimens with 7,12-dimethylbenz(a)anthracene (DMBA) and 12-O-tetradecanoylphorbol-13-acetate (TPA) chemical treatment as initiators and promoters. Additionally, the UVB exposure has been applied. The DMBA was applied 2 times/week in the first 2 weeks and after that was used TPA 2 times/week. After DMBA application the mice were exposed to UV-lamp 2 times/week, 5 min/day to accelerate the pathologic process. The UMFT Bioethical Committee agrees the protocol and institutional guidelines were followed in the handling and care the animals. For the biopsy preparation: the skin was excised from the most damaged skin area.

Thin excised skin layer tissue samples were immersed in 10% formalin mixed with the colloidal silver solution 1/1 v/v. The same procedure was applied for tested organs like liver or lung. Representative FT-SERS spectra collected from three different skin samples from NMRI mice specimen (UVB+DMBA treated), are shown in the Fig. 3. The results were correlated with haematoxilin-eosin histology evaluation. The spectra collected from the tissue are always SERS spectra, measured only at positions where silver nanoparticles are present after incubation or penetrating the cells membrane. The presence of the band at 225 cm-1characteristic for the Ag-molecule bond within the SERS process support this supposition. The dominant bands are observed at 2927, 1574, 1332, 1229, 1131 and 481 cm-1 and are tentatively assigned to collagen, nucleic acids and with less extent to proteins along the pathology evolution.

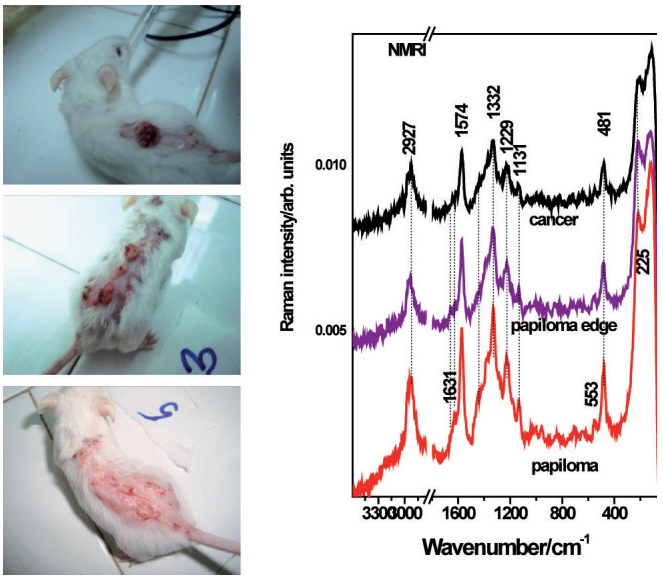

Fig. 3. NIR-SERS spectra collected from three different skin samples from NMRI (UVB+DMBA treated), as shown on each spectrum; excitation 1064 nm, 140 mW. Several animal models in pathology evolution from melanoma (top), melanoma and papiloma (center) and carcinoma (bottom).

As shown in the Fig. 3, the SERS signal is dominated by the nucleic acids bands, revealing for the first time the evidence of the subtle changes at the DNA and RNA level.

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SYNTHESIS AND SEARCH FOR NEW NANODIMENSIONAL ANTITUBERCULAR AGENTS

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Appearance of dramatic health problems such as multi-drug resistance and other different forms of diseases have made the research for new anti-TB drugs even more urgent. It is well known what important role metal ions play in biological phenomena and their presence can dramatically influence on the substance's properties. Co-ordination to a metal ion, as has often been reported, enhances the anti-mycobacterial and anti-proliferative properties of several ligands. Our recent researches in the framework of SCOPES and DAAD programmes have been focused on the modelling of antitubercular systems which are potential for targeted drug delivery.

This problem can be solved in several ways:

- 1) Direct drug administration;
- 2) Attaching of anti-TB agents (coordination compounds) to the surface of MNs (magnetic nanoparticles);
- 3) Design of new Single Molecule Magnets (SMMs) with enhanced blocking temperature (BT) and antimycobacterial properties. SMMs are metal-organic clusters that display purely individual magnetic properties, with each molecule able to be individually magnetised [1].

It is known from the literature that oleic acid and oleylamine play significant roles in the formation of the nanoparticles, probably because they prevent the formation of agglomerates as the particles are stabilised by the surfactants which related to surface of iron oxide. With this in mind we decided to look for other surfactants to produce iron oxide nanosystems. We have elaborated a strategy for making Fe2O3 nanoparticles with the size of about 1.0-1.5 nm (amorphous and maghemite) and 5-6 nm (amorphous) using sunflower oil. Such vegetable oils are rich in different long chain components and represent a cheap and accessible source of the long chain fatty acids, such as oleic acid, for nanoparticle synthesis. All the samples were synthesized according to published synthetic protocol [2]. The reaction was protected under argon in order to avoid any undesired side-reactions. The nanoparticles (N1) were dispersed into nonpolar or weakly polar hydrocarbon solvents such as hexane or toluene. It was observed that in the absence of hexadecylamine or using dodecylamine instead in the synthetic method leads to the separation of an oil fraction (N2) of nanoparticles, which makes the further study difficult. To extract Fe2O3 nanoparticles from this oil fraction the multifunctional ligands 4-(5-sulfanyl-1,3,4-oxadiazol-2-yl)phenol (L1) and 2-(5-sulfanyl-1,3,4-oxadiazol-2-yl)phenol (L2) were used (Figure 1a). These reagents refer to the well-known anti-tuberculosis class of substances [3].

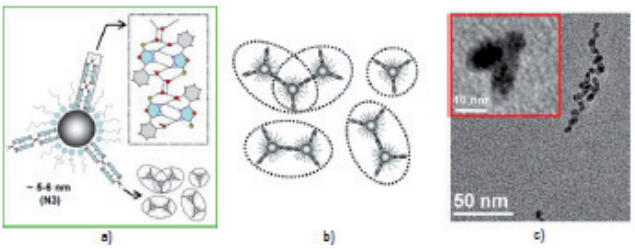

Fig 1: Schematic representation of N3 nanoparticles (a) and (b); TEM images of nanoparticles N3 deposited from their hexane dispersions (c).

We have observed that the position of OH-group in the ligand molecule plays an important role in efficiency of nanoparticles extraction from the oil phase: a good result was obtained only when L2 was used (N3). We think that in this case an important role plays the presence of hydrogen bonds which are found in the crystal lattice of L2 (Fig 1a, insert; [2]). Furthermore we suppose that pairs of L2 molecules between the surfactants lead to further agglomeration of the nanoparticles reinforced by the presence of NH···N (S1) or NH···S interparticle hydrogen bonds (Figure 1b). We suppose that agglomeration take place because it explains appearance of a big number of "rugby-ball-form" particles (up to ~6 nm in diameter) in the TEM analysis (Figure 1c). The synthesized nanoparticles have been also characterized by 57Fe Mössbauer spectroscopy as this technique is considered to be a very informative method, when used with an applied magnetic field, to distinguish and identify the nanostructured Fe2O3 particles and mineral phase [2].

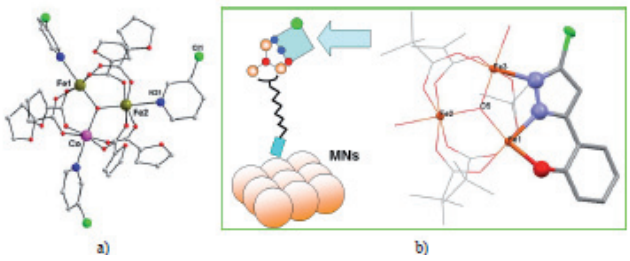

Figure 2: a) Molecular structure of coordination compound with {Fe2CoO} core [4]; b) Perspective view of "ligand-in-cluster"-"magnetic nanoparticle" system

In our recent work [4], we have also demonstrated the synthesis of some iron(III)-cobalt(II) containing compounds (Figure 2a) with antimycobacterial activity between 0.82÷4.00 μg/ml and results of cytotoxicity which are acceptable for their further screening. Such coordination compounds or other systems further can be attached to the surface of magnetic nanoparticles (Figure 2b).

Chemists, biochemists, and chemical engineers are all looking beyond traditional polymer networks to find other innovative drug transport systems. Our further researches on the synthesis and design of new antitubercular agents will be continued.

ACKNOWLEDGEMENTS

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TARGETED FUNCTIONAL GENOMICS SCREEN FOR METASTASIS AND EMT MODULATORS IN BREAST CANCER

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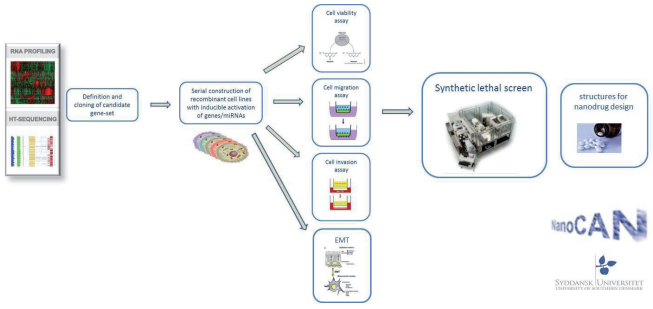
Targeted gene silencing using RNA interference (RNAi) approaches holds great therapeutic possibilities in the treatment of cancer, so that short interfering RNAs (siRNAs) will presumably represent an important component of the next generation of nanodrugs.

Synthetic lethal screening techniques have been successfully applied to identify short interfering RNAs (siRNAs) that sensitize cancer cells for specific drugs or exert differential killing activity on different cell types. Metastatic spread still represents the major cause of death among women diagnosed with breast cancer, so that causing synthetic lethality in breast cancer cells would represent a desirable goal.

To recover novel starting points for synthetic lethal screens, we selected a set of 100 candidate genes and miRNAs from available molecular profiles of metastatic breast cancer and subject these to systematic functional studies regarding their impact on cancer growth, cancer cell migration and invasion as well as their ability to cause the so-called epithelial-mesenchymal transition (EMT). EMT is a change in cell phenotype associated with increased metastatic potential and with cancer stem cell-like characteristics. 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, roughly 80% of the candidate genes/miRNAs have been cloned and 50 stable cell lines in MCF7 breast cancer cells have been constructed and analyzed with regard to cancer growth. So far, this yielded 5 genes that had a substantial impact on the viability of the breast cancer cells. Migration and invasion assays have been initiated. This strategy may yield interesting new targets for nanodrug discovery and design.



FOCUSSING OF MAGNETIC NANOPARTICLES (MNP) IN CANCEROUS TISSUES TO USE FOR HYPERTHERMIA AND LOCAL DRUG RELEASE

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BACKGROUND AND AIM

Drug carriers containing superparamagnetic nanoparticles (MNP) can be used for magnetic drug targeting to improve local chemotherapy. After being loaded with chemotherapeutic agents and injected into the circulatory system, the drug carriers can be directed to a specific body compartment using a magnetic field that acts on the MNP. The application of a high-frequency magnetic field enables the particles to release the drug and to generate enough heat to be used for hyperthermia applications.

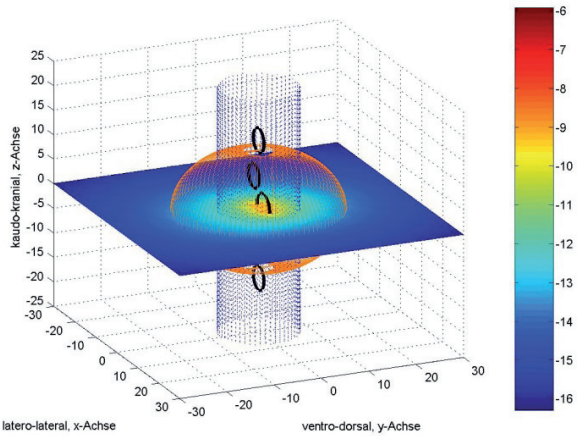
MATERIALS AND METHODS

We virtually designed, built and tested multiple coil configurations in the oesophagus for later treatment of oesophageal cancer. We mathematically modeled the oesophagus and the neighboring structures and then virtually placed the coil arrays within. Pathological reports of excised human tissue were evaluated and the magnetic susceptibility of the tissue was determined. We then analyzed the profile and strength of the magnetic field while varying the spatial parameters of the setup to find the coil configuration that fulfilled our requirements best.

RESULTS

Comparison with known magnetic fields of different coil arrays

proofed that our analysis of the profile and strength of the magnetic field had a relative error of 10-3 %. Figure 1 shows the optimized coil formation within the oesophagus and the resulting magnetic field.



The targeting efficiency of the MNP could be increased by a factor of nearly 8 purely by geometrical optimization.

DISCUSSION AND CONCLUSIONS

With our program to virtually design coil configurations and measure the resulting magnetic field we could increase the targeting efficiency of the MNP a lot. It´s wide variability allows the optimization of magnetic drug targeting for many other tumors. The resulting optimized coil formation needs to be proofed in an animal model in the future.

NATURAL IMMUNE REACTIONS AGAINST INTRAVENOUS NANOFORMULATIONS

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Nanoimmunosafety focuses on immunological factors and mechanisms that can be involved in adverse reactions caused by various nanomaterials and nanomedicines. In this study, we have investigated human serum complement (C) activation caused by intravenous cytostatic drug formulations, such as Caelyx (liposomal Doxorubicin), Ambisome (liposomal Amphotericin B), as well as Paclitaxel and Taxotere. The effect of these products and their placebo formulations such as placebo liposomes and micellar surfactants, Cremophor EL or Polysorbate 80, were compared.

Here we report that (i) Caelyx, Ambisome, Paclitaxel and Taxotere were capable of inducing C activating enzymes, C3- and C5 convertases in normal human sera, and production of both anaphylatoxins and the C-derived membrane attack complex, SC5b-9; (ii) these medicines and their placebo nanoformulations activated the C system in similar extent; moreover, (iii) serum C reactions induced by Paclitaxel/Taxotere and their placebo formulations exhibited a significant linear correlation.

Collectively, these results demonstrated that liposomal/micellar nanocomplexes of cytostatic drugs can be recognized by the natural immune system via C-related pattern recognition. C reaction opsonizes the nanodrugs and produces anaphylatoxins. Opsonization is a critical factor determining pharmacokinetic properties and cellular uptake, i.e. targeting, of the nanodrugs. Production of anaphylatoxins may lead to liberation of several other danger signals, inflammatory and cardiovascular mediators, which can provoke a transient systemic immunostimulation and/or harmful, immediate adverse reactions, hypersensitivity in susceptible patients during or shortly after the intravenous administration of these nanomedicines.

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TYR-DEPHOSHORYLATION AS MOLECULAR SWITCH TO INDUCE MEMBRANE LEAKAGE OF A CELL-PENETRATING PEPTIDE

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Cell-penetrating peptides (CPPs) are polycationic peptides that enter biological cells at low micromolar concentrations. Furthermore, they are able to carry various (macro)molecules along with, such as nucleic acids or even proteins. These properties make them promising vectors for intracellular drug delivery.

In vitro studies propose endosomal pathways as major uptake mechanism. However, in order to reach the cytoplasm, the CPPs have to escape from the endosome before lysosomal degradation. Unilamellar vesicles provide a reproducible model to investigate such a translocation across a lipid bilayer.

We have investigated dephosphorylation of a CPP as a molecular switch to trigger the conversion of the membrane inactive CPP into its active form. Phosphorylated Tyr was introduced by substitution of Ala10 in p2AL, a more hydrophobic mutant of the well known CPP penetratin (pAntp).

Our results show that p2AL‘s destabilizing effect on the lipid bilayer was modulated by the phosphorylated Tyr residue, resulting in the peptide termed pA(pY)L, which showed considerably reduced membrane permeation. However, its unphosphorylated counterpart, pAYL, still induced membrane permeation similar to p2AL. Importantly, the membrane permeation of pAYL was restored by enzymatic dephosphorylation of pA(pY)L, using an alkaline phosphatase. Thus, Tyr-phosphorylation of pAYL is a powerful molecular switch to trigger the conversion of the membrane-inactive peptide pA(pY)L into its membrane-lytic form pAYL.

STRUCTURE CONTROLLED G5G2.5 TECTO-DENDRIMERS: CYTOTOXICITY, UPTAKE AND TRANSEPITHELIAL TRANSPORT ACROSS CACO-2 CELL MONOLAYERS

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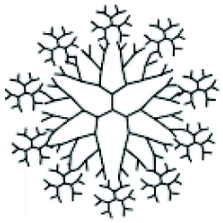
INTRODUCTION

Anionic poly(amido amine) (PAMAM) dendrimers generations (G) 1.5 to 3.5 are acknowledged to cross epithelial barriers by a combination of transcellular and paracellular routes. These small dendrimers are said to induce a transient opening of tight junctions, thereby enhancing their own transport via the paracellular pathway. This unique property makes them promising tools capable of increasing the oral bioavailability of poorly permeable or poorly soluble molecules. On the other hand, PAMAM dendrimers can be used as bricks to construct a new class of polymers known as tecto-dendrimers. These polymers exhibit a higher architectonic order than single dendrimers and are composed of a central dendrimer with multiple dendrimers attached to its periphery (figure 1). Up to the moment however, the main aspects of their interaction with biological structures remain unknown. Thus in the present work we have synthesized PAMAM G5 core and G2.5 shell tecto-dendrimers (G5G2.5), determined their cytotoxicity and cell uptake, and finally screened their own transepithelial transport across Caco-2 cell monolayers.



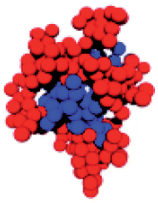


Fig. 1. Schematic representation of a tecto-dendrimer

METHODS

Briefly, the synthesis of the saturated tecto dendrimers was done by combining an amine-terminated core reagent (PAMAM dendrimer generation G5), with an excess of a carboxylic acid terminated dendrimeric shell reagent G2.5; the addition of carbodiimide was required to induce a covalent bond. The resultant product was purified by dialysis and size exclusion chromatography (SEC) to remove the excess shell reagent and lyophilized as a white crystalline solid. The final yield achieved was 50 % w/w.

Formation and characterization of G5G2.5 were analyzed by polyacrylamide gel electrophoresis (PAGE), MALDI-TOF-MS, SEC, high performance liquid chromatography (HPLC), atomic force microscopy (AFM) and dynamic light scattering (DLS).

Cytotoxicity on Caco-2 cells was determined as function of concentration upon 24h incubation by mitochondrial succinate dehydrogenase activity employing a tetrazolium salt (MTT) and lactate dehydrogenase (LDH) leakage.

Kinetics of Caco-2 uptake was determined by flow cytometry. For that purpose, first G5G2.5, G2.5 and G6.5 were labeled with fluorescein isothiocyanate isomer I (FITC). Caco-2 cells grown at 80-90% confluence into 6-well plates were incubated with 20 µM of G5G2.5-FITC, G2.5-FITC or G6.5-FITC for 0.5, 1, 3, 5 h at 37 °C. Extracellular FITC was quenched with Trypan Blue and cells were analyzed by FACSCalibur flow cytometer.

Transport of G5G2.5-FITC, G2.5-FITC and G6.5-FITC was determined across Caco-2 monolayers as models of the intestinal epithelial barrier. Caco-2 cells were seed onto 12-well Transwell filters of 0.4 mm mean pore size with 1 cm2 surface area and maintained under grown conditions for around 20 days. Transepithelial electrical resistant (TEER) and Lucifer yellow transport were measured to ensure the integrity of the monolayers. Monolayers with TEER 400-600 Ω·cm2 and Lucifer yellow transport less than 0.1% were incubated for 5 hours with 20 µM of samples. Percentage of transport was calculated with regards of the initial fluorescence in the upper compartment.

RESULTS

G5G2.5 showed to be structurally stable, monodisperse, with a molecular size distribution of 8 ± 1.5 nm determined by DLS and 16.9 ± 4.3 nm (height 1.12 ± 0.2 nm) determined by AFM (figure 2), and Z potential of -10 mV. Molecular weight of G5G2.5 determined by MALDI-TOF-MS was 98000 Da, consistent with data obtained by PAGE. Shell saturation levels of 85-90% were observed, corresponding with 10-11 G2.5 shell dendrimers surrounding the G5 core as expected from Mansfield-Tomalia-Rakesh´s equation (theoric N máx: 11.7).

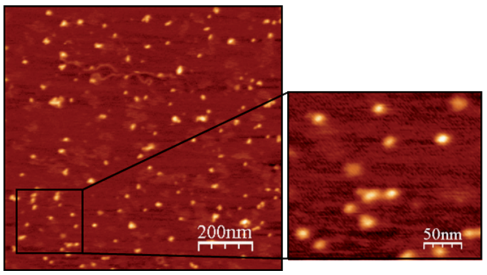
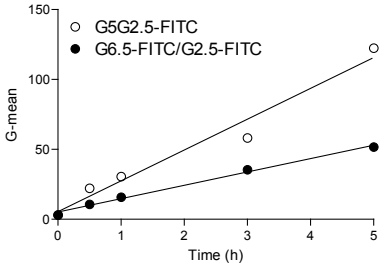


Fig. 2 AFM contact mode of G5G2.5

Cytotoxicity determinations showed that the cationic G5 dendrimers used as core decreased 80 % viability by and caused almost 100% LDH leakage at 14 mM on Caco-2 cells, meanwhile G5G2.5, G6.5 and G2.5 were non cytotoxic up to 150 mM upon 24h incubation.

Uptake of G5G2.5-FITC, G2.5-FITC and G6.5-FITC by Caco-2 cells increased linearly with time up to 5h of incubation (figure 3). By comparing the slops of the regression curves it was shown that the rate of internalization of G5G2.5-FITC was 2.3 fold higher than those of G6.5-FITC and G2.5-FITC.

Finally, G2.5-FITC showed higher transport than G6.5-FITC and G5G2.5-FITC across Caco-2 monolayer after 5 h incubation (figure 4), and all samples reduced around 75% TEER values after incubation.



G5G2.5-FITC
Slope 22.04
Y-intercept 5.383
R2 0.9666
G6.5-FITC/G2.5-FITC
Slope 9.556
Y-intercept 5.180
R2 0.9912

Fig. 3. G5G2.5-FITC and G6.5-FITC uptake by Caco-2 cells as a function of incubation time

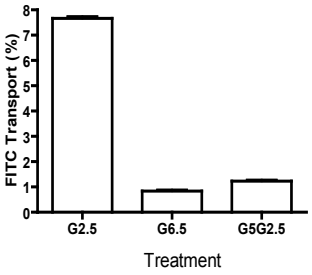


Fig. 4. Percent of transport of G2.5-FITC, G6.5- FITC and G5G2.5-FITC across Caco-2 monolayers

CONCLUSIONS

Up to our knowledge, this is the first report of interaction between cells and tecto dendrimers. The exhibition of multiple G2.5 dendrimers on the surface of G5G2.5 could lead us to speculate that tecto dendrimers could overcome the single G2.5 capacity of increasing their own trans epithelial transport across Caco-2 cell monolayers. However, our results showed that although less cytotoxic than core cationic dendrimers, tecto dendrimers did not increase their own trans epithelial transport. However, all dendritic polymers reduced the TEER and did not cause significant release of LDH. This could be interpreted as the induction of a tight junction perturbation, in the absence of massive cell damage. Further work should be aimed to explore the internalization pathway of G5G2.5, which is responsible for their higher internalization rate in comparison to G6.5 and G2.5. On the other hand, if well the higher size of G5G2.5 and G6.5 (8 nm) as compared to G2.5 (3 nm) could impair their passage across tight junctions, probably an increased permeability of reporter molecules could make profit of the resultant TEER reduction.

ANTIMICROBIAL NANO-PARTICLE-SYSTEMS – WORKING MECHANISM, SAFETY, EXPERIMENTAL EVIDENCE AND MATHEMATICAL MODELING

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In recent years some nano-particles with antimicrobial effects were developed and successfully tested within biological experiments. Especially precious metals show sophisticated effects with potentials for additional functionalization. In this context it is of importance, to be aware, that the working mechanism is not only depending on the particle type, but also a function of the overall-system-formulation.

Therefore ICT has developed antimicrobial nano-particle-systems based on nano-silver and nano-magnetite in combination with biocompatible polymers, which show strong synergetic effects.

The dominating working mechanism is a surface interaction of the metal-nano-particles with structure-proteins of the cell-walls of microorganisms. The nano-safety-concept follows the principle, that nano-particles are embedded in the polymer matrix, so that an uncontrolled release is prohibited.

The antimicrobial effect is detected by macroscopic surface-examination, where the treated samples are not colonized or microorganisms are directly destroyed.

The mathematical modeling of the kinetics of microbial growth can done by fitting experimental CFU(t)-data [colony forming units] with adequate exponential functions.

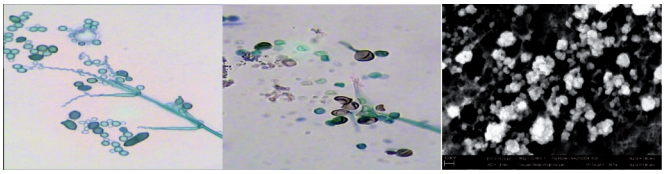

Figure 1 a, b, c:U Macroscopic pictures of fungal spores (a-b), which are inoculated on a surface (a) and killed by the nano-system (b). SEM-record of the nano-Ag-system (c)

IDENTIFICATION OF SYNTHETIC LETHAL SIRNAS FOR PERSONALIZED TREATMENT OF ADVANCED MELANOMA

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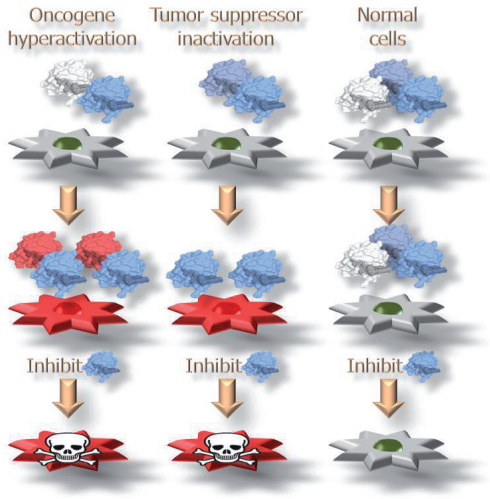
Synthetic lethal drugs have the ability to selectively kill cells with a specific molecular alteration, such as a cancer-specific mutation. Nanodrugs based on this principle not only bear the promise of prospectively low adverse side effects. They also represent an excellent starting point for personalized cancer nanomedicine, because prospective responders can be identified prior to treatment by analyzing the presence of the molecular alteration in the tumor cells.

At the molecular level, this is based on the phenomenon that certain mutations causing cancer require the collateral upregulation of other pathways to avoid apoptosis. This is referred to a pathway or oncogene addiction. Accordingly, inhibition of these pathways causes apoptosis only in the presence of the original mutation, i.e. synthetic lethality.

Here, we report on the identification of a synthetic lethal drug target (referred to here as SLT1) required for survival of melanoma cells with a specific mutation in an oncogene. SLT1-targeting siRNAs selectively killed in vitro melanoma cells, in which mutations of the oncogene and SLT1 upregulation coincided, but not melanoma cells lacking this specific molecular fingerprint. This may provide potential starting points for the development of new nanodrugs for personalized treatment of advanced melanoma.



SOLVOTHERMAL SYNTHESIS OF NONSTOICHIOMETRIC HYDROXYAPATITE NANOPARTICLES

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Today, the most common approach for regrow of bone in case of large bone gaps is to use autografts. In this case two or more operations are needed, with increased risk, suffering and costs for the patient. There is also an emerging market for hydroxyapatite (HAp) and calcium phosphate (TCP) in the form of paste or granulates used to fill small bone gaps. For large bone gaps bone regrowth scaffolds are being developed, but still exist unsolved problems like: low regrowth rate, poor mechanical properties of the scaffolds, high risk of inflammatory processes and slow or no resorbtion. Therefore the main objectives of the current regeneration medicine projects is to develop the technology for a bioactive scaffold with improved comparing to the state of the art control of shape, mechanical properties, bioactivity and resorbability. One of the ways to achieve these goals is to produce nonstoichiometric nanoparticles of hydroxyapatite with grain size lower than 10 nm and shape close to the natural HAp which will be used as material for bioactive, mechanically strong scaffolds.

The Institute of High Pressure Physics of the Polish Academy of Sciences (IHPP) is an expert in synthesis of doped nanoparticles with narrow size distribution, at relatively low temperatures by using Microwave Solvothermal Synthesis (MSS) technology. The MSS technology permits synthesis of nanoparticles with precise control of the reaction time, temperature and pressure. IHPP and the Faculty of Materials Science, Warsaw University of Technology, created a joint Center for Bionanomaterials and started cooperation to develop materials for bone regrowth scaffolds.

Thanks to unique worldwide reactors for microwave solvothermal synthesis of nanoparticles, IHPP is able to synthesize unique HAp nanoparticles using the standard reaction:

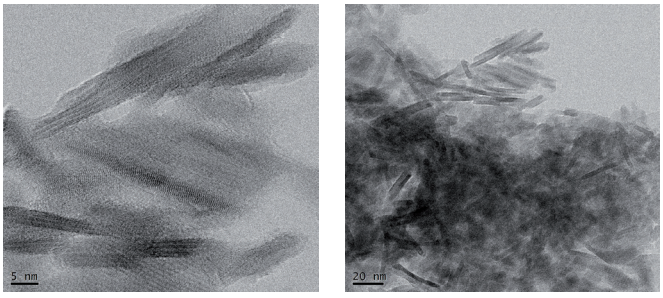

Fig 1: TEM pictures of n=HAP produced at IHPP using the MSS technology

$$Ca(OH)2 + H3PO4 \rightarrow HAp + H2O$$

The reaction is carried out in water solution in time lower than 5 minutes. The specific surface is 250m2/g, the average grain size 9 nm with shape in the form of platelets mimicking the natural bone particles. Fig 1 shows HREM pictures of the nanoparticles produced at IHPP using the MSS technology.

APPLICATION OF TOPICAL MUCOADHESIVE GANCICLOVIR NANOCARRIERS IN EFFECTIVE TREATMENT OF OCULAR VIRAL INFECTION

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The purpose of present study is to evaluate the ocular retention behavior and enhanced ocular bioavailability of Ganciclovir (GCV) through mucoadhesive nanocarriers. Developed chitosan coated niosomes (GCV-CN), chitosan nanoparticles (GCV-CNP) and mucoadhesive nanoemulsion (GCV-CNE) having the dispersion size of 80.03± 1.02nm (GCV-CN), 59.41nm± 1.47nm (GCV-CNP), 22.7± 1.11nm (GCV-CNE) with narrow distribution (PI, < 0.3). Furthermore, the zeta potentials greater than +30mV indicate the stable nature of these nanosized dispersion phases. Sustained release effect of the GCV loaded nanocarriers were investigated goat eye cornea emphasized the increase in permeability parameters with these nanocarriers. Furthermore, the in-vivo performance of the developed carriers were studied through corneal retention behavior and bioavailability study by Gama scintigraphy and UPLC method respectively on rabbits. Results revealed that selected nanocarriers showed excellent retention (> 8hr) over the non mucoadhesive GCV-solution (10min) and more than 3841.9±20.0ng.h/mL ocular bioavailability over the GCV-solution (AUC 0-t, 459.6±11.2 ng.h/mL). In addition, ocular irritation potential of the developed formulation was studied on rabbit eye by IR camera, and it was found that no significant changes in temperature on the corneal surface that confirm its non-irritant nature for ocular delivery. These results suggested that GCV-CN, GCV-CNP and GCV-CNE are potential vehicles for improved ocular delivery of GCV in ocular viral infections.

KEY WORDS

Mucoadhesive nanocarriers, ocular viral infections, Gamma scintigraphy, Ocular irritation

INTRODUCTION

Ganciclovir (GCV) is a synthetic acyclic nucleoside analog of 2'-deoxyguanosine, which exhibits antiviral activity against herpes simplex virus and cytomegalovirus at relatively low inhibitory concentrations [1, 2]. Current conventional treatment involves oral administration of GCV at a dose of 3.0-5g/day. Such a high dose results in dose-related toxicity like bone marrow suppression and neutropenia [2]. Therefore targeting of ocular viral infections through topical route is valuable, but is limited in case of GCV delivery due to poor ocular availability owing to its hydrophilic character and rapid elimination. Development of mucoadhesive GCV nanoformulations for the treatment of ocular infections is worthwhile since they are expected to prolong the pre-ocular retention and increase the ocular bioavailability. So, the aim of the present work was to investigate the comparative potential of different mucoadhesive nanocarriers (chitosan coated niosome, chitosan nanoparticle and chitosan containing nanoemulsion) for the topical ocular delivery of Ganciclovir in the form of eye drops. The in-vivo performance of the developed carriers were studied through corneal retention behavior and bioavailability study by Gama scintigraphy and UPLC method respectively on rabbits. Additionally, extensive ocular irritation studies were also done to assess the nontoxic nature of developed nanocarriers.

METHODS

Preparation of GCV nanocarriers. GCV mucoadhesive nanoemulsions (GCV-CNE) was prepared by aqueous titration technique using Triacetin, Tween 20 and diethylene glycol monoethylether as oil, surfactant and cosurfactant respectively under chitosan solution as aqueous phase. Chitosan nanoparticles (GCV-CNP) were prepared according to the ionotropic gelation method with slight modification [3]. GCV mucoadhesive niosome (GCV-CN) was prepared by the reverse-phase evaporation technique [4].

Characterization of GCV nanocarriers. The mean particle size and zeta potentials to evaluate the stability of the dispersion system of GCV nano-carriers were determined by photon correlation spectroscopy using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK).

In-vivo study. Corneal retention study by γ- scintigraphy and Ocular pharmacokinetic study. The pre-corneal retention of GCV nanocarriers were assessed by γ- scintigraphy. 99mTc-labelled GCV loaded nanocarriers was compared with 99mTc-labelled GCV solution. A total of 20 µL of the labelled formulations were instilled into the culde-sac of the left eye, and the eye was manually blinked three times to distribute the formulation over the cornea. The rabbit was positioned below the head of the gamma camera and clearance of the formulations from the eye was followed for 30 min by dynamic imaging using gamma camera (Millenium VG, GE, USA).

Four groups, each having seven rabbits (2.25±0.25 kg), were used in pharmacokinetic study. In both the eyes, a single topical instillation (50 µL) of GCV-solution, GCV nanocarriers dose equivalent to 0.3% w/v of GCV were added and 50 µL of the aqueous humor was and analyzed for GCV by UPLC.

RESULTS AND DISCUSSION

The particle size distribution studies showed that all of the selected nanocarriers GCV-CN, GCV-CNP and GCV-CNE were evenly round in shape with mean particle size in the range of 15-200nm with narrow distribution (PI, < 0.3). The zeta potentials which are indicative of stability of dispersion system with value greater than +30mV indicate the stable nature of these nanosized dispersions. The values of transcorneal flux for selected formulations were observed between 102.59 to 217.32 µg cm-2 h-1 in in-vitro transcorneal permeation study which is 11 fold greater than the control solution (20.213 µg cm-2 h-1). γ- Scientygraphy study was carried out to study the ocular retention performance of the mucoadhesive Nanocarriers (Figure 1). Results revel that selected nanosystems showed excellent retention (> 8hr) over the non mucoadhesive solution (10min).

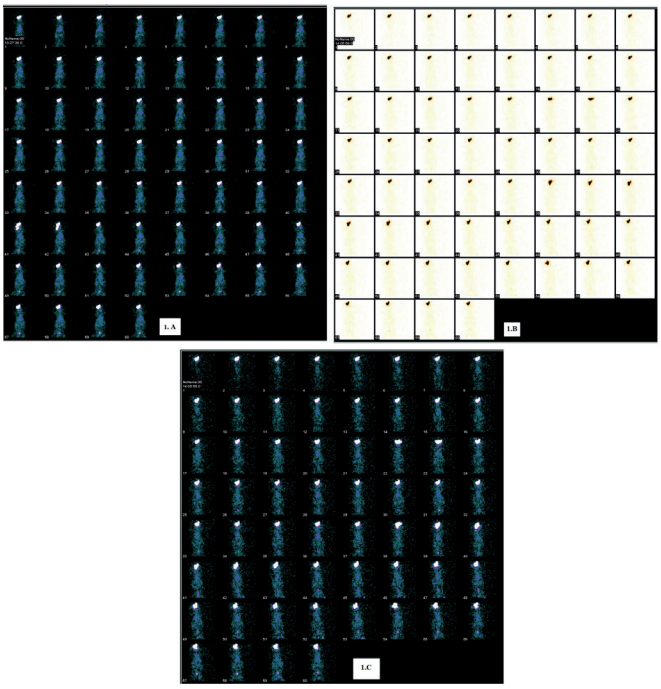

Figure 1: Dynamic radio-image of the whole rabbit showing the decrease of radioactivity in the eye and appearance in the body in 30 min after instillation of 99mTc-GCV containing A) GCV mucoadhesive nanoemulsions (GCV-CNE) B) Chitosan nanoparticles (GCV-CNP) and C) GCV mucoadhesive niosome (GCV-CN).

Pharmacokinetics study revealed that GCV mucoadhesive nanoemulsions (GCV-CNE) (AUC0→t, 4690.7±21.8 ng.hr/mL) Chitosan nanoparticles (GCV-CNP) (AUC0→t, 3318.1±29.4ng.h/mL) and GCV

mucoadhesive niosome (GCV-CN) 2816.3±29.4ng.h/mL provided approximately 6 fold increase in the relative ocular bioavailability compared with GCV solution. This could be attributed to increased precorneal retention of the GCV nanoformulations owing to presence of mucoadhesive chitosan and increased corneal penetration of nanosized particles. Furthermore, in Draize rabbit eye irritation test, non-significant redness were seen in the test eyes and only 2ºC rise in temperature above normal (35±0.7 ºc) were found on corneal surface on measurement by IR- camera. These results revealed that there were no any sign of local inflammation in eye and the studied formulations were nonirritant and nontoxic in nature. The achieved results may be useful for formulation development of GCV, which could be effective in the treatment of ocular infections by topical instillation. These results suggested that these nanosystems are potential vehicles for improved ocular delivery of Ganciclovir in ocular viral infections.

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NANOFIBER MEMBRANES AS A DRUG CARRIER SYSTEM FOR SUBLINGUAL APPLICATION

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Nanofiber nonwoven membranes have been recently studied as novel carriers of drugs. The retention of active substances within the membrane carriers of that type is generally influenced by a number of factors such as interaction of the drugs with polymeric nanofibers, thickness of the nanofibers layer, choice of the polymer.

The aim of the study was to find technological limits of preparation of nanofiber membranes based on different types of biopolymers (polylactid acid, polyacrylic acid, polycaprolactone, chitosane) in the term of the maximal drug load capacity and the release profiles of three model drugs of different chemical-physical characteristics. The drugs were added into the pertinent polymer solution before the process and consecutively transformed into the nanofibres of the unwoven membranes.

All the nanofiber membranes were prepared by industrial electrospinning technology NanospiderTM which enables large scale and economical production of nanofibers.

The important values of the total releasable amount of the drugs under in vitro conditions and the release in vitro characteristics of drugs were measured in the short and/or long-time release experiments are presented.

The relative amount of drugs that are realistically available for release were measured at levels from 15 % to 60 % that we consider to be interesting from practical point of view. The extend of the release kinetics is sufficiently wide for different therapeutic purposes.

We conclude that the used method of electrospinning is suitable possible way of drug-loaded membrane carriers manufacturing.

The authors would like to acknowledge the Ministry of Industry and Trade No. MPO TIP FR-TI1/0436 and the Ministry of Education No. MSM 0021620822 for financial support.

IN VIVO AND IN VITRO STUDIES ON THE EFFICIENCY AND TOXICITY OF SBCC IMPLANT PARTICLES ON B16 MELANOMA

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BACKGROUND

Some progress in cancer research was possible in recent years mainly due to important advances in nanotechnology. However, clinical use of nanomaterials is still hindered by limitations. In search for better performance and control of inoculated materials, the efficiency and toxicity of proprietary SBCC implant particles was assessed. These implants were designed to continuously liberate chemical antitumoral agents on-site,thus acting as local sources of nanoagents.

B16 Murine melanoma type tumoral cells were subjected to SBCC particles using in vivo and in vitro experimental models. The weight, viability and tumoral dimension were monitored. MTT Assay and Flow Cytometry were used to complete this investigation. A reduction of tumoral volume and a 54% survival rate in the treated animals compared to the controls was obtained. Also, a strong anti-proliferative activity, a high ability to produce apoptosis and no macroscopic toxicity were observed. The results show that the SBCC implants are effective against B16 melanoma cells, while there is no toxicity associated.

Keywords: apoptosis, B16 murine melanoma, cancer research, flow cytometry, in vivo

INTRODUCTION

Chemotherapy is associated with local and systemic side-effects and therefore better alternatives are under continuous investigation. Some progress in cancer research was possible in recent years mainly due to important advances in nanotechnology. From biofunctionalized quantum dots or nanotubes [1], nanoantennas [2] and nanoshells to magnetic nanoheaters and nanoparticle carriers [3], the fast development of nanomaterials has lead to important achievements in the fight against cancer [4]. Despite of the high spending in cancer nanotechnology research, there are still many issues and chalenges to be addressed before using nanomaterials in clinical practice [5]. The size of the nanoparticles has great benefits in the interference with intracellular activity, but their size induces limitations like: poor selective toxicity, poor penetration control within the tumor and also poor localization control within the body. Moreover, their aggregation in vital organs leads to organ toxicity and death [4]. We imagined that ideal antitumoral agents would be effective nontoxic microparticles of sizes sufficiently large so that, when implanted into the affected area, no free circulation within the body is possible. Knowing that normal vasculature is 8-10 μm in diameter and tumor vasculature ranges from 20 to 100 μm, we have used SBCC implant particles of 400 μm in size for investigations on antitumoral effect [6]. The particles continuously liberate antitumoral chemical agents on-site, at a nanometric level.

MATERIALS AND METHODS

B16 Murine melanoma type tumoral cells were subjected to SBCC particles using in vivo and in vitro experimental models. The weight, viability and tumoral dimension were monitored. MTT Assay and Flow Cytometry were used to complete this investigation.

Six groups of SPF C57BL6 mice weighing 18-20.1 g were used in these experiments, according to the protocol presented in Table 1.

Table 1. Implant particles (IP), Tumoral cells (TC)

Group 1-control			Group 2-positive control			Group 3		
No. of mice	Day 1	Day 11	No. of mice	Day 1	Day 11	No. of mice	Day 1	Day 11
5	-	-	13	$2.5x10^5$ TC cells/100µl/mouse	-	13	TC + IP simultaneously	-

Group 4			Group 5			Group 6		
No. of mice	Day 1	Day 11	No. of mice	Day 1	Day 11	No. of mice	Day 1	Day 11
15	TC	IP - peritumoral	14	TC	IP - intratumoral	5	-	MP

The treated mice received 5 implant particles each, subcutaneously, either simultaneously with the tumoral cells, peritumorally or intratumorally. All 5 particles were innoculated in the same place. The last day of our experiment was the day when all animals in the positive control group were dead (Day 23). Finally, the mice were euthanized and dissected. Anatomopathologycal examination was also performed in all animals both on the dead ones and at the end of experiment.

RESULTS AND DISCUSSION

The percentage of animals with measurable tumor 11 days after innoculation is presented in Figure 1. It is a striking result the fact that visible tumors appeared only in 22% of the mice of Group 3 and also the lowest percentage of dead animals at the end of experiment was observed in this Group (Figure 2).

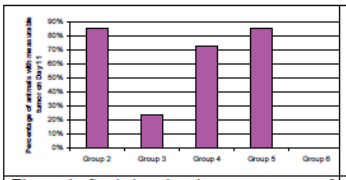

Figure 1. Statistics showing percentages of animals with measurable tumor on Day 11

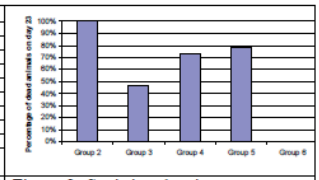

Figure 2. Statistics showing percentages of dead animals on Day 23

Figure 1. Statistics showing percentages of animals with measurable tumor on Day 11. Figure 2. Statistics showing percentages of dead animals on Day 23

A low local toxicity of the healthy cells is ensured by the immune system which walls-off the SBCC implants by forming a granuloma of fatty tissue arround them, as evidenced in our in vivo experiment (Figure 3). Dissection of the granuloma revealed the and the surrounding fatty tissue.

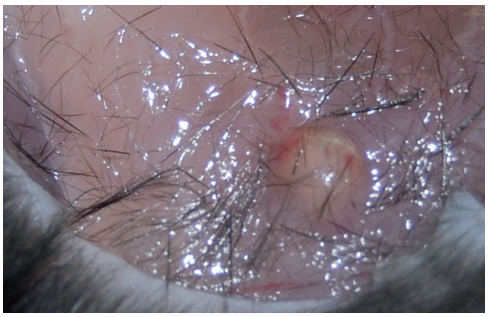

Figure 3. Photo showing the granuloma formed in healthy tissue due to th presence of SBCC particles, three weeks after inoculation.

The results showed that the efficiency of the SBCC particles increases proportionally with the number of particles, as revealed by MTT analysis (Figure 4).

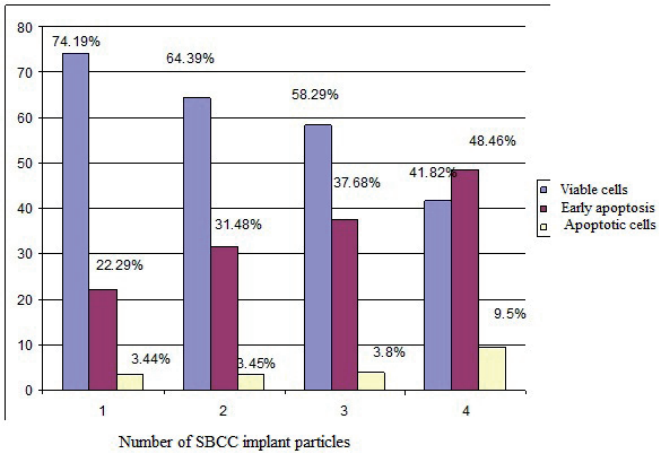

Figure 4. In vitro analysis

We observed the efficiency of SBCC implants to reduce tumor growth while safely remaining at the inoculation site. The size of the SBCC implant particles does not allow free circulation within the body, their agglomeration in vital organs being thus avoided, in contrast with nanomaterials. These implants were designed to continuously liberate chemical antitumoral agents on-site,thus acting as local sources of nanoagents.

The SBCC implant particles may represent a therapeutic potential against cancer.

ACKNOWLEDGEMENTS

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PREPARATION OF CHITOSAN NANOPARTICLES CONTAINING FMD VIRUS AS A NEW CARRIER FOR INTRANASAL VACCINE DELIVERY

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INTRODUCTION

Mucosal vaccination holds great promise as an approach to needle-free vaccination. Indeed, the nasal mucosa is the first site of contact with inhaled antigens, and the nasal-associated lymphatic tissue (NALT) at the base of the nasal cavity is important in the defense of the mucosal surface [1]. The aim of this study was to investigate the ability of chitosan (CS) nanoparticles with different molecular weights as a vehicle for the nasal administration of foot and mouth disease viruses (FMDv) as a model antigen to enhance both the systemic and mucosal immune responses after nasal administration in guinea pig. Moreover, our goal was to evaluate if a trimethyl derivative of CS (TMC) could affect the efficacy of the carrier for the delivery of the vaccine.

METHOD

CS nanoparticles were prepared according to the modified method of Alonso et al, [2] based on the ionotropic gelation of CS upon contact with the sodium tripoly phosphate (NaTPP) anions. For the association of FMDv with CS nanoparticles, 1ml FMDv solution (5×105 TCID50) was incorporated in the NaTPP solution. Nanoparticles were elicited by centrifugation at 16,000×g on a glycerol bed for 40 min at 4°C. Supernatants were discarded and nanoparticles were resuspended in 2ml deionized water for their in-vitro characterizations. The mean particle size and zeta potential of nanoparticles were deter-

mined by Dynamic Light Scattering (DLS) technique using a Zetasizer (Malvern Instruments, United Kingdom). The morphology and diameter of nanoparticles were examined by transmission electron microscopy. The in-vivo evaluation of the CS formulations was assessed in guinea pig following intranasal immunization.

RESULTS AND DISCUSSION

Results showed that nanoparticles with high loading efficacy (>83%), particle size within the range of 300–400 nm with positive charges (between 3.2 mV and12.95 mV) were obtained.

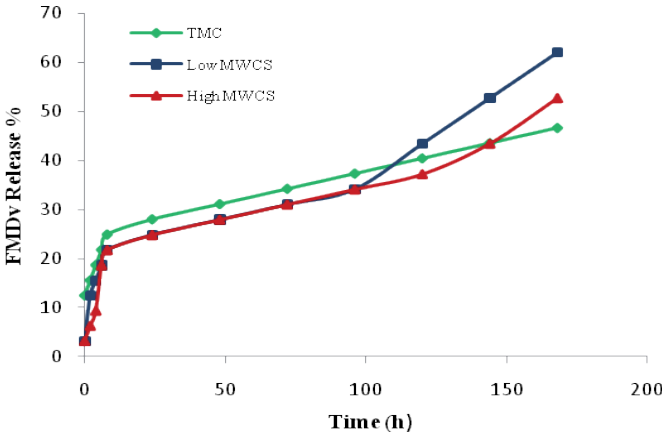

Fig.1. In vitro release of FMDv from nanoparticles made of TMC(●), low Mw CS(■) and high Mw CS(▲).

In-vitro release studies showed an initial burst over the first 8 h followed by an extended release of FMD viruses for up to 8 days (Fig.1). Antibody responses after intranasal administration of CS nanoparticles to guinea pig have been shown in figures 2 and 3. Enhanced immune responses were obtained with intranasal (IN) application of nanoparticle formulations.

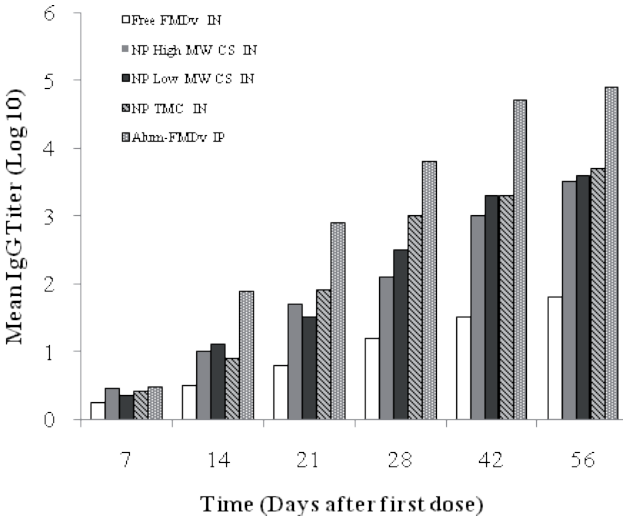

Fig. 2 Anti FMDv IgG end-point titers in guinea pig serum after intranasal administration of 5×106 TCID50 incorporated in CS nanoparticles on days 0, 7 and 14 (n=5).

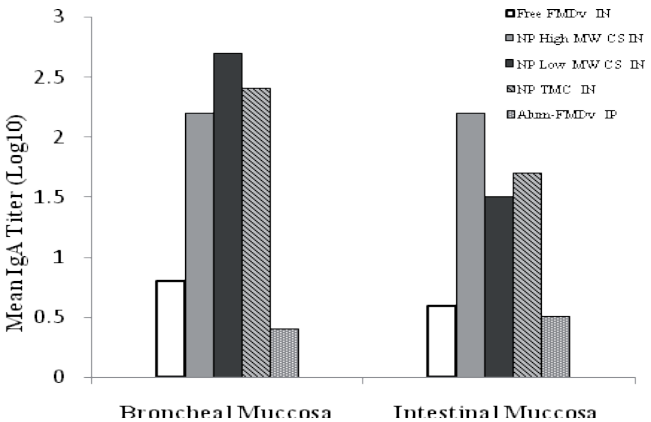

Fig. 3 Anti FMDv IgA end-point titers in guinea pig tissues after intranasal administration of 5×106 TCID50 incorporated in CS nanoparticles on days 0, 7 and 14 (n=5).

Following intranasal administration, FMDv-loaded nanoparticles elicited an increasing and enhanced humoral immunogenic response (IgG titers), as compared to the injection vaccine. Similarly, the mucosal response (IgA levels) achieved at 60 days post-administration was significantly higher for the FMDv-loaded CS nanoparticles than for the injection vaccine. Interestingly, TMC nanoparticles induced higher serum IgG titer when compared to those prepared with other chitosans. However, the highest IgA level was obtained by high Mw CS. Nanoparticle formulations developed in this study can be used as promising adjuvant/delivery systems for mucosal immunization.

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PREPARATION AND CHARACTERIZATION OF TRASTUZUMAB-PEG-NANOLIPOSOMES FOR TARGETING BREAST TUMOR CELLS

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INTRODUCTION

Stealth liposomes, nano-sized vesicular bilayer membranes comprising PEGylated phospholipids, are regarded as carriers for prolonged drug delivery and passive targeting to tumor cells. To promote specificity of the carrier, mAb-tagged liposomes named immunoliposomes were developed by post-insertion method. The developed carrier benefits active targeting of antiapoptotic HER-2 receptor in breast cancer cells; moreover, bioconjugation to these nanocarriers may improve in-vivo fate of anti-HER-2 mAb (trastuzumab). The main concern of the study is how the liposome composition could influence the degree of antibody incorporation into liposomal structure by post-insertion method. By the way, the incorporation of rhodamine-PE probe may be intended for reporting drug delivery and bioluminescent imaging purposes.

METHODS

Stealth liposomes of different lipid compositions including DPPC, DPPG, PEG-PE and cholesterol were prepared at different mole ratios and sized by extrusion through 100 nm membranes. Rhodamine-PE at 0.5% mole was added to the lipid mix if applicable.

PEG-PE-maleimide micelles were prepared by thin layer hydration followed by 5 min ultrasonic homogenization in HEPES-buffered saline (pH=6.5). Trastuzumab, recombinant humanized mAb against HER-2, was activated by Traut's reagent for 1 hour at different conditions. The thiolated antibody was purified by ultra-filtration (cut-off 10,000 Da) according to manufacturer instruction. The degrees of thiolation and protein recovery were determined by Ellman assay and Bradford reagent protein quantification, respectively. The thiolated trastuzumab was coupled to PEG-PE-maleimide micelles through thioether linkage at different conditions. The reaction was optimized according to response-surface methodology to obtain monothiolated antibody and the complete consumption of thiol by excess maleimide residues.

Immuno-liposomes were prepared by post-insertion method of combining the trastuzumab-PEG-PE micelle and nanoliposomes. The particle size distribution and mAb conjugation yield were determined by laser light scattering and Ellman/Bradford assay.

The degrees of antibody-micelle incorporation into the liposomes were determined by the lipid mixing assay [3]. The developed carriers were compared regarding their cell association in SK-BR-3 (3+ HER-2) and MCF-7 (1+ HER-2) cells using Tecan fluorescence microplate reader and epifluorescent microscope.

RESULTS

Trastuzumab was monothiolated at optimum condition of pH=7.3, Traut's reagent/trastuzumab mole ratio = 40 and incubation time= 1 hour. The purification was almost complete as confirmed by HP-SEC and Bradford assay (Fig.1).

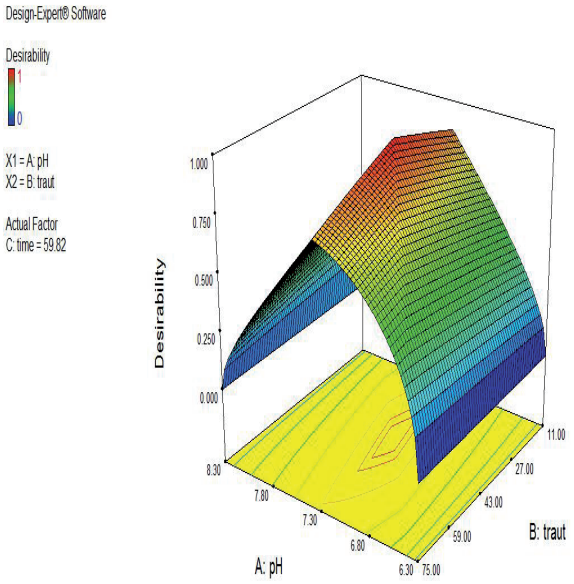

Fig. 1. Effects of pH and traut,s/trastuzumab mole ratio on degree of antibody thiolation

Stealth liposomes were homogeneous with mean sizes in range of 100-110 nm. The particles demonstrated uni-lamellar vesicular structure with about 85-90% lipid recoveries (Fig.2).

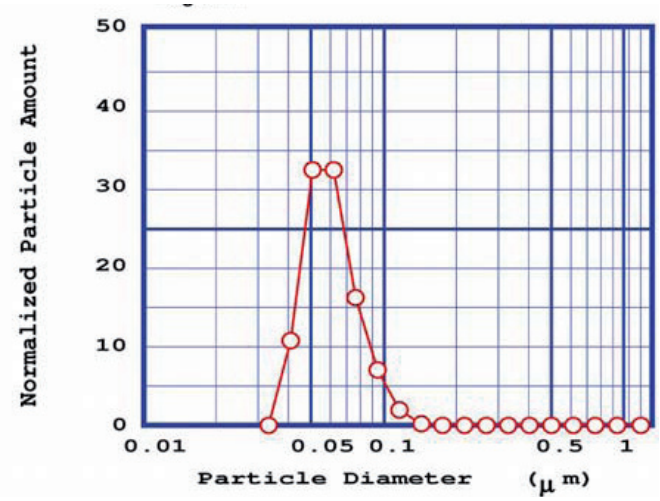

Fig. 2. Particle size distribution of the lipsomes (F4 formulation)

PEG-PE micelles were prepared at concentration about 100-fold above CMC (<10 μM) represented a colloidal dispersion with particle size ranging from 5-50 nm. The maleimide-micelle was conjugated to the monothiolated trastuzumab almost rapidly and thoroughly at maleimide / thiol ratios more than 3. SDS-PAGE confirmed that the integrity of trastuzumab was preserved during thiolation and micelle conjugation reactions.

Post-insertion method proved to be efficient method for functionalization of the liposomes, but depends on the lipid composition (Fig. 4).

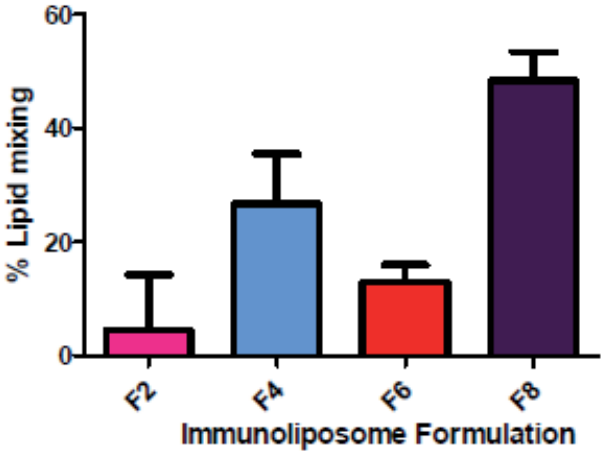

Fig. 4. Lipid mixing percentages for different liposome formulations: F2 (DPPC, PEG-DOPE, Rh-DOPE), F4 (DPPC, CHOL, PEG-DOPE, Rh-DOPE), F6 (DPPC, CHOL, Rh-DOPE), F8 (DPPC, DPPG, Rh-DOPE).

The results of lipid mixing assay were in agreement with that of cell association study. The cell binding was more pronounced for immunoliposomes of F4 and F8 formulations in SK-BR-3 (3+ HER-2) than MCF-7 cells (1+ HER-2).

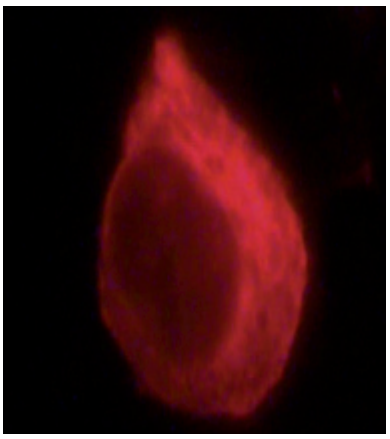

Fig.5. SK-BR-3 cell association of the rhodamine labeled F4 immunoliposomes (DPPC, CHOL, PEG-DOPE, Rh-DOPE)

CONCLUSION

The bioconjugates of Stealth nanoliposomes expected to be useful for active targeted cellular delivery of their cargo such as biopharmaceuticals and for simultaneous diagnostic purposes. The liposomes comprising are more capable of being merged with the immune-micelles.

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EXPERIMENTAL DATA ON THE BIOAVAILABILITY AND BLOOD BRAIN BARRIER PENETRATION OF A SYSTEMICALLY ADMINISTERED SILICA NANOPARTICLE

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The blood brain barrier penetration and availability of silica nanoparticles (SNP) in mice was studied in order to determine the possible uses as a drug carrier.

METHODS

Fluorescent silica nanoparticles (SNP+Glucose+PEG) were administered i.p. A different group of 6 mice received a similar volume

of glucose solution as control. The animals were sacrificed at 1h and samples of brain were harvested and prepared for analysis by fluorescence confocal microscopy.

Coronal sections were cut using a freezing microtome (CM 1850; Leica Microsystems, Germany). Sections were collected, mounted on slides and examined in a Laser Scanning Confocal Microscope TCS SPE (Leica Microsystems, Germany), 480 nm excitation and 610-630 nm emission.

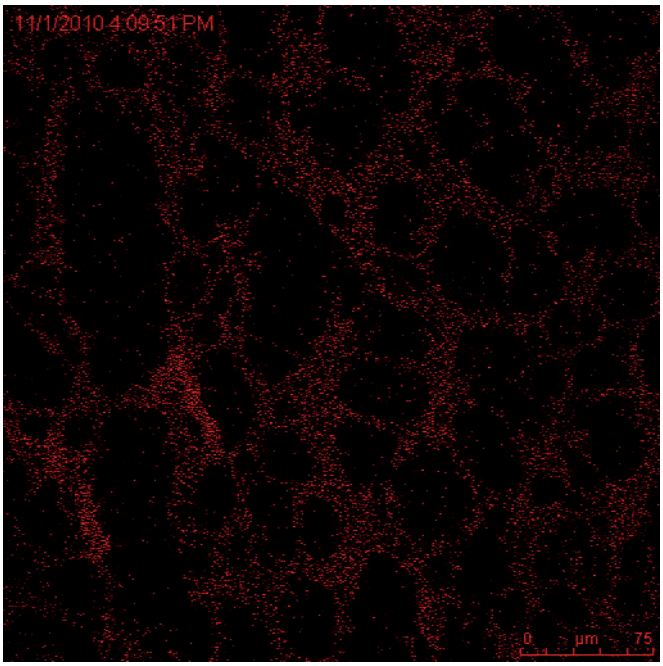
For positive control, the injected glucose SNP solutions were also examined under microscope.

RESULTS

Compared to the control sections, the sectiones from SNP+Glucose+PEG treated animals displayed displayed fluorescence of significant magnitude. Therefore, following i.p. administration, our new SNP penetrated the blood brain barrier. The results support the idea of using our SNP as container for modular drug delivery system with usage in pain control drugs.

CONCLUSIONS

The tested SNP is a possible candidate as a delivery system for modern analgesic drugs specifically targeting the central nervous system.



REPORTER CELLS FOR CANCER STEM CELL-TARGETING NANODRUG SCREENS

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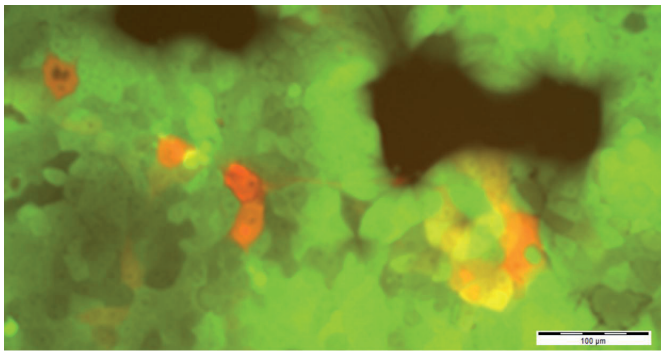
The cancer stem cell concept assumes that cancers are sustained by a small subpopulation of cells known as cancer stem cells (CSCs). These CSCs are thought to be resistant against conventional chemotherapies and to cause tumor relapse and metastasis. Therefore it is of high priority to specifically address these CSCs for the development of new strategies for cancer treatment. However, the big obstacle which currently hampers research on the CSCs is their low frequency within a tumor and their ability to undergo asymmetric cell division. The latter causes that only one of the two cells resulting from division retains the identity of the stem cell. CSCs can be identified through mammosphere formation under non-adhesive cell culture conditions and by defined surface markers. But even in a sorted population after short time in culture the percentage of CSCs will rapidly decrease due to asymmetric cell division, making it difficult to trace the fate of these cells, especially in large scale drug discovery approaches.

Here we report on the construction of a stable breast cancer cell line, in which non-CSCs display green fluorescence, while CSCs display red or blue fluorescence. Thus, a decrease or expansion of the CSC population can, for example, be determined by automated high-throughput fluorescence microscopy. This opens possibilities for future systematic screens for nanodrugs directed against CSCs.



IMMUNOTOXICITY TESTING OF NANODRUGS, NANOCARRIERS – NEW IN VIVO APPROACHES

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Nanotechnology is delivering new therapeutic and diagnostic products with improved kinetic properties with increasing rate. 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 make these products attractive, and 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.

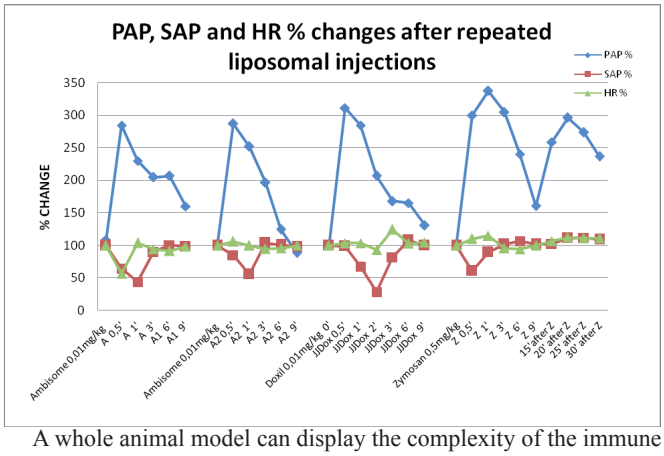
The size of these new formulations is in the 50-200 nm range, and the human immune system readily recognizes them as foreign materials, and triggers an immune response. The triggered response can be an inmediate hypersensitivity reaction (HSR) and/or an antigen-specific immune response, which may cause antibody production against the drug thereby altering its pharmacokinetic properties.

This presentation deals with the acute immunotoxic properties of nanodrugs/nanocarriers, presenting sensitive in vivo animal models to detect this potentially life-threatening side-effect. This pseudoallergic reaction (also called anaphylactoid, idiosyncratic or infusion reaction) correlates with the activation of the complement (C) system rationalizing the distinction of a novel subcategory of type I allergic reactions, called C activation-related pseudoallergy (CARPA)1,2. The reaction is accompanied by rapid release of inflammatory cytokines too. 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 hypersensitivity reaction occurs rapidly, it is dose dependent and usually shows spontaneous resolution.

The occurance rate is high (2-40%) and the reaction occasionally may be 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, Poly(amidoamine) dendrimeres and polyethylenimine block copolymers.

The reaction can be tested in an animal model, a pig CARPA model. Pigs provide a sensitive model for human pseudoallergic reactions. In case of intravenous application of liposomal drugs like Doxil or AmBisome in pigs, CARPA reactions occur in about 90% of animals. In humans moderate to severe HSRs occur in 45% of patients following Doxil3 treatment and in about 36% according to an MTD study4 with AmBisome. Pigs show all characteristics of severe human CARPA reactions: the reaction starts after about 1 min of drug infusion, 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 (T, ST elevation, extrasystoles), dyspnoe, flush. Occasionally, respiratory or cardiac arrest leads to a fatal outcome. In most cases the reaction spontaneously ceases after 6-15 minutes 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.

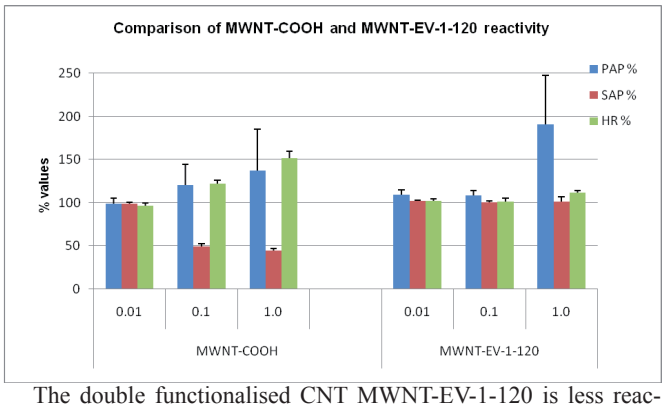


A whole animal model can display the complexity of the immune system and mirror the functional responses observed in human HSR reactions. The phenomenon can be explained with activation of the complement system on the surface of lipid particles, leading to anaphylatoxins (C5a and C3a) liberation and subsequent release reactions of mast cells, basophils and possibly other inflammatory cells in blood.

Different polymers and other gene and drug delivery systems, similarly to liposomes may also evoke immune side effects. These nanocarriers may deliver poorly soluble pharmaceuticals or may deliver drugs to earlier unreachable sites, intracellular targets. Such delivery systems are e.g. the PEGylated derivatives of poly(ethylene imine), poly(amidoamine) dendrimeres or carbon nanotubes. These systems can be constructed as multifunctional delivery systems, carrying the desired constructs to intracellular targets. The pig model is suitable also to testing these materials regarding their immunotoxicological character.

As as an example, reactions of carbon nanotubes (CNTs) are demonstrated in the figure below. CNTs are unique materials in that they appear to be able to move freely through biological tissues and enter cells and able to deliver antibodies for intracellular targets for cancer therapy. 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 profiles5,6. In the next figure different f-CNTs are tested in the pig model. The expriment clearly demonstrates that the functionalisation leads to different reactogenicity.

In mongrel dogs the CARPA reactions are similar, but are usually evoked by 10x higher doses, and the reactions occur only in about 30-40% of animals, but dogs are sensitive also to micellar carriers. These models are able to detect the adverse immunitoxicological reactions of nano-products already early in the development phase, preventing later clinical reactions and giving clues for succesful functionalisation.



The double functionalised CNT MWNT-EV-1-120 is less reactogenic, evokes PAP increase only with 10x higher dose without SAP, HR or ECG alterations.

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OBSERVATION OF MACROPHAGE RESPONSE TO COBALT/CHROMIUM METAL NANOPARTICLES: IN VITRO AND IN RETRIEVED TISSUE FROM METAL-ON-METAL HIP REPLACEMENT

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Metal and their alloys have been widely used as implantable materials and prostheses in orthopaedic surgery. However, concerns exist as the metal nanoparticles released from wear of the prostheses cause clinical complications and in some cases result in catastrophic host tissue responses. The mechanism of nanotoxicity and cellular responses to wear metal nanoparticles are largely unknown. The aim of this study was to characterise macrophage phagocytosed cobalt/chromium metal nanoparticles both in vitro and in retrieved tissue, and investigate the consequent cytotoxicity. Two types of macrophage cell lines, murine RAW246.7 and human THP-1s were used for in vitro study, and tissues retrieved from pseudotumour patients caused by metal-on-metal hip resurfacing (MoMHR) were used for ex vivo observation.

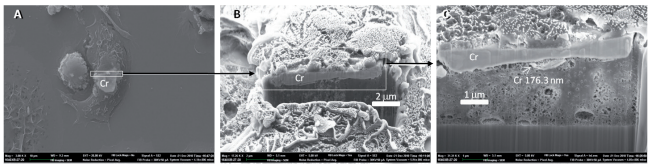


Figure 1. The FIB/SEM/EDS system for detection of THP-1 macrophage phagocytosis of Cr metal micro- and nano-sized particles.

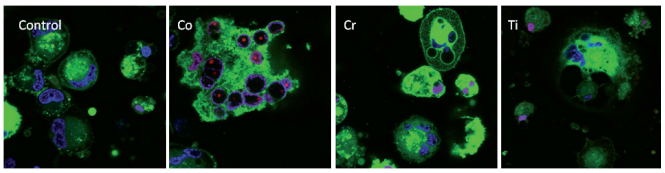
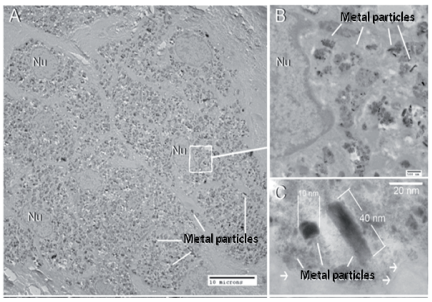


Figure 2. Confocal micrograph of nanotoxicity of Co, Cr and Ti metal nanoparticles to THP-1 macrophages

Transmission electron microscopy (TEM), scanning electron microscopy (SEM) in combination with backscatter, energy-disperse X-ray spectrometer (EDS), focused ion beam (FIB) were employed to characterise phagocytosed metal nanoparticles. Alamar blue assay, cell viability assays in addition to confocal microscopy in combination with imaging analysis were employed to study the cytotoxiticy in vitro. The results showed that macrophages phagocytosed cobalt and chromium nanoparticles in vitro and the phagocytosed metal particles were confirmed by backscatter SEM+EDS and FIB+EDS (Figure 1). These particles were toxic to macrophages (Figure 2) at a dose dependent manner.



The analysis of retrieved tissue from revision of MoMHR showed that cobalt/chromium metal nanoparticles were observed exclusively in living macrophages and fragments of dead macrophages

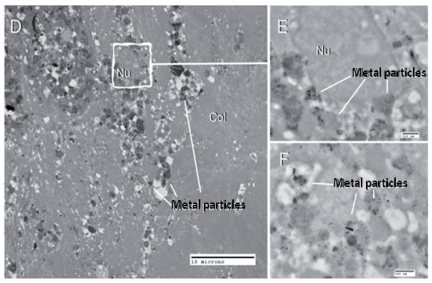


Figure 3) , but they were not seen within either live or dead fibroblasts. Dead fibroblasts were associated with dead and disintegrated macrophages and were not directly in contact with metal particles; chromium but not cobalt was the predominant component remaining in tissue. We conclude that as an important type of innate immune cells and phagocytes, macrophages play a key role in metal nanoparticles related cytotoxicity. Metal nanoparticles are taken up mainly by macrophages. They corrode in an acidic environment of the phagosomes. Cobalt that is more soluble than chromium may release inside macrophages to cause death of individual nanoparticle-overloaded macrophages. It is then released into the local environment and results in death of fibroblasts and is subsequently leached from the tissue.

Figure 3 Transmission electron micrographs of macrophage-phagocytosed Co/Cr metal nanoparticles (unpublished data)

APOLIPOPROTEIN A-1: THERMODYNAMIC CHARACTERISTICS OF THERMAL PROTEIN UNFOLDING

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Atherosclerosis is a clinical syndrome affecting a large number of people worldwide. Since blood levels of high-density lipoprotein (HDL) are inversely correlated to cardiovascular diseases, factors leading to increased HDL levels are atheroprotective. Apolipoprotein A-1, a 28.2 kDa protein, is the main protein constituent of HDL and facilitates its transport through the bloodstream by stabilizing the hydrophobic lipid core within the aqueous phase.

Although a lot of research has been done in this field, the existing literature reveals diverging answers on a number of elementary structural problems. We investigated thermal protein unfolding and used highly purified recombinant human Apo A-1 for our studies. Circular dichroism spectroscopy (CD) was used to determine secondary structure and shows predominantly alpha-helical structure between 25 - 85 °C. After cooling down the sample from 85 °C to 25 °C the spectrum was identical to the first one at this temperature, meaning the protein refolds reversibly.

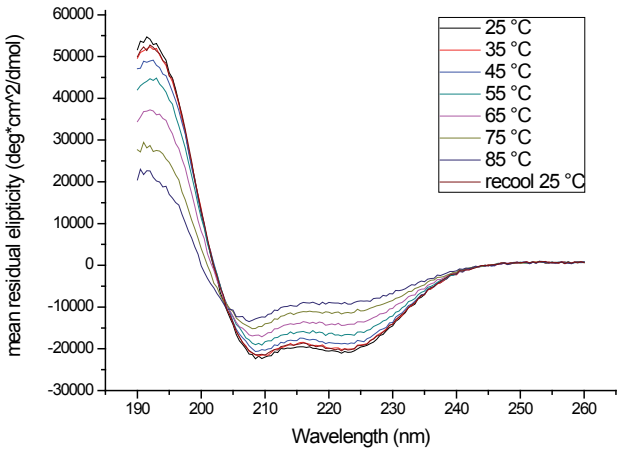

Figure 1: Far-UV-CD spectra of Apo A-I for temperatures between 25 °C (black line) to 85 °C (dark blue line) recorded at 10 °C steps. The Apo A-I concentration was 0.5 mg/mL (~17.6 µM) in buff-

er (100 mM NaF, 10 mM sodium phosphate, pH 7.4). Measurements at a lower concentration of 0.1 mg/mL (3.5 µM) yielded similar spectra in the range of 25 °C to 65 °C.

By heating up, approximately 33 % of the helical structure is lost, while the fraction of beta-sheet and random coiled increases from 26 % to 50 %, respectively from 19 % to 28 %. Thermodynamics of the helix -> sheet/random coiled transition was measured using differential scanning calorimetry (DSC). We found the maximum of molar heat capacity at 53 °C and a transition enthalpy of ΔUNH = 430 kJ/mol (102.7 kcal/mol). This is in excellent agreement with a theoretical analysis using the Zimm-Bragg model, which is normally used only for small peptides. In this cooperative model, the nucleation parameter for the first step of transition and the length of the secondary structural element plays the main role. The nucleation parameter was determined to be σ = 2.7 x 10-5, and the number of residues involved in the transition was calculated as n = 85. Analog to the CD data the DSC curves were completely reversible up to 80 °C.

CFTR SPECIFIC EFFECTS OF CPT-CAMP AND FORSKOLIN ON CELL METABOLISM

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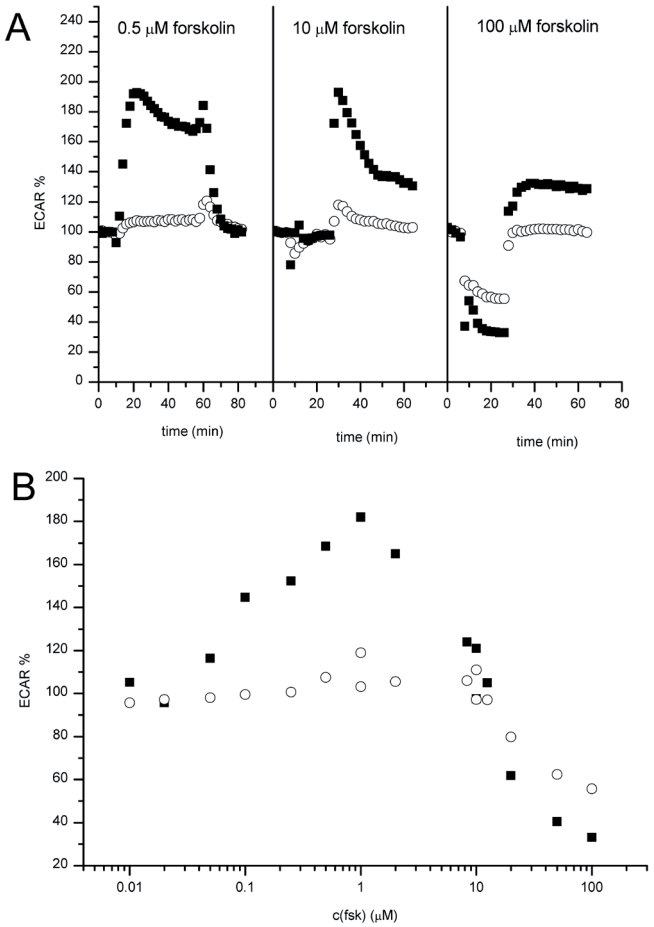

CFTR (ABCC7) is the only ABC protein known that functions as an ion channel. Defective CFTR causes severe diseases like cystic fibrosis and secretory diarrhea. It is known that the channel function is regulated in many ways including phosphorylation by protein kinase A and the presence of ATP. Furthermore there is evidence for ATPase activity similar to other ABC transporters. Nevertheless literature seems somewhat contradictory on the velocity of this ATP hydrolysis and on the influence of protein kinase A on ATPase activity. As membrane proteins can behave differently in purified reconstituted systems and native environments, we wanted to address this problem using micophysiometry[1] applied in our lab to measure the effect of drugs on ATP hydrolysis by ABC transporters in living cells. Microphysiometry was shown to allow monitoring the cellular ATP consumption rate by measuring the extracellular acidification and oxygen consumption rate, respectively, in real time in MDR1 transfected cells[2]. As stimulation of PKA by agents like forskolin or CPT-cAMP seems to be necessary for channel function of CFTR it is a prerequisite for our approach to understand the effects of these agents on cell metabolism. We found that both substances have specific effects on extracellular acidification and oxygen consumption rates of CHO cells stably expressing functional CFTR which cannot be observed in non-transfected cells or cells expressing CFTR-ΔF508. Both molecules seem to have at least two effects in the concentration range (0.1 µM ≤ c(CPT-cAMP) ≤ 400 µM and 0.01 µM ≤ c(forskolin) ≤ 100 µM) investigated. There is not only stimulation of activity as seen in electrophysiology experiments, but also inhibition at higher concentrations. This results in bell-shaped activation profiles resembling those seen for ABC transporter substrates. Furthermore forskolin seems to have an additional CFTR independent inhibitory effect on cell metabolism at concentrations c > 10 µM which is most likely due to inhibition of glucose import. The results for forskolin are shown in Fig. 1.

Figure 1: Effects of forskolin on extracellular acidification rates (ECAR).

■ CHO CFTR, □ CHO CFTRΔF508; A: Normalized ECAR vs. time. Black bars indicate forskolin application. Stimulation at low and inhibition at high concentration could be observed in functional CFTR expressing cells. B: Normalized ECAR vs. forskolin concentration. Up to c(fsk) = 10 µM a bell-shaped CFTR-specific activation profile could be observed. At higher concentrations c(fsk) > 10 µM an unspecific inhibition seems to mask other effects.

We could also show that these effects cannot be completely suppressed by inhibition of protein kinase A, suggesting that especially forskolin has additional CFTR dependent effects in living cells, that might even include direct interactions with the transmembrane domains of CFTR. Microphysiometry can thus be used to measure CFTR specific effects on cell metabolism and allows monitoring the influence of drugs on CFTR function in a native environment. As we could easily distinguish between CFTR specific effects and general effects, i.e. decreased glucose import, that may indirectly influence CFTR mediated chloride currents and may then lead to misinterpretations of experiments using only ion channel conductance as readout. The present analysis shows that microphysiometry successfully complements electrophysiology as well as ATPase activity measurements in purified systems.

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