CLINAM
European Foundation for Clinical Nanomedicine

CLINAM 7/ 2014

nanomedicine
EUROPEAN TECHNOLOGY PLATFORM

7th Conference and Exhibition, June 23-25, 2014

Supported by the Swiss Confederation, Swiss Department of Economic Affairs, Education and Research

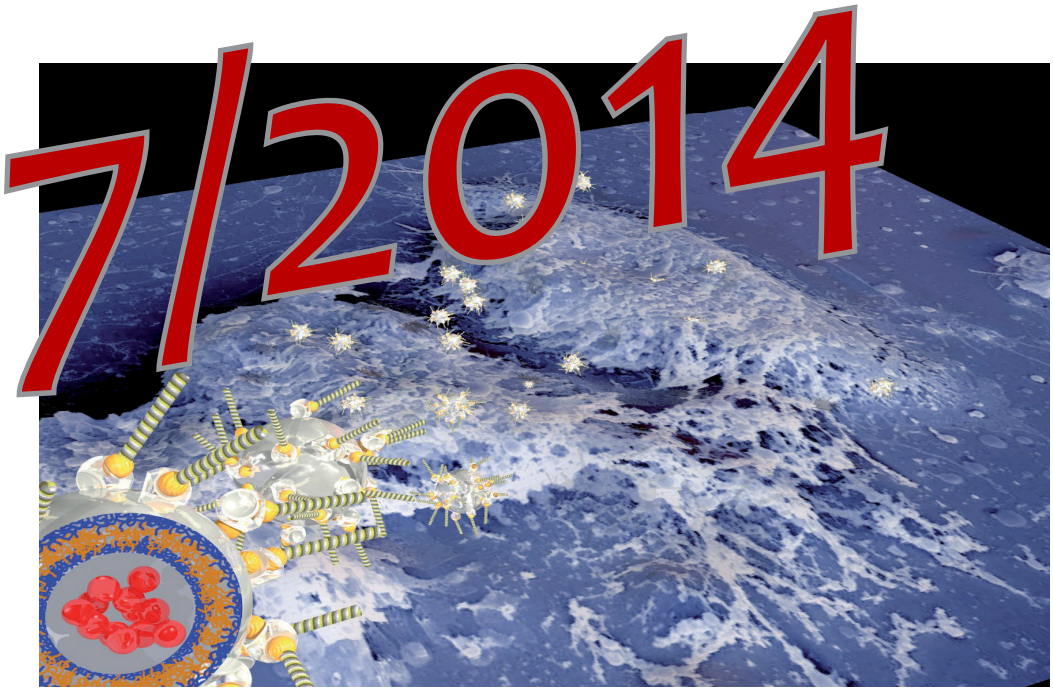
The European Summit for Clinical Nanomedicine and Targeted Medicine

Paving the Way to Personalized Diagnostics & Therapy

Main Sections of the Summit

- **7th European Conference for Clinical Nanomedicine:**
- **5rd ETP Nanomedicine Brokerage Session**
- **4nd ETP Nanomedicine Policy – Industry Table**
- **7th Foyer Exhibition & University Village**

CONFERENCE PROCEEDINGS



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

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

CLINAM European Foundation for Clinical Nanomedicine

CLINAM 6/14 with ETPN

European Summit for Clinical Nanomedicine and Targeted Medicine

Basel, Switzerland, Monday, June 23, 09.00 h – Wednesday, June 25, 2013, 17.45 h

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INTERVENTION ABSTRACTS

POSTER ABSTRACTS

INTRODUCTION BY THE RECTOR OF THE UNIVERSITY OF BASEL

Dear Nanomedicine community,
dear members of the CLINAM 7/ 2014 European Summit for Clinical Nanomedicine and Targeted Medicine

On behalf of the University of Basel, I would like to warmly welcome you to Basel. You are the scientific community of the field of Nanomedicine from 34 countries in the world, who have gathered in our city to attend the CLINAM-Summit. All stakeholder groups are represented here, because the field of Nanomedicine is highly interdisciplinary and promises many mutually rewarding discussions.

In its long history which spans over 550 years and has experienced a variety of scientific innovations, our University of Basel has remained faithful to its openness for new perspectives, attracting scientists from all over the world, some of whom chose to stay with us for their entire academic career. Also thanks to the presence of big pharmaceutical companies, our city has developed into a true cluster for Life Sciences.

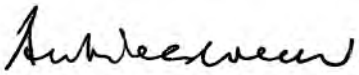
In 1971, we founded the Biozentrum of our University, which is today our largest science department and has acquired a stellar reputation among peers. The primary focus of this interdisciplinary institute was always basic molecular and biomedical research and teaching; today, scientists from more than 30 nations are engaged in investigating the molecular basis of biological processes. This Biozentrum was founded with the vision of developing an interdisciplinary research facility. It is this attention for interdisciplinary perspectives that continues to generate important results.

If you want a demonstration of the excellence of research at the Biozentrum in your field, take a closer look at the University Village table “Switzerland”, where you will see an example from Nanomedicine which was developed in the Center for Cellular Imaging and Nanoanalytics of the Biozentrum. The innovative methods to analyze the proteome of individual cells which are developed by the combination of microfluidics, cantilever technology and electron microscopy are the result of the research team of Prof. Henning Stahlberg.

When developing the concept of the CLINAM summit, Beat Löffler recognized the University of Basel’s great strengths in Life Sciences and of Nanophysics, with its world-famous Nanoscience Institute which can be seen as a major research center in this field, thus continuing a tradition initiated by the later Nobel laureates Heinrich Rohrer and Gerd Binning, the scanning tunneling microscopy, further developed by our own Professor Christoph Gerber to the system of Atomic Force Microscopy (AFM). The Swiss Nanoscience Institute (SNI) developed from the National Center of Competence in Research (NCCR) “Nanoscale Science” constitutes one of the focal areas at our university.

Pioneer work in the field of Nanomedicine has been conducted for many years by Prof. Patrick Hunziker, whose research includes the application of intelligent nanomaterials for medical application in a range of models and disease contexts. His work is also shown at the Swiss table of the University Village.

In wishing you a fruitful and interesting meeting, I would like to wish CLINAM a continued intense collaboration with the University of Basel and all the best for its future scientific endeavors.



Prof. Dr. Antonio Loprieno
Rector of the University of Basel

CURRICULA VITAE OF SPEAKERS

Johann S. Ach

Center for Bioethics & Center for Advances Study in Bioethics
University of Münster
Geiststraße 24-26, D-48151 Münster
Tel. 0251 83 23559
eMail: ach@uni-muenster.de

Johann S. Ach, Priv.-Doz. Dr. phil., studied philosophy, theology and sociology in Augsburg und Münster and adult education in Kaiserslautern. 1991 Magister. 1997 PhD (moral problems of animal experimentation in biomedical sciences); 2010 Habilitation (‚Praktische Philosophie mit besonderer Berücksichtigung der Angewandten Ethik'). 1993 - 2000 research assistant at the Department of Philosophy at the University of Münster; from 2000 to 2002 research assistant at the Study commission ‚Recht und Ethik der modernen Medizin' (law and ethics of modern medicine) at the German parliament. Since October 2003 managing director of the Center for Bioethics at the University of Münster; since November 2009 in addition academic coordinator of the Center for Advanced Study in Bioethics ‚Theoretische Grundfragen der Normenbegründung in Medizinethik und Biopolitik' at the University of Münster.

Surveying activities for the Office of Technology Assessment at the German Bundestag, the Federal Department of the Environment, Transport, Energy and Communications of the Swiss confederation, the Stem Cell Network NRW, the ethics board of Nano2Life and foodwatch (an independent, non-profit organization that exposes food-industry practices).

Lectureships at the Universities of Münster, Osnabrück, Berlin and Rostock. Lecturer at the Institute for Ethics, History and Philosophy of Medicine and at the Department for Philosophy at the University of Münster.

MAIN AREAS OF WORK

Ethics and applied ethics (autonomy in bioethics); ethical issues in modern medicine (regenerative medicine, nanomedicine, human enhancement); animal ethics.

MEMBERSHIPS AND FUNCTIONS

Academy for Ethics in Medicine; Nanomed Round Table (2009); Photonics4Life, WP 11 International Contacts and Cooperation, Regulatory and Ethical Issues (2009-2012); Standing Committee on Education and Ethics der International Society on Optics Within Life Sciences (seit 2012).

PUBLICATIONS (selection)

- *Warum man Lassie nicht quälen darf. Tierversuche und moralischer Individualismus* (Harald Fischer: Erlangen 1999)
- *Ethik der Organtransplantation* (gemeinsam mit Michael Anderheiden und Michael Quante, Harald Fischer: Erlangen 2000)
- *Bioethik: Disziplin und Diskurs. Zur Selbstaufklärung angewandter Ethik* (gemeinsam mit Christa Runtenberg, Campus:Frankfurt/M. 2002)

Coeditor of

- *Herausforderung der Bioethik* (frommann-holzboog: Stuttgart-Bad Cannstatt 1993)
- *Hirntod und Organverpflanzung* (frommann-holzboog: Stuttgart-Bad Cannstatt 1997; zweite, erw. Auflage 1999)
- *Hello Dolly?* Über *das Klonen* (Suhrkamp: Frankfurt/M. 1998)
- *No Body is Perfect. Baumaßnahmen am menschlichen Körper* (transcript: Bielefeld 2006)
- *Nanobiotechnology, Nanomedicine and Human Enhancement* (Lit.: Berlin 2008)
- *Die Frage nach dem Tier. Interdisziplinäre Perspektiven auf das Mensch-Tier-Verhältnis* (Lit.: Berlin 2010)
- *Im Dienste der Schönheit. Interdisziplinäre Perspektiven auf die Ästhetische Chirurgie* (Lit.: Berlin 2011)
- *Proceed with caution? Concept und application of the precautionary principle in nanobiotechnology* (Lit.: Berlin 2012)
- *Grenzen der Selbstbestimmung in der Medizin* (mentis: Münster 2013)
- *wissen.ethik.leben. Themen und Positionen der Bioethik* (mentis: Münster 2014)

SELECTED PAPERS

- *Nano-Food, Nano-Medizin, Nano-Implantate: Ausgewählte ethische Fragen und Probleme der Nanobiotechnologie* (In: Jahrbuch für Recht und Ethik 15, 2007, 153-170)
- *Ethische Dimensionen der Nanobiotechnologie* (In: Hoeren, Thomas u.a. eds.: Leitfaden zum deutsch-brasilianischen Technologietransfer in ausgewählten Bereichen der Biotechnologie. Münster 2007, 75-78);
- *Cognitive Enhancement – was spricht dagegen?* (In: zur debatte 2/2010, 37-38); Human Enhancement (In: Grunwald, A. ed.: Handbuch Technikethik. Stuttgart/Weimar: Metzler 2013).

Rafik Ait Sarkouh

Ph.D

Rafik holds a doctorate in medicinal chemistry, specialized in drug delivery, from Curie Institute (Paris) and Paris Descartes (Paris5). He also holds a doctorate in molecular biology from Imperial College (London) and a Master degree in organic and bio-organic chemistry from the Pierre and Marie Curie University (Paris-).
His research is based on an interdisciplinary approach, combining biology and chemistry.
His scientific interests are focused on molecular and therapeutic innovation in the fields of medicinal chemistry, including development of new bio-conjugation techniques.
He also contributes to the elucidation and understanding of basic mechanisms of cell growth, particularly in the context of the fight against cancer.
In 2013 he joined Nanobiotix, where he works as clinical research associate in the Clinical Department taking care in clinical studies form phase 1 to 3.

Christoph Alexiou

Prof. 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.

Theresa Mary Allen

PhD, FRCS
Professor Emeritus of Pharmacology and Oncology, University of Alberta, Edmonton, AB
Strategic Advisor, Drug Delivery, Centre for Drug Research & Development, Vancouver, BC
Adjunct Professor, Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC

EDUCATION

R.T., Medical Technology, 1961 Ottawa General Hospital; B.Sc. Honors, Biochemistry, 1965, University of Ottawa; Ph.D., Oceanography, 1971, Dalhousie University.

RESEARCH

Theresa Allen is a Professor Emeritus of Pharmacology and an adjunct professor of Oncology at the University of Alberta. She has been active in the drug-delivery field for over 30 years, has over 230 peer-reviewed publications, many highly cited, and is an inventor on several patents. The product Doxil,Ò the first anticancer nanomedicines,came out of pioneering research in her laboratory at the University of Alberta. She has also been active in the area of new drugs from natural products, resulting in two drugs proceeding into Phase II clinical trials. Her recent work in developing ligand-targeted therapeutics for small molecule therapeutics and gene medicines are at the leading edge of this new field and the methods she developed are widely used throughout the field. Her former graduate students occupy senior positions in academia and industry throughout the world.
Dr. Allen is a founding member and strategic advisor of the Centre for Drug Research & Development (CDRD, www.cdrd.ca), which is a novel hybrid organization devoted to advancing promising medical discoveries from academia to a commercially attractive stage. CDRD evolved from the recognition by the Founders of the pressing need to improve the translation of medical discoveries made in the universities and teaching hospitals into new drugs and technologies that result in global economic and health benefits. CDRD identifies, advances, and commercializes innovative life science discoveries via a unique structure that connects scientists, health care providers, and industry.

HONORS AND AWARDS

Governor General's award for highest average graduation from high school; Gold Key award for highest average B.Sc. in Biochemistry; Fisheries Research Board of Canada grant for 5 years for Ph.D. in oceanography; Defence Research Board of Canada grant for 1 year for post-doctoral research in Neurochemistry; Killam Professor, U. of Alberta, 1995-96; McCalla Research Professor, U. of Alberta, 1998-99; finalist ASTech Award for Leadership in Alberta Technology, 1999; Cygnus Award (Controlled Release Society) for excellence in guiding graduate student research; Novartis award 2000 (Pharmacological Society of Canada) for significant contributions to the advancement and extension of knowledge in Pharmacology; winner ASTech Award for Leadership in Alberta Technology, 2001; winner Alec Bangham International Award for contributions to liposome research, 2002; winner Leadership Award from the Canadian Society for Pharmaceutical Sciences, 2004; elected a Fellow of the Royal Society of Canada, 2005. Elected to the College of Fellow of the Controlled Release Society, 2012

María José Alonso

María José Alonso is full professor of Biopharmaceutics and Pharmaceutical Technology at the University of Santiago de Compostela (USC).
She has made critical contributions to the design and development of novel nanostructures for the targeted delivery of drugs and vaccines and to the understanding of the interaction of nanoparticles with mucosal barriers.
She has been the coordinator and PI of several consortia and cooperative projects financed by the WHO, the "Bill ad Melinda Gates Foundation" and the European Commission. Currently, she is coordinating the TRANS-INT consortium (oral peptide delivery).
She is the author of more than 200 international scientific contributions with more than 9000 cites (H factor 57). Because of the quality of her papers she has been among the TOP TEN in Pharmacology, according to the Times Higher Education international ranking (2011). She has also been the inventor of 16 patents.
She is part of the scientific boards of a number of societies and a member of the executive board of the Controlled Release Society. She has also received 15 Awards, among them the "King Jaume I Award" given to the best researcher in the area of new technologies in Spain and the Maurice Marie Janot Award 2014 (APGI).

Daniel G. Anderson

Dr. Daniel G. Anderson is the Goldblith Associate Professor at the Massachusetts Institute of Technology, and a member of the Department of Chemical Engineering, the Institute of Medical Engineering and Science, and the David H. Koch Institute for Integrative Cancer. He received his PhD in molecular genetics from the University of California at Davis. At MIT, he pioneered the use of robotic methods for the development of smart biomaterials for drug delivery and medical devices. His work has led to the first methods rapid synthesis, formulation, analysis, and biological evaluation of large libraries of biomaterials for use in medical devices, cell therapy and drug delivery. In particular, the advanced drug delivery systems he has developed provide new methods for nanoparticulate drug delivery, non-viral gene therapy, siRNA delivery, and vaccines. His work has resulted in the publication of over 190 papers, patents and patent applications. These patents have led to a number of licenses to pharmaceutical, chemical and biotechnology companies, and a number of products that have been commercialized or are in clinical development.

Christopher Anzalone

Dr. Christopher Anzalone is President and CEO of Arrowhead Research Corporation, a targeted therapeutics company primarily focused on developing RNAi drugs. Dr. Anzalone has a wealth of experience in nanotechnology, biotechnology, company-building, and venture capital. Prior to joining Arrowhead, Dr. Anzalone founded and built The Benet Group, a private equity firm focused on creating and building new nanobiotechnology companies from university-generated science. Prior to Benet, Dr. Anzalone was a partner at the Washington DC-based private equity firm Galway Partners, LLC where he was in charge building new business ventures. Dr. Anzalone holds a Ph.D. and M.A. in Biology from UCLA and a B.A. in Government from Lawrence University.

Werner Arber

born 1929, Swiss citizen

EDUCATION

Studies of natural sciences at the Swiss Polytechnical School in Zürich (diploma) and at the University of Geneva (PhD). Postdoctoral stay at the University of Southern California in Los Angeles.

EXPERIENCE

- 1960/70 Research assistant, later associate professor in Molecular Biology at the University of Geneva.
- 1970/71 Visiting research Professor, University of California, Berkeley
- 1971/96 Full Professor of Molecular Microbiology, Biozentrum, University of Basel
- 1986/88 Rector of the University of Basel
- 1996/99 President of the International Council for Science (ICSU)
- 1978 Nobel Prize in Medicine/Physiology for the discovery of restriction enzymes and their application to problems of molecular genetics
- 1981 Member and since 2011 Präsident of the Pontifical Academy of Sciences

Research topics: Microbial genetics, restriction enzymes, horizontal gene transfer, mobile genetic elements, site-specific recombination, molecular mechanisms of microbial evolution.

Marianne Ashford

Upon completion of her PhD into Oral Drug Delivery Systems to the Colon, in the Department of Pharmacy and Pharmaceutical Science, the University of Manchester, UK, Marianne joined ICI Pharmaceuticals in Cheshire (later to become Zeneca and then AstraZeneca). Marianne worked in a Pharmaceutical Research group initially looking at formulation approaches for poorly soluble compounds and building up the biopharmaceutics capability. She became Team Leader and then Associate Director of a Preformulation and Biopharmaceutics group evaluating the product design characteristics of candidate drugs in the Oncology, Inflammation and Cardiovascular therapy areas, supplying pre-clinical formulations as well as providing solid state science and biopharmaceutics support across the Discovery and Development portfolio. In 2005, Marianne moved to a project management role leading the pharmaceutical development of a number of AstraZeneca's Oncology development drugs at all stages of clinical development.
In 2011, Marianne returned to a scientific role focused on exploiting drug delivery approaches to improve the therapeutic index of medicines and in particular, has worked closely with the Oncology teams to initiate a number of joint projects and collaborations in the Nanomedicine area. Marianne has authored a number of book chapters, research papers and patents in the pharmaceutical science arena and is keen to use her scientific knowledge and experience to improve therapies for patients.

Lajos (Lou) P. Balogh

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

Dr. Lajos (Lou) Balogh is the Editor-in-Chief of the journal Nanomedicine: Nanotechnology, Biology and Medicine (Elsevier, 2012 Impact Factor=6.93, 5-year IF=7.46, www.nanomedjournal.com) and member of numerous USA, European, and International expert committees including the Steering Committee of the American National Standard Institute Nanotechnology Panel and the US Technical Advisory Committee to the International Standard Organization on Nanotechnology (TC-229). Lou is one of the five Founders of the American Society for Nanomedicine. (http://www.amsocnanomed.org)
Lou is Chief Scientific Advisor and Principal of AA Nanomedicine & Nanotechnology Consultants, North Andover, MA (baloghl@prodigy.net), providing expert advice, scientific evaluation, and feasibility assessments for nanomedicine related R&D projects, business plans, as well as technology due diligence for private companies, government agencies, and investors in Nanomedicine, Nanobiotechnology, and Nanotechnology since 2000.
Dr. Balogh is the former Co-Director of the NanoBiotechnology Center and Director of Nanotechnology Research in the Department of Radiation Medicine at the Roswell Park Cancer Institute, Buffalo, NY. He received his Ph.D. with honors from the Kossuth L. University in Hungary in Chemical Technology and was invited to the University of Massachusetts Lowell as a Visiting Professor in 1991. Later he worked at the Michigan Molecular Institute as a senior scientist, and had faculty appointments at the University of Michigan, Ann Arbor, and later at the University at Buffalo, SUNY. Dr. Balogh is an Adjunct Professor of Pharmaceutical Sciences at Northeastern University, Boston, MA, and Professor and Distinguished International Scientist of the Chinese Academy of Sciences. He authored or coauthored over 150 scientific publications and six book chapters, delivered more than 120 presentations was awarded 12 patents in various disciplines, including nanotechnology and nanomedicine.

Stephan Barcikowski

Prof. Dr.-Ing., Chair of Technical Chemistry I, University of Duisburg-Essen, and Center for Nanointegration Duisburg-Essen (CENIDE), Universitaetsstr. 7, 45141 Essen, Germany
Tel.: +49 (0)201 - 183 3150
Internet: http://www.uni-due.de/barcikowski/
YouTube Channel: http://youtube.com/nanofunction
Email: stephan.barcikowski@uni-due.de

Stephan Barcikowski studied chemistry in Braunschweig and Hannover, after which he worked for the industrial laser manufacturer Rofin-Sinar Laser AG and received his doctorate award in Mechanical Engineering. At Laser Zentrum Hannover e.V. (LZH), a private-non-profit research center, Barcikowski built up the Nanomaterials group and the research group "Nanoparticles" in the Cluster of Excellence "REBIRTH", and led the institute's Material Processing Department. In 2010, he co-founded the company Particular GmbH and organized the 1st international conference on laser ablation in liquid ANGEL. In 2011, he accepted the call to the Chair of Technical Chemistry I at the University of Duisburg-Essen.
He received the "first prize for scientific work" by the German Foundation of Industrial Research (Stiftung Industrieforschung). In 2012, he has been nominated for the Berthold Leibinger Innovation Award and in 2013, he received the Faculties' price for best teaching.
Stephan Barcikowski is working on applications of liquid-assisted laser materials processing in chemistry. His research fields reach from up-scaling process technology for laser-based nanomaterial synthesis to the functionalization of nanoparticles and nanocomposites for biotechnology, biomedicine and energy science.
Prof. Barcikowski is Editor-in-Chief of the peer-reviewed journal "BioNanoMaterials", and has served as Guest Editor for the Journal of Physical Chemistry Chemical Physics, ANGEL Themed Issue (11 articles, 115 pages), 2013, Vol. 15, 9, 3009-3114 as well as the Journal of Physical Chemistry, ANGEL Themed Issue (26 articles, 95 pages), 2011, Vol. 115, 12, 4985-5180.
He has more than 400 publications, including 90 reviewed papers and 14 patent files. Recently, he launched a scientific video channel 'nanofunction' on youtube.

Yechezkel (Chezy) Barenholz

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

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

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

His applied research centers around the development of drug delivery systems (DDS) and drugs based on such DDS including low molecular weight anti-cancer, anti-inflammatory, and local anesthetic drugs, as well as delivery systems for peptides, proteins, nucleic acids, and vaccines. This is exemplified by Doxil®, which was based on his invention and was developed to an FDA- and worldwide-approved anti-cancer drug by Professor Barenholz together with the oncologist Professor Alberto Gabizon, and SEQUUS Pharmaceuticals, Menlo Park CA, USA. Doxil® (Caelyx® in Europe) is the first FDA-approved nano drug and the first FDA-approved liposomal drug (1995). It is distributed today all over the world by Johnson and Johnson. Doxil sales exceeds half a billion dollars a year. Professor Barenholz, with the help of others, based on his inventions, founded the following start-up companies: 1. NasVax Ltd (now a public company on the Israeli stock market), a vaccine developing company, based on, among others VaxiSomeTM , a Barenholz-invented polycationic sphingolipid adjuvant; 2. Moebius Medical, which develops a liposome-based medical device for treatment of osteoarthritis. Moebius finished successfully her first clinical trial and are now preparing for a large pivotal clinical trials; LipoCure Ltd for the development of liposomal nano drugs based on Professor Barenholz' inventions for treatment of cancer and inflammatory diseases [rheumatoid arthritis (RA) and multiple sclerosis (MS)], as well as for special liposomes remote loaded with local anesthetics for prolonging analgesia duration. Two of the liposomal drugs under development in LipoCure are in final preparation for clinical trials.

Professor Barenholz is a coauthor of more than 360 scientific publications having altogether more than 10,000 citations. He is a co-inventor in more than 30 approved patent families. He was an executive editor of Progress in Lipid Research, an editor of 4 Special Issues, and is on the editorial board of 5 scientific journals.

Professor Barenholz was awarded the following prizes and awards: the Donders Chair Professor at the Faculty of Pharmacy, University of Utrecht, The Netherlands {1992); the Kaye award for innovation, twice (1995 & 1997) at the Hebrew University, Jerusalem, Israel; the international Alec D. Bangham (the founder of the Liposome field) award (1998); the Teva Founders Prize (2001), Israel; an Honorary Doctor degree for "outstanding contributions to lipid membrane research and highly innovative achievements in nanomedicine" from the Technical University of Denmark (DTU) in 2012, (Copenhagen, Denmark); and the international Controlled Release Society's (CRS) most prestigious CRS Founders Award for 2012. In 2003 Professor Barenholz founded (from Doxil royalties) the "Barenholz Prizes" for Israeli Ph.D. students to encourage excellence and innovation in applied science.

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

Thierry Bastogne

Dr., 45 years (Born July, 1968)
Professor in the Université de Lorraine
Prime d'Excellence Scientifique (rang A) since 2012
Phone: +33 383 684 473
Email: thierry.bastogne@univ-lorraine.fr

DEGREES AND TITLES

• Habilitation à Diriger des Recherches, Experimental Modeling of Interconnected Systems - Applications in Systems Biology, Université Henri Poincaré, Nancy, 2008

• Doctor of Philosophy in System Control Engineering, Université Henri Poincaré, Nancy, 1997

RESEARCH INTEREST

Topic: Modeling and control of tumors with nanoparticles activated by ionizing and non-ionizing radiations. My research activity is shared between two scientific projects:

• Research leader of project team "Biologie intégrative : cybErnétique des thérApies par rayonneMent" at CRAN (Centre de Recherche en Automatique de Nancy), Univ. de Lorraine - CNRS UMR 7039

• Member of the INRIA team BIGS (BIology, Genetics and Statistics), INRIA Nancy Grand-Est

Keywords: Data-driven modeling, Control theory, Systems Biology, Cancerology, Biostatistics

MAJOR ACHIEVEMENTS (RESEARCH & EDUCATION)

• More than 60 international publications in peer-refereed journals and conferences

• Thematic mobility and Visiting positions:

- Centre de Lutte Contre le Cancer de Lorraine (Centre Alexis Vautrin, Nancy) (2005-2006)
- Centre de Recherche Public de la Santé, NorLux Neuro-Oncology Laboratory (Luxembourg), S. Niclou (2011-2012)

• Coordinator and member of European & National scientific projects in nano-medicine & systems biology:

- Nano-Xrays (2011-2014) Nanoparticles-based X ray-induced photodynamic therapy in glioblastoma multiforme, INCa.
- PDTX (2010-2013) Active Nanoplatforms for Photodynamic Therapy, ANR.
- Target-PDT (2009-2013) Photodynamic Therapy using photosensitizer-doped targeted organic nanoparticles, FP7 ERA-NET EuroNanoMed: European Innovative RTD Projects Proposals in Nanomedicine
- Nano-VTP (2008-2011) Nanoparticles for Imaging and Vascular Photodynamic Treatment of Brain Tumors, ANR

• Co-founder of the start-up CYBERnano: CRO in nano-cancerology (www.cybernano.eu)

FIVE RECENT PUBLICATIONS

[1] H. Benachour, T. Bastogne, M. Toussaint, Y. Chemli, A. Sève, C. Frochot, F. Lux, O. Tillement, R. Vanderesse, and M. Barberi-Heyob. Real-time monitoring of photocytotoxicity in nanoparticles-based photodynamic therapy: A model-based approach. PLoS ONE, 7(11):e48617, 2012.

[2] H. Benachour, A. Sève, T. Bastogne, C. Frochot, R. Vanderesse, J. Jasniewski, I. Miladi, C. Billotey, O. Tillement, F. Lux, and M. Barberi-Heyob. Multifunctional peptide-conjugated hybrid silica nanoparticles for photodynamic therapy and MRI. Theranostics, 2(9):889–904, 2012.

[3] S. Dobre, T. Bastogne, C. Profeta, M. Barberi-Heyob, and A. Richard. Limits of variance-based sensitivity analysis for non-identiability testing in high dimensional dynamic models. Automatica, 48(11):2740–2749, 2012.

[4] R. Keinj, T. Bastogne, and P. Vallois. Tumor growth modeling based on cell and tumor lifespans. Journal Theoretical of Biology, 312:76–86, 2012.

[5] J. Mriouah, C. Boura, M. Thomassin, T. Bastogne, B. Faivre, and M. Barberi-Heyob. Tumor vascular responses to anti-vascular and -angiogenic strategies: looking for suitable models. Trends in Biotechnology, 30(12):649–658, September 2012

Patrick Baumann

Patric Baumann was born in 1986 in Switzerland and got his master degree in Nanoscience 2010 at the University of Basel. He started 2010 at the Department of Chemistry of the University of Basel in the group of Prof. Wolfgang Meier and Prof. Cornelia Palivan his PhD. As part of the national research program 62 “smart materials” he was developing a nanoreactor for photodynamic therapy. He currently is finishing his PhD thesis and will further work for the pharma industry.

Raj Bawa

Dr. Raj Bawa is president of Bawa Biotech LLC, a biotech/pharma consultancy and patent law firm he founded in 2002. He is an inventor, entrepreneur, professor and a registered patent agent licensed to practice before the U.S. Patent & Trademark Office. Trained as a biochemist and microbiologist, he has been an active researcher for the past two decades. He has extensive expertise in pharmaceutical sciences, biotechnology, nanomedicine, drug delivery, medical devices and biodefense-related scientific, FDA regulatory and patent law issues. Since 1999, he has held various adjunct faculty appointments at Rensselaer Polytechnic Institute in Troy, NY where he currently is an adjunct professor of biological sciences. Since 2004, he has been an adjunct associate professor of natural and applied sciences at the Extended Learning Institute of NVCC in Annandale, VA. Since 2012, he has been a scientific advisor to Teva Pharmaceutical Industries, Ltd., Israel. He previously served as patent legal advisor at Sequoia Pharmaceuticals, Gaithersburg, MD and as senior scientist at SynerGene Therapeutics, Inc., Potomac, MD. He recently served as principle investigator of two National Cancer Institute/SBIR contracts titled “Targeted nanocomplexed iron oxide for early detection with concurrent hyperthermia treatment of cancer” and “A targeted nanocomplex for early detection of lung cancer.”

He has served as an advisor, consultant or expert to numerous global corporations, US government (NIH and NSF), law firms, universities, non-profits and NGOs. In the 1990s, Dr. Bawa held various positions at the US Patent and Trademark Office, including primary examiner (6 years) and instructor at the US Patent Academy. He is a life member of Sigma Xi, founding director of the American Society for Nanomedicine, co-chair of the Nanotech Committee of the American Bar Association and Global Advisory Council Member of the World Future Society. He has authored over 100 publications, co-edited two texts, presented or chaired at over 200 conferences worldwide and serves on the editorial boards of 14 peer-reviewed journals. Some of the awards he has received include Innovations Prize from the Institution of Mechanical Engineers, London (2008); Appreciation Award from the Undersecretary of Commerce, Washington, DC (2001); Key Award from Rensselaer (2005) and The Lifetime Achievement Award in Translational Nanomedicine from the American Society for Nanomedicine (2014).

François Berger

CLINATEC director
Professor of cell Biology and oncology in Grenoble medical university
Director of the Brain nanomedicine Group, INSERM U 836 CEA-Leti- MINATEC Campus, 17, rue des Martyrs, 38054 Grenoble Cedex 9, France; francois.berger@cea.fr, www.leti.fr
Tél. + 33 4 38 78 15 18, Mobile + 33 6 16 13 20 47

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

Research area: neuro-oncology, neurosciences, biomarkers, nanomedicine

EDUCATION

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

PROFESSIONAL CAREER

- Since 2011: director of CLINATEC INSTITUE, CEA, Grenoble; (Innovative Applications of Micro-Nano-Technologies to Medicine)
- Since 2009: scientific advisor of the French technology Institute associating all the public research agencies in France (CEA, INSERM, CNRS, INRIA)
- Since 2004: Head of the INSERM research laboratory “Brain nanomedicine group” evaluated A plus in 2010 by the national evaluation agency.
- Since 2000: Professor of Cell Biology and Oncology- clinical activity in the field of neuro-oncology
- 1999-2004: Head of the neuro-oncology group in the INSERM research laboratory of AL Benabid.
- 1994- 1995 Assistant in cell biology and neuro-oncology

RECENT PUBLICATIONS

- Nissou MF, El Atifi M, Guttin A, Godfraind C, Salon C, Garcion E, van der Sanden B, Issartel JP, Berger F, Wion D. Hypoxia-induced expression of VE-cadherin and filamin B in glioma cell cultures and pseudopalisade structures. J Neurooncol. 2013 Mar 31.
- Zaccaria A, Bouamrani A, Selek L, El Atifi M, Hesse AM, Juhem A, Ratel D, Mathieu H, Coute Y, Bruley C, Garin J, Benabid AL, Chabardes S, Piallat B, Berger F. A micro-silicon chip for in vivo cerebral imprint in monkey. ACS Chem Neurosci. 2013 Mar 20;4(3):385-92.
- Perles-Barbacaru AT, Berger F, Lahrech H. Quantitative rapid steady state T(1) magnetic resonance imaging for cerebral blood volume mapping in mice: Lengthened measurement time window with intraperitoneal Gd-DOTA injection. Magn Reson Med.
- Lages E, Guttin A, El Atifi M, Ramus C, Ipas H, Dupré I, Rolland D, Salon C,Godfraind C, deFraipont F, Dhobb M, Pelletier L, Wion D, Gay E, Berger F,Issartel JP. MicroRNA and target protein patterns reveal physiopathological features of glioma subtypes. PLoS One. 2011;6(5):e20600.

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

Iwan Bertholjotti

Iwan Bertholjotti started his professional career at Lonza in Visp with an apprenticeship as Laboratory Technician which he successfully finished in 1989. Afterwards he studied Chemical Engineering at the Valais University of Applied Sciences. He received his diploma as Chem. Ing. FH in 1992.
As Project leader he managed from 1993 until 1997 several product development and transfers in the field of adhesives and sealants for the company Sika in Zürich.
Since 1997 he works for Lonza in Visp in different functions and technology areas to deliver products to the Pharma market. As development chemist he developed Pharma API (Active Pharmaceutical Ingredients) processes to ensure a smooth transfer into different facilities of the Lonza Manufacturing network in Switzerland and USA. As plant chemist he managed different campaigns with different chemical technologies (e.g. low temperature reactions, continues flow technology, Peptides) to deliver product on time and with the right quality (GMP) to various Pharma customers. As plant manager he was responsible to build a new organization for the new build clinical API supply facility in Visp. In 2006 he moved to the new build Conjugates organization to manage the program to introduce the Conjugation Technology to manufacture Antibody Drug Conjugates (ADC) at Lonza. In 2010 he became the head of the business team Conjugates to ensure a profitable and sustainable growth of this new technology area. The successful start of the conjugates program and business was honored by the Lonza CEO Innovation Award 2012. In all the years in the industry a constant training program was in place to develop the technical, personality and leadership skills. One external training program successfully completed was the program for technical entrepreneurship (Technischer Unternehmer) at the University of St. Gallen. 2013 until today he leads the Lonza Pharma Program Management Organization for the Chemical Technology with Program Managers in Europe and China. The Chemical Technology includes beside the traditional Small Molecule Synthesis also the technology areas of Peptide Synthesis and Conjugation Biomolecules with Cytotoxic compounds. The program management organization is responsible to manage customer programs to deliver on time with the right quantity and quality.

Gerd Binnig

Gerd Binnig studied physics at the Johann Wolfgang Goethe University in Frankfurt and completed his Ph.D. in 1978. Since 1978, he has been a research staff member of the IBM Zurich Research Laboratory, interrupted by a sabbatical at the IBM Almaden Research Center (1985/86) and a guest professorship at Stanford University (1985-88). From 1987 to 1995, he headed an IBM Physics group at the University of Munich, from which he received an honorary professorship in 1987. For the development of the Scanning Tunneling Microscope (STM), which he invented together with Heinrich Rohrer, he received numerous awards including the Nobel Prize in Physics in 1986. The STM, and the Atomic Force Microscope (AFM), which he invented later and developed together with Calvin Quate and Christoph Gerber, were essential for nanotechnology to emerge. Later he developed a model to describe complex systems and founded in 2000 the company Definiens, where he together with others developed the novel computer language CNL. Today CNL is widely used for complex image analysis.

Patrick Boisseau

CEA-Leti, Minatec Campus, Grenoble, France
Email: patrick.boisseau@cea.fr
Cell: +33 607 477 131

EXPERIENCE

M. Patrick BOISSEAU joined the French Atomic Energy and Alternative Energies Commission (CEA) in 1987 to work for 7 years as academic research fellow in plant biology. He then spent 4 years at the Foresight & Strategy Division at the CEA headquarters as expert on strategy in life sciences and environment.
From 2001 to 2004, he was committed to the design, organisation and funding of the NanoBio innovation cluster in Grenoble. The NanoBio center is an integral part of the Minatec Innovation Center, the model for France's competitive clusters and #1 European centre for micro- and nanotechnologies.
From 2004 till 2008, he was coordinator of the European network of excellence in nanobiotechnology, Nano2Life (www.nano2life.org). This network of excellence integrates 23 full academic partners and 41 associate companies (>400 scientists) in a comprehensive joint programme of activity. Since then, he has been part of more than 12 European projects and main coordinator of 5 Framework Projects

Since 2012, he has been elected Chairman of the European Technology Platforms on Nanomedicine that he joined in 2005.
Since 2008, he has been Programme Manager on nanomedicine, at CEA-Leti."; 15 scientists are devoted there to the preclinical and clinical development of Lipidots® lipid nanocarriers.
Since 2012 he is head of the Strategic Planning on Healthcare at CEATech.
Patrick Boisseau is graduate of the Institut National Agronomique (1983) and of the Ecole Nationale du Génie Rural, des Eaux et des Forêts(1985). He holds a Master's Degree in Human Nutrition (2005).

MANDATES

- Foundation in 2004 of the nanobiotech section at the European Federation of Biotech.
- Member In 2004-2005 of the steering committee of the European Science Foundation Forward Look on Nanomedicine, responsible for the working group on "nanodiagnostics."
- Chairman since late 2012 of the European Technology Platform on Nanomedicine.
- Board Member in charge of Development of the European Platform on PhotoDynamic Medicine since 2011
- Experts for several international organizations such as the European Science Foundation, and the European Commission. Reviewers of numerous articles.

Gerrit Borchard

Ph.D.

Gerrit Borchard is a licensed pharmacist and obtained his Ph.D. in pharmaceutical technology from the University of Frankfurt (Germany) for his thesis on the interaction of colloidal drug carrier systems with the immune system. After holding several academic posts, including a lecturer position at Saarland University (Germany) and Assistant and Associate Professorships at Leiden University (The Netherlands), he joined Enzon Pharmaceuticals, Inc. (USA) as Vice President Research. In 2005, he was appointed Full Professor of Biopharmaceutics at the University of Geneva (Switzerland), and Scientific Director of the Centre Pharmapeptides in Archamps (France), an international center for biopharmaceutical research and training.
In the past, Prof. Borchard has served as Scientific Advisor for the Controlled Release Society (CRS), as Scientific Secretary of the Eu-

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

Ulrich Brinkmann

Ph.D.
Senior Principle Scientist, Scientific Director
Roche Pharma Research and Early Development- Large Molecule Research
Penzberg, FRG

Dr. Ulrich Brinkmann heads a New Technology / Protein Engineering unit within Roche Pharma Research in Penzberg, FRG. His Ph.D thesis covered development of expression systems to produce recombinant reteplase. Subsequently, he held positions as Postoc and Associate Scientist at the NIH/NCI (Ira Pastan Lab) focusing on antibody stabilization/engineering and recombinant immunotoxins for cancer therapy. Prior to joining Roche, he served as CSO in Functional Genetics and Pharmacogenetics companies, Xantos and Epidauros (now Beckmann Coulter) respectively. Ulrich Brinkmann is author and inventor of numerous publications and patents in the field of antibody engineering.

RELATED PUBLICATIONS

• Schneider B, Grote M, John M, Haas A, Bramlage B, Ickenstein LM, Jahn-Hofmann K, Bauss F, Cheng W, Croasdale R, Daub K, Dill S, Hoffmann E, Lau W, Burtscher H, Ludtke JL, Metz S, Mundigl O, Neal ZC, Scheuer W, Stracke J, Herweijer H, Brinkmann U Targeted siRNA Delivery and mRNA Knockdown Mediated by Bispecific Digoxigenin-binding Antibodies. Molecular therapy. Nucleic acids; 2012 Sep 18;1:e46 PMID:23344238

• Metz S, Panke C, Haas AK, Schanzer J, Lau W, Croasdale R, Hoffmann E, Schneider B, Auer J, Gassner C, Bossenmaier B, Umana P, Sustmann C, Brinkmann U Bispecific antibody derivatives with restricted binding functionalities that are activated by proteolytic processing. Protein engineering, design & selection : PEDS; Sep 13; 2012

• Haas AK, Maisel D, Adelmann J, von Schwerin C, Kahnt I, Brinkmann U Human-protein-derived peptides for intracellular delivery of biomolecules. The Biochemical journal; Mar 15;442(3):583-93 2012

• Metz S, Haas AK, Daub K, Croasdale R, Stracke J, Lau W, Georges G, Josel HP, Dziadek S, Hopfner KP, Lammens A, Scheuer W, Hoffmann E, Mundigl O, Brinkmann U Bispecific digoxigenin-binding antibodies for targeted payload delivery. Proceedings of the National Academy of Sciences of the United States of America; 2011 May 17;108(20):8194-9

Donald Bruce

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

Reto Brun

Reto Brun is a well-known parasitologist who mainly worked on malaria and African sleeping sickness. His main interest is drug discovery and development for those diseases. At the Swiss Tropical and Public Health Institute he established a Screening Centre for protozoan parasites which was involved in the discovery of most of the clinical candidates for malaria and sleeping sickness. He is also a founder of the Eastern Africa Network for Trypanosomiasis which is doing research and control of sleeping sickness. As a Professor at the University of Basel he supervised over 60 MSc and PhD students and as an author he published over 500 research articles and book chapters.

Zbigniew Brzozka

D.Sc., Tenured Professsor

Department of Microbioanalytics, Faculty of Chemistry, Warsaw University of Technology
Noakowskiego 3, 00-664 Warsaw, Poland
Tel./Fax +48 22 234 5427
e-mail: brzozka@ch.pw.edu.pl;
http://csrg.ch.pw.edu.pl

EDUCATION

• 1998 Tenured professor of analytical chemistry in chemical sciences – January 1998
• 1990 Dr.Sc. in chemical sciences -Ion Selective Electrodes Based on PVC Membranes – November 1990
• 1977-1982 Ph.D. in analytical chemistry - Extraction of Cu(II), Fe(III) and Co(II) by naphtenic acids, with honor - October 1982
• 1972-1977 M.Sc. in polymer chemistry and technology - Perylene radical cations as catalysts for polymerisation reactions, The 1st Class Honour Prize - 1977

RESEARCH EXPERIENCE

a) Professional career

Department of Analytical Chemistry, Warsaw University of Technology:

- 1977 junior assistant,
- 1978-1979 research-teaching assistant,
- 1980-1982 senior research-teaching assistant,
- 1982-1992 research associate (tenure track staff member),
- 1988-1999 head of the Instrumental Analysis Lab for undergraduate students.
- 1992-1998 associate professor in analytical chemistry,
- 1998-till now full professor of analytical chemistry

Institute of Biocybernetics and Biomed. Engineering Polish Academy of Sciences

- 1994-1998 associate professor (half position)
- 1998 -2002 full professor (half position)

Within the period August 1999 – December 2002 has been employed in Institute of Electron Technology at Warsaw (half position of full professor).

b) Current positions and duties

Warsaw University of Technology, Faculty of Chemistry, Institute of Biotechnology, Departament of Microbioanalytics:

- Tenured professor – full position
- Dean of Faculty since September 2008.

c) Initiated and establish collaborations with foreign research center and universities

Joint research work, seminars and lectures at following foreign research center and universities:

- fellowship holder of ETH Zurich in Prof. W.Simon's group – within 1984 – 1985
- research work in Prof. W.Simon's group supported by the Swiss National Science Fundation and by Orion Research, Inc – between August 1986 – December 1986
- postdoc position in Prof. D.N. Reinhoudt's group (University of Twente) – within 1991– 1992
- research coordinator in Prof. D.N. Reinhoudt's group (sensor project supported by the Royal Dutch Science Fundations (NWO,STW), by Priva and Sentron, Inc. – between September 1992 - June 1993
- visiting professor of MESA Institute and University of Twente – within 1993 – 1994
- co-initiator and coordinator of research colaboration with University of Twente (The Netherlands) (formal agreement between University of Twente and Warsaw University of Technology has been signed in January 1995).
- co-initiator of current research collaboration with Technical University Denmark (DTU) (with prof. Jorg Kutter - ChemLabChip Group)
- co-initiator of current research collaboration with Lund University (with prof. Thomas Laurell - Nanobiotechnology Group)
- co-initiator of current research collaboration with Groningen Research Institute of Pharmacy, University of Groningen (with prof. Sabeth Verpoorte - Analytical Chemistry and Pharmaceutical Analysis Group)

d) Awards

- 1991 The Minister of Science and Higher Education Award For The Scientific Achievements
- 1994 The Rector Award For The Scientific Achievements
- 1998 1st Degree Team Award of the Rector of WUT For The Scientific Achievements
- 1999 2nd Degree Team Award of the Rector of WUT For The Scientific Achievements
- 2002 The Rector Award for The Teaching Achievements
- 2004, twice 1st Degree Team Award of the Rector of WUT For The Scientific Achievements
- 2005 1st Degree Team Award of the Rector of WUT For The Scientific Achievements
- 2003-2006 Professor Subvention of The Foundation for Polish Science (exact sciences)
- 2010 – Wiktor Kemula Medal (Honour Award of Polish Socierty of Chemistry)

e) Summary of scientific activity

Book Chapters: 20

Peer Reviewed Papers (with IF): 98, cumulative IF of all publications is 247,3.

Cumulative cited index of 87 publications is 2016, Hirsch index is 27

Conferences: over 160 (4 plenary, 5 keynote, 10 invited, 12 oral in selected international conferences)

Patents: 11 (+ 7 during the review process)

f) Summary of scientific activity

The current research interests focus on:

Miniaturized analytical systems (Lab-on-a-Chip) for monitoring of bioanalytes. Emphasis is now also being laid on the applications of polymer microfabrication technologies to microchemical analysis, i.e. the integrated microchips with optical and electrochemical detection principles dedicated to early diagnostics of genetic diseases as a novel approach to reliably diagnose patients, and protect them from mistaken diagnoses and disorder progress. Another field of interest of the partners will be the development of polymeric chips for human cell culture in unique, in vivo-mimicking microenvironment where studies of cellular growth and responses to external factors are conducted for drug screening and toxicology applications.

Peter Burckhardt

Peter Burckhardt is CEO of „EVA – the Basel life sciences start-up agency" since 2006. In addition he is CEO of the BASEL INKUBATOR. He has an education as a chemist and achieved his PhD at the University of Basel. In 1984 he joined Ciba-Geigy where he started his career as a research chemist in Pharma Research. Later he held various management positions in research, development, manufacturing, and communications in Ciba-Geigy / Novartis. From 1996 – 1999 he was CEO of Novartis Animal Health Germany. From 2001 to 2006 he worked as a consultant in human resources. As CEO of EVA he is member of the board in several portfolio companies. Since 2009, Peter Burckhardt is president of the BioValley Business Angels Club BioBAC.

Manuela Calin

Institute of Cellular Biology and Pathology "N. Simionescu" (ICBP)

Current position: PhD, scientific researcher grade I, head of Nanotherapy laboratory and member of the Scientific Council of the ICBP "N. Simionescu".

EDUCATION

- 2011-2013- postdoctoral position in "Biomaterials: nanocarriers with controlled drug release", Institute of Macromolecular Chemistry "Petru Poni", Iasi
- 2005-PhD in Biological Sciences with Distinction: Summa cum Laude, Supervisor Prof. Maya Simionescu
- 1996- master of Science in Biophysics, University of Bucharest;
- 1995- graduated physicist, Faculty of Physics, University of Bucharest.

EXPERTISE

Long experience in liposome preparation and characterization techniques; targeting of liposomes by coupling ligands to their surface, cell culture, biochemistry techniques, immunological methods, enzymatic assays, fluorimetry, molecular biology: transfection, RT-PCR; fluorescence and electron microscopy.

PROJECT COORDINATOR

Coordinator of 5 national research projects; Research & Development Coordinator of EuroNanoMed ERA-NET project NANODIATER, 2011-2014; colaborator in 3 international and 12 national research projects.

PRIZES

1) Constantin Velican Award of the Romanian Society for Cell Biology, 2012

2) First prize offered by Romanian Society of Cell Biology, 2011

3) Prize of Excellence offered by Romanian Medical Association, 2010
4) First prize offered by Romanian Academy of Medical Sciences, 2008
5) Prize offered by Romanian Academy and Institute of Cellular Biology and Pathology " N. Simionescu" for the scientific activities in the European Community framework programme FP6, 2006
6) Prize of Excellence, Annual conference of National Society of Cellular Biology, 2003
7) "Agora Diabetologica" Prize, XXVII National Congress of Diabetes, Nutrition and Metabolic Diseases with international participation,Bucharest 2002
8) First prize sponsored by Nature Publishing Group at European Life Scientist Organization Meeting, France, 2002.

PUBLISHED PAPERS

32 from which 24 published in ISI cited journals with impact factors and 8 in journals without impact factor, 1 book chapter at Wiley and Sons, NY, USA; cited >550 times; average citations per paper: 17.33; h-index: 12.

LIST OF FIVE RELEVANT PUBLICATIONS WITHIN THE LAST FIVE YEARS

- Durdureanu-Angheluta A et al., Heparin-anthranoid conjugates associated with nanomagnetite particles and their cytotoxic effect on Cancer Cells, J. Biomed. Nanotechnol. 10, 131-142 (2014)
- Simion V et al., Development of curcumin-loaded poly (hydroxybutyrate-co-hydroxyvalerate) nanoparticles as anti-inflammatory carriers to human activated endothelial cells, Journal of Nanoparticle Research, vol 15: 2108 (2013).
- Gan AM et al., Cross-talk between activated monocytes and smooth muscle cells activates the STAT3 pathway and induces resistin and reactive oxygen species production, J Cell Biochem 114(10):2273-83 (2013)
- Pirvulescu M et al, A novel pro-inflammatory mechanism of action of resistin in human endothelial cells: up-regulation of SOCS3 expression through STAT3 activation, Biochem Biophys Res Commun 422: 321-326 (2012)
- Voinea Calin M et al., Effect of depletion of monocytes/macrophages on early aortic valve lesion in experimental hyperlipidemia, Cell Tissue Res 336 (2009) 237-48

Chris Cannizzaro

Dr., Physical Science Officer, National Nanotechnology Initiative, Global Issues Coordinator, US Department of State, Washington, DC (USA)

Dr. Chris Cannizzaro represents the U.S. Department of State on the National Science and Technology Council's (NSTC) Nanoscale Science, Engineering, and Technology (NSET) subcommittee and serves as the Global Issues Coordinator for the U.S. National Nanotechnology Initiative (NNI). He helped develop the 2014 NNI Strategic Plan and the 2011 NNI Environment Health and Safety (EHS) Research Strategy, and often represents the NNI in bilateral science and technology meetings and through interactions with business groups. Prior to joining the State Department he was a research Assistant Research Professor in the Department of Biomedical Engineering at Tufts University with interests in tissue engineering, biomaterials, microfluidics, and nanomedicine, a member of the NIH-funded Tissue Engineering Resource Center, and a Research Affiliate in the Harvard-MIT Division of Health Sciences and Technology. Chris received a B.S. from the University of Massachusetts, Amherst and a Ph.D. from the Swiss Federal Institute of Technology (EPFL), both in Chemical Engineering. In addition he was a Fulbright Scholar at the University of Milan, a Visiting Scholar at the Hawaii Natural Energy Institute, and a Postdoctoral Associate at the Massachusetts Institute of Technology.

Bob Carr

Dr Bob Carr, until recently Chief Technical Officer and Founder of NanoSight having previously worked for 20 years at a leading government research establishment in Wiltshire, UK before founding NanoSight in 2002/3. His background is in biodetection techniques employing laser optics and microsystems leading to 200+ publications/ Conf Reports/talks/patents, etc. Current interests include multiparameter nanoparticle detection and analysis techniques for application in the biotech and industrial chemical sectors. NanoSight has recently been acquired by Malvern Instruments, UK.

Nam-Joon Cho

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

Wan-Seob Cho

D.V.M., Ph.D
Lab of Toxicology, Medicinal Biotechnology
School of Natural Resources and Life Science,
Dong-A University, Busan, South Korea
E-mail: chows77@hotmail.com
wcho@dau.ac.kr

EDUCATION

2002.3-2004.2	Seoul Nat. University	Veterinary Pathology	Ph.D.
2000.3-2002.2	Seoul Nat. University	Veterinary Pathology	M.S.
1996.3-2000.2	Seoul Nat. University	Veterinary Medicine	D.V.M.

EMPLOYMENT

- Mar. 2012-present: Assistant Professor, Dong-A University, South Korea
- Nov. 2011-Feb. 2012: Scientist (scientific officer), National Institute of Food and Drug Safety Evaluation, Korea Food and Drug Administration (KFDA).
- Nov. 2008-Nov. 2011: Post-doc fellow, MRC/University of Edinburgh Centre for Inflammation Research, ELEGI Colt Laboratory, Queen's Medical Research Institute. Edinburgh (Supervisor: Professor Ken Donaldson).

• Oct. 2002-Nov. 2008: Scientist (scientific officer), Division of Toxicological Research, National Institute of Toxicological Research, Korea Food and Drug Administration (KFDA).

SELECTED PUBLICATIONS

1. **Cho WS**, Duffin R, Bradley M, Megson IL, MacNee W, Lee JK, Jeong J, Donaldson K. Predictive value of in vitro assays depends on the mechanism of toxicity of metal oxide nanoparticles. Part Fibre Toxicol. 2013 Oct 25;10(1):55.
2. **Cho WS**, Kang BC, Lee JK, Jeong J, Che JH, Seok SH. Comparative absorption, distribution, and excretion of titanium dioxide and zinc oxide nanoparticles after repeated oral administration. Part Fibre Toxicol. 2013 Mar 26;10:9.
3. **Cho WS**, Thielbeer F, Duffin R, Johansson EM, Megson IL, MacNee W, Bradley M, Donaldson K. Surface functionalization affects the zeta potential, coronal stability and membranolytic activity of polymeric nanoparticles. Nanotoxicology. 2014 Mar;8(2):202-11.
4. Donaldson K, Schinwald A, Murphy F, **Cho WS**, Duffin R, Tran L, Poland C. The biologically effective dose in inhalation nanotoxicology. Acc Chem Res. 2013 Mar 19;46(3):723-32.
5. **Cho WS**, Dart K, Donaldson K, Howie SEM. Adjuvanticity and toxicity of cobalt oxide nanoparticles as an alternative vaccine adjuvant. Nanomedicine, 2012 Oct 7(10); 1495-1505.
6. **Cho WS**, Thielbeer F, Duffin R, Bradley M, Megson IL, MacNee W, Donaldson K. Zeta potential and solubility to toxic ions as mechanisms of lung inflammation caused by metal/metal-oxide nanoparticles. Toxicological Sciences, 2012 Apr 126(2): 469-477.
7. **Cho WS**, Duffin R, Howie SEM, Scotton CJ, Wallace WAH, MacNee W, Bradley M, Megson IL, Donaldson K. Progressive severe lung injury by zinc oxide nanoparticles; the role of Zn2+ inside lysosomes. Particle and Fibre Toxicology, 2011 Sep 6;8(1): 27.
8. **Cho WS**, Duffin R, Bradley M, Megson IL, MacNee W, Howie SEM, Donaldson K. NiO and Co3O4 nanoparticles induce lung DTH-like responses and alveolar lipoproteinosis. European Respiratory Journal, 2012, 39(3): 546-57.
9. **Cho WS**, Duffin R, Poland CA, Duschl A, Oostingh, GJ, MacNee W, Bardley M, Megson IL, Donaldson K. Differential pro-inflammatory effects of metal oxide nanoparticles and their soluble ions in vitro and in vivo; zinc and copper nanoparticles, but not their ions, recruit eosinophils to the lungs. Nanotoxicology, 2012 6(1): 22-35.
10. **Cho WS**, Duffin R, Poland CA, Howie SEM, MacNee W, Bardley M, Megson IL, Donaldson K. Metal oxide nanoparticles induce unique inflammatory footprints in the lung; import implications for nanoparticle testing. Environ. Health. Perspective. 2010, Dec; 118(12): 1699-1706.
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12. **Cho WS**, Cho M, Jeong J, Choi M, Han BS, Shin HS, Hong J, Chung BH, Jeong J, Cho MH. Size-dependent tissue kinetics of PEG-coated gold nanoparticles. Toxicology and Applied Pharmacology, 2010, May; 15; 245(1), 116-123.
13. **Cho WS**, Kim S, Han BS, Son WC, Jeong J. Comparison of gene expression profiles in mice liver following intravenous injection of 4 and 100 nm-sized PEG-coated gold nanoparticles. Toxicology Letters, 2009, Dec; 191(1), 96-102.
14. **Cho WS**, Cho M, Kim SR, Choi M, Lee JY, Han BS, Park SN, Yu MK, Jon S, and Jeong J. Pulmonary toxicity and kinetic study of Cy5.5-conjugated superparamagnetic iron oxide nanoparticles by optical imaging. Toxicology and Applied Pharmacology, 2009, Aug; 15; 239(1), 106-115.
15. **Cho WS**, Cho M, Jeong J, Choi M, Cho HY, Han BS, Kim SH, Kim HO, Kim YT, Chung BH, Jeong J. Acute toxicity and pharmacokinetics of 13 nm-sized PEG-coated gold nanoparticles. Toxicology and Applied Pharmacology. 2009, Apr; 1;236(1): 16-24.

Tomasz Ciach

PhD, Professor at
Warsaw University of Technology
Faculty of Chemical and Process Engineering
Warynskiego str. 1
00-645 Warsaw, POLAND
t.ciach@ichip.pw.edu.pl
www.biomedlab.ichip.pw.edu.pl

EDUCATION

Chemical Engineering at Warsaw University of Technology, Biology at Warsaw University, doctorate at Warsaw University of Technology. Currently head of Biotechnology Division at the Faculty of Chemical Engineering, Warsaw University of Technology, Warsaw, Poland.

SCIENTIFIC INTEREST

Drug delivery systems: various types of drug delivery devices, implants or injectable / ihaleble systems designed to deliver the drug in the proper place and rate. This also includes nanoparticles for anticancer drug delivery and cancer diagnosis.
Medical devices and implants for bone, skin and vasculature regeneration.
Surface modification of medical devices; advanced antibacterial, biocompatible, antitrombogenic and cell anchoring surface modification systems for permanent implantebale medical devices.

Aaron Ciechanover

Aaron Ciechanover was born in Haifa, Israel in 1947. He is a Distinguished Research Professor in the Technion - Israel Institute of Technology in Haifa. He received his M.Sc. (1971) and M.D. (1973) from the Hebrew University in Jerusalem. He then completed his national service (1973-1976) as military physician, and continued his studies to obtain a doctorate in biological sciences in the Faculty of Medicine in the Technion (D.Sc.; 1982). There, as a graduate student with Dr. Avram Hershko and in collaboration with Dr. Irwin A. Rose from the Fox Chase Cancer Center in Philadelphia, USA, they discovered that covalent attachment of ubiquitin to a target protein signals it for degradation. They deciphered the mechanism of conjugation, described the general proteolytic functions of the system, and proposed a model according to which this modification serves as a recognition signal for a specific downstream protease. As a post doctoral fellow with Dr. Harvey Lodish at the M.I.T., he continued his studies on the ubiquitin system and made additional important discoveries. Along the years it has become clear that ubiquitin-mediated proteolysis plays major roles in numerous cellular processes, and aberrations in the system underlie the pathogenetic mechanisms of many diseases, among them certain malignancies and neurodegenerative disorders. Consequently, the system has become an important platform for drug development. Among the numerous prizes Ciechanover received are the 2000 Albert Lasker Award, the 2003 Israel Prize, and the 2004 Nobel Prize (Chemistry; shared with Drs. Hershko and Rose). Among many academies, Ciechanover is member of the Israeli National Academy of Sciences and Humanities, the American Academy of Arts and Sciences (Foreign Fellow), the American Philosophical Society, the National Academy of Sciences of the USA and the Institute of Medicine of the National Academies of the USA (Foreign Associate), the Pontifical Academy of Sciences at the Vatican, the Chinese Academy of Sciences (CAS; Foreign Member), and the Russian Academy of Sciences (Foreign Member).

Andy Clark

Andy Clark received a Bachelor's degree in Natural Sciences from the University of Cambridge (UK) in 1987. He then studied for a PhD at the University of Birmingham (UK), investigating the control of insulin gene expression. After receiving his PhD in 1992, Andy remained in Birmingham, continuing his studies as a postdoctoral fellow for another year, then worked for three years as a postdoctoral fellow at the Cancer Research UK labs in London, investigating effects of cell signalling on gene expression. In 1996 he was appointed as a lecturer at the Kennedy Institute of Rheumatology in London, where he remained for the next 16 years, being promoted to senior lecturer then reader in Cell Signalling. In 2012 Andy accepted a chair at the University of Birmingham (UK), where he is now Professor of Inflammation Biology and heads a group of nine research scientists. Andy's interests focus on the mechanisms employed by the immune system to prevent excessive inflammation and to promote the resolution of inflammatory responses. He contends that the chronic inflammatory diseases that create enormous health-economic burdens throughout the world may be best understood in terms of failures of endogenous mechanisms that exist to limit inflammation or promote its resolution. Furthermore, a better understanding of those control mechanisms is likely to lead to the development of more effective treatments for inflammatory diseases.

Patrick Couvreur

Pr Patrick Couvreur is Full Professor of Pharmacy at the Paris-Sud University and holder of the chair of "Innovation Technologique" (2009-2010) at the prestigious « Collège de France ». He is appointed as a Senior Member of the "Institut Universitaire de France". He is also the recipient of an "ERC Advanced Grant" (2010-2015).
Pr Patrick COUVREUR's contributions in the field of drug delivery and targeting are highly recognized around the world with more than 450 peer review research publications (H-index 73 and over 20,000 citations). His research is interdisciplinary, at the interface between Physico-Chemistry of Colloids, Polymer Chemistry, Material Science, Molecular and Cellular Biology and Experimental Pharmacology.
Patrick COUVREUR's research has led to the funding of two start-up companies (Bioalliance and Medsqual). The major scientific contribution of Patrick COUVREUR to the Pharmaceutical Sciences is also recognized by numerous international (the "2004 Pharmaceutical Sciences World Congress Award", the prestigious "Host Madsen Medal", the "European Pharmaceutical Scientist Award" of the European Federation of Pharmaceutical Sciences and the European Inventor Award 2013 given by the European Patent Office) and national awards (the "Prix Galien 2009" and the "Médaille de l'Innovation 2012 of the CNRS). His appointment as a member of six academies (Académie des Technologies, Académie de Médecine and Académie de Pharmacie in France, as well as the Académie Royale de Médecine in Belgium, the Royal Academy of Pharmacy in Spain and the US National Academy, Institute of Medicine in USA) is another recognition of major scientific and scholarly contributions of Patrick Couvreur.

Tom Crabbe

UCB, External Discovery Solutions

I have worked as a researcher, project leader and department head in preclinical drug discovery at Celltech (from 1987) and UCB (from 2004). I currently lead UCB's External Discovery Solutions team, which aims to bring scientists together in order to accelerate drug discovery in areas of unmet patient need.

Kenneth Dawson

Prof. Dawson is Chair of Physical Chemistry, Chairman of the National BioNanoscience Action, and co-ordinator of the European Infrastructure in the arena. He has experience in the management of large scale EU projects, including multi-sectoral cross-disciplinary research projects, and other international programs. He has received several international prizes, including the 2007 Cozzarelli prize from the National Academy of Sciences USA, as well as IBM, Packard, Canon, Sloan and Dreyfus prizes.

Prof. Dawson's professional roles include representing Ireland on various international bodies, the OECD and ISO working groups on standards for Nanotechnology. He is currently Editor of Current Opinion in Colloid Science, Senior Editor of Physica, Associate Editor of Journal of Nanoparticle Research, and former President of the European Colloid and Interface Society. He has been an advisor on nanoscience matters in the EU New Risk Committee of the European Commission, and the Advisory group of the European Medicines Agency.

Neil P. Desai

PhD

Neil Desai is currently Vice President of Strategic Platforms at Celgene Corp. Prior to its acquisition by Celgene in Oct 2010, he was Sr. Vice President of Global Research and Development at Abraxis Bioscience, in Los Angeles, California, USA, where he led the development of Abraxane®, the company's flagship product and considered to be the first true nanotherapeutic. Dr. Desai is an inventor of Abraxis' nanoparticle-albumin bound (nab®) drug delivery platform and was responsible for company's product pipeline and the development its intellectual property portfolio. This platform has been clinically proven with global approvals for Abraxane® in metastatic breast cancer, non-small cell lung cancer and most recently for pancreatic cancer. Abraxis was acquired by Celgene in 2010 for approximately $3 billion. Prior to his positions at Abraxis, Dr. Desai was Senior Director of Biopolymer Research at VivoRx, Inc and VivoRx Pharmaceuticals, Inc. (predecessor companies of Abraxis), where he worked on the early discovery and development of Abraxane, developed novel encapsulation systems for living cells and was part of the team that performed the world's first successful encapsulated islet cell transplant in a diabetic patient. Dr. Desai has more than 25 years of experience in the research and development of novel therapeutic delivery systems withover 100 issued patents, over 40 peer-reviewed publications and book chapters, and over 200 presentations at scientific meetings. He is a reviewer for several scientific journals in the area of cancer therapeutics and drug delivery. He is an active participant in FDA and EU Nanotechnology initiatives and a member of the Steering Committee for the NCI Alliance for Nanotechnology in Cancer. He is also the founder and CEO of AADi, LLC, a clinical stage company developing a nanotechnology therapeutic in the oncology and cardiovascular fields. Dr. Desai holds an M.S and Ph.D. in Chemical Engineering from the University of Texas at Austin, USA, and a B.S. in Chemical Engineering from the University Institute of Chemical Technology in Mumbai, India.

Jacques Descotes

Prof. Jacques Descotes, M.D. Pharm.D. Ph.D is Medical toxicologist Professor of Medical Pharmacology and Toxicology. He is author of over 350 scientific papers and 10 books dedicated to immunotoxicology and non-clinical or clinical toxicology He serves as Head of the Poison Control and Toxicovigilance Centre of Lyon. He also serves as Chairman of the Poison Center and Pharmacovigilance Unit in Lyon (France) and also as Member of Scientific Board at ERYtech Pharma SA.

Prof. Descotes serves as Member of Advisory Board for Animascope. He is an expert of clinical and pre-clinical safety for health products. He is Founding President and Scientific Director of Summerschool in Immunotoxicology (since 1992) - Fellow US Academy of Toxicological Sciences -Eurotox Registered Toxicologist & recipient of 2003 Eurotox Annual Award -Scientific Advisor, Immunotoxicology Technical Committee (HESI, ILSI, Washington DC) -Recipient of 2010 Society of Toxicology Vos Award for Career Achievement in Immunotoxicology.

Wouter Driessen

Wouter Driessen is a chemist at iThera Medical and a guest scientist at the Institute for Biological and Medical Imaging (IBMI) at the Helmholtz Center in Munich, Germany. He received his M.S. in pharmaceutical sciences from Utrecht University and his doctorate in Pharmaceutics from the University of Florida, Gainesville (2007). After completing a Keck Postdoctoral Fellowship at the MD Anderson Cancer Center in Houston, TX, during which he worked in the areas of one-step target/lead discovery by in vivo combinatorial screening of peptide libraries and applications, such as biomarker imaging, drug delivery and nanotechnology he joined iThera Medical where he is working on the identification and development of imaging probes for photoacoustics and the advancement of imaging applications, especially in oncology and pharmacokinetic imaging.

Admire Dube

Ph.D.

PROFILE

Dr. Admire Dube is a pharmaceutical scientist whose research focuses on nanomedicines for infectious diseases, in particular targeted nanomedicines and multifunctional nanomedicines for malaria and tuberculosis.

EDUCATION

• Ph.D. Pharmaceutical Sciences 2011
Monash University, Australia
Thesis title: Assessment of the impact of biopolymeric nanoparticles on the oral absorption of green tea catechins
• Magister Pharmaceuticae 2006
University of the Western Cape, South Africa
Thesis title: The design, preparation and evaluation of Artemisia afra in tea bag dosage form suitable for use in clinical trials
• Bachelor of Pharmacy (Honours) 2003
University of Zimbabwe

WORK EXPERIENCE (PAST THREE YEARS)

Senior Researcher-Encapsulation and Delivery group, CSIR, South Africa 2013 to present
Developing nanoparticle delivery systems for drugs used in malaria and tuberculosis (TB), translatable to human clinical trials. Activities include co-development of product development plans, execution of experiments, project management, supervision of junior scientific staff (Post-doctoral research fellow) and grant writing. Focus is on strategic applied research to generate commercially feasible intellectual property and transferrable technology demonstrators. Projects of note I have initiated and I am working on include multifunctional nanoparticles for TB treatment, and liver targeted oral nanoformulations for malaria prophylaxis.
Post-doctoral research fellow, University at Buffalo, USA 2011-2013
Investigated biopolymeric nanoparticles for targeted TB drug delivery and concurrent immunotherapy. This work was conducted in the laboratory of Distinguished Professor Paras N. Prasad at the Institute for Lasers, Photonics and Biophotonics, and Professor Gene Morse at the New York State Centre of Excellence in Bioinformatics and Life Sciences.

PUBLICATIONS

1. Dube, A., Reynolds, J., Wing-Cheung, Law, Prasad, P.N., Morse, G.E. Multimodal Nanoparticles that Provide Immunomodulation and Intracellular Drug Delivery for Infectious Diseases. In Press: Nanomedicine Nanotechnology, Biology and Medicine. 2013; xx:1-8, http://dx.doi.org/ 10.1016/j.nano.2013.11.012.
2. Dube, A., Y. Lemmer, R. Hayeshi, M. Balogun, P. Labuschagne, H. Swai and L. Kalombo (2013). State of the art and future directions in nanomedicine for tuberculosis. Expert Opinion on Drug Delivery 10(12): 1725-1734.
3. Dube, A., Nicolazzo, J.A., Larson, I. (2011). Chitosan-tripolyphosphate nanoparticles enhance the plasma exposure of EGCG in mice through an enhancement in the intestinal stability. European Journal of Pharma ceutical Sciences, 44(3), 422-426.
4. Dube, A., Nicolazzo, J.A., Larson, I (2011). Assessment of plasma concentrations of (-)-epigallocatechin gallate in mice at a dose reflecting consumption of a standard green tea beverage, Food Chemistry, 128(1), 7-13.
5. Dube, A., Nicolazzo, J.A., Larson, I (2010). Chitosan nanoparticles enhance the intestinal absorption of the green tea catechins (+)-catechin and (-)-epigallocatechin gallate, European Journal of Pharmaceutical Sciences, 41(2), 219-225.
6. Dube, A., Ng, K., Nicolazzo, J.A., Larson, I. (2010). Effective use of reducing agents and nanoparticle encapsulation in stabilizing catechins in alkaline solution, Food Chemistry, 122(3), 662-667.
7. Dube, A., Manthata, L.N., Syce, J.A. (2007). The design and evaluation of placebo material for crude herbals: Artemisia afra herb as a model. Phytotherapy Research, 21(5), 448-451.

Rutledge Ellis-Behnke

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

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

Previously he was Associate Professor in the Faculty of Medicine at the University of Hong Kong, as well as Associate Director of the Technology Transfer Office.
Ellis-Behnke is redefining tissue engineering for nanomedicine. His research is focused on reconnecting the disconnected parts of the brain—with the goal of being able to provide a prescription to restore quality of life after brain or spinal cord trauma, or stroke. In animals he was the first to repair the brain showing reversal of blindness; to stop bleeding in less than 15 seconds without clotting; to preserve stem cells; and to immobilize prostate cancer stem cells.
Ellis-Behnke is an advisor to, and co-founder of, Arch Therapeutics. He has multiple worldwide patent applications and his “Nano Neuro Knitting” and “Immediate Hemostasis” technologies have each been licensed to companies for translation to humans. Technology Review named his “Nanohealing” discoveries one of the “Top 10 Emerging Technologies.”

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

Prior to returning to school to pursue his PhD, Ellis-Behnke held various management positions including Senior Vice President of Huntingdon Engineering and Environmental, a public company for testing and consulting services; and in 1995 was co-founder/CEO of one of the first internet companies in the world to do online commerce.

In addition to his work in neuroscience and nanomedicine, Ellis-Behnke introduced the TabletPC to MIT in 2001 and the University of Hong Kong in 2005, as part of the migration to the paperless classroom to deliver all course material and texts to the students digitally. At both MIT and the University of Hong Kong the students learned 25% more material; and the bottom 25% of the class improved by one letter grade.

Ellis-Behnke is Associate Editor for Nanomedicine: Nanotechnology, Biology and Medicine; and also Frontiers in Neurotrauma. He is a founding board member of the International Society of Nanomedicine; and is on the Scientific Advisory Board of the Glaucoma Foundation.

Bengt Fadeel

Bengt Fadeel is Professor of Medical Inflammation Research at the Institute of Environmental Medicine, Karolinska Institutet, Stockholm, and Adjunct Professor of Environmental and Occupational Health, University of Pittsburgh. He received his M.D. and Ph.D. degrees from Karolinska Institutet. He is Director of the Nanosafety & Nanomedicine Laboratory and Head of the Division of Molecular Toxicology at the Institute of Environmental Medicine. Dr. Fadeel is a Fellow of the US Academy of Toxicological Sciences (ATS) since 2012. He is the past coordinator of FP7-NANOMMUNE and currently engaged in several other nanosafety projects including FP7-MARINA, FP7-NANOREG, FP7-NANOSOLUTIONS, FP7-SUN, and FP7-eNANOMAPPER, as well as the Flagship Project GRAPHENE. Dr. Fadeel is Editor of "Adverse Effects of Engineered Nanomaterials: Exposure, Toxicology, and Impact on Human Health" (Elsevier, 2012) and "Handbook of Safety Assessment of Nanomaterials: From Toxicological Testing to Personalized Medicine" (Pan Stanford, 2014). He served as co-chair of the 7th International Nanotoxicology Congress in Antalya, Turkey (2014).

Omid Farokhzad

Omid Farokhzad is an Associate Professor at Harvard Medical School (HMS) and a physicianscientist at Brigham and Women's Hospital (BWH). Dr. Farokhzad directs the Laboratory of Nanomedicine and Biomaterials at BWH. He is a faculty member of the Brigham Research Institute Cancer Research Center and a member of the Dana Farber/Harvard Cancer Center. Dr. Farokhzad's research is focused on the development of therapeutic nanoparticle technologies; most notably, he pioneered the high throughput combinatorial development and screening of multifunctional nanoparticles for medical applications. Dr. Farokhzad has authored approximately 100 papers and holds more than 125 issued/pending US and International patents. The technologies that Dr. Farokhzad has developed with collaborators at HMS and MIT have formed the basis for: 1) a new class of targeted nanoparticles for treatment of important human diseases including cancer and cardiovascular disease; 2) a new class of synthetic nanoparticle vaccines for prophylactic and therapeutic applications and 3) a new class of integrative combination nanomedicines for synergistic treatment of cancers, inflammation/pain, and infectious diseases. These technologies formed the foundational platform for the launch of three biotechnology companies: BIND Therapeutics (NASDAQ: BIND), Selecta Biosciences, Blend Therapeutics, which are translating the aforementioned academic innovations toward commercialization and societal impact. Dr. Farokhzad was elected to the College of the Fellows of the American Institute of Medical and biological Engineering. He was a recipient of the 2013 RUSNANOPRIZE, the largest international nanotechnology prize, for the development and industrialization of nanoparticle technologies for medical applications. In 2013, the Boston Globe selected him among the top innovators in Massachusetts and the Boston Business Journal selected him among the Health Care Champions for his innovations. In 2012, he was among the regional Ernst & Young Entrepreneur of the Year awardees. Dr. Farokhzad completed his postgraduate clinical and post-doctoral research trainings, respectively, at the BWH/HMS and MIT in the laboratory of Institute Professor Robert Langer. He received his M.D. and M.A. from Boston University School of Medicine.

Xavier Fernàndez Busquets

Associate Researcher, Head of Nanomalaria Joint Unit, Institute for Bioengineering of Catalonia (IBEC), Barcelona Science Park, Baldiri Reixac 10-12, E-08028 Barcelona, Spain. www.ibecbarcelona.eu.

Assistant Research Professor, Head of Nanomalaria Joint Unit, Barcelona Centre for International Health Research (CRESIB, Hospital Clínic-Universitat de Barcelona), Rosselló 132, E-08036 Barcelona, Spain. www.cresib.cat.

Coordinator, Biomolecular Interactions Group, Nanoscience and Nanotechnology Institute (IN2UB), University of Barcelona, Martí i Franquès 1, E-08028 Barcelona, Spain.
+34 93 227 5400 (ext. 4581); xfernandez_busquets@ub.edu

Current research: Nanobiomedicine

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

ACADEMIC BACKGROUND

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

POSITIONS HELD

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

PEER-REVIEWED PUBLICATIONS: 64; **CONFERENCE CONTRIBUTIONS**: 118

Anne C Field

Ph.D Pharmacology, (1986) University College, London
Bachelor of Science (Hons), (1983) Physiology, King's College, London

PROFESSIONAL EXPERIENCE

Therapeutic Goods Administration, Canberra, Australia (1994 to date)
I am currently engaged as a Senior Toxicologist in the Toxicology Section, Office of Scientific Evaluation. My main role is to provide scientific and toxicological advice based on nonclinical (animal) data submitted to support applications to register or vary the conditions of registration for prescription medicines covering all therapeutic areas. In addition, my role in the Toxicology Section requires me to provide advice on a range of toxicological issues relating to therapeutic products as they arise, including issues relating to process or solvent impurities in medicinal products.
I have also have had a number of years of experience working in the non-prescription medicines and complementary medicines sections of the TGA. This has provided me with a broader understanding of the TGA's risk based regulatory framework, since these medicines are generally subject to lower levels of regulatory control, owing to the lower level of inherent risk associated with such products.

ACADEMIC CAREER

Prior to joining the TGA I worked as a Postdoctoral Fellow at the John Curtin School of Medical Research (JCSMR), Australian National University, studying voltage gated ionic currents in excitable tissues. Following my promotion to a Research Fellowship, I investigated mechanisms underlying long-term potentiation in the hippocampus (a model for learning and memory) using quantal analysis of synaptic transmission. Prior to this, I undertook research on the mechanisms underlying α-adrenoceptor mediated changes in ionic permeability in the liver. These studies, carried out in the Department of Pharmacology, University College, London, formed the basis of my doctoral thesis.
Key publications resulting from my scientific research are provided below. I also presented the results of my research at conferences in the United Kingdom and Australia, and I was responsible for running a neuroscience research seminar program at the JCSMR. In addition, I was involved in teaching medical and neuroscience students.

MEMBERSHIP OF PROFESSIONAL BODIES

Member of the Australian Society for Clinical and Experimental Pharmacology and Toxicology (ASCEPT)

RESEARCH PUBLICATIONS

• **Field, A.C.** and Jenkinson, D.H. (1987).The effect of noradrenaline on the ion permeability of isolated mammalian hepatocytes, studied by intracellular recording. *Journal of Physiology* **392** 493-512.

• **Field, A.C.,** Hill, C. and Lamb, G.D. (1988). Asymmetric charge movement and calcium currents in ventricular myocytes of neonatal rat. *Journal of Physiology* **406:** 277-97.

• Stricker, C., **Field, A.C.** and Redman, S.J. (1994). Probabalistic secretion of quanta at excitatory synapses on CA1 pyramidal neurones. *Advances in Second Messenger and Phosphoprotein Research* **29:** 323-40.

• Stricker C., Cowan, A.I., **Field, A.C.,** and Redman, S.J. (1996). Changes in quantal parameters of EPSCs in rat CA1 neurones *in vitro* after the induction of long-term potentiation. *Journal of Physiology* **490:** 419-41.

• Stricker C., Cowan, A.I., **Field, A.C.,** and Redman, S.J. (1996). Analysis of NMDA-independent long-term potentiation induced at CA3-CA1 synapses in rat hippocampus *in vitro*. *Journal of Physiology* **490:** 443-54.

Alke Fink

Tel. +41 (26) 300-9501
FAX +41 (26) 300-9624
Email: alke.fink@unifr.ch

Professor Alke Fink received her Ph.D. in Chemistry from the University of Ulm, Germany in 1999. After a first post-doc at the University of Gainesville, Florida, she joined the Institute of Materials Science at the Ecole Polytechnique Federale de Lausanne (EPFL) first as a post-doc, then as senior scientists. Alke Fink became Associate Swiss National Science Foundation Professor in the Department of Chemistry at the University of Fribourg in 2009 and Full Professor in 2011 at the Adolphe Merkle Institute, Switzerland. She shares her position at the institute with her colleague, Prof Rothen-Rutishauser, a biologist by training. Together, they chair the highly interdisciplinary BioNanomaterials group. Her research focuses mostly on inorganic nanoparticles, their syntheses, surfaces, and interactions with biological cells. She has published numerous peer-reviewed papers, acquired substantial personal and project funding, received patents, and was invited to join Academia Net, a database of excellent women academics in 2012.

Gina L. Fiore

Dr. Gina L. Fiore is a maître-assistante/ project leader at the Adolphe Merkle Institute in Fribourg, Switzerland. She received a B.Sc. in Molecular Biology from West Chester University of Pennsylvania in 2003. She then went on to receive a PhD in Chemistry in 2008 from the University of Virginia where she worked with Cassandra Fraser on polymeric biomaterials for biomedical applications. After which, she moved to the Department of Chemistry as a postdoctoral associate with Marc Hillmyer at the University of Minnesota to work on biodegradable plastics. In 2010, she then moved to Switzerland to join Christoph Weder and the Polymer Chemistry and Materials group. Her research interests focus on the applications of stimuli-responsive polymeric materials that combine the unique properties of metal-complexes (e.g., luminescence, pH responsiveness, catalysis) with the ease of processing of polymers. Her team works on functional polymers, luminescent materials, bio-renewable and biodegradable polymers, reversible adhesives, self-healing materials, hydrogels, micelles, biomaterials, and cell imaging and drug delivery vectors.

Andreas Fisch

Andreas Fisch holds a position as Senior Fellow in the Pharmaceutical Development of parenteral dosage forms at Novartis Pharma AG in Basel, Switzerland. He is leading several projects in the Technology Platform Parenteral Nanomedicine.
He received his PhD in Pharmacy from Mainz University, Germany, in 1992 after completing this graduate research at the Institute of Immunology in Mainz on MCH class II restricted antigen presentation. He continued his academic career in Clinical Pharmacology at the University Hospital of Mainz on blood cell and tissue interactions.
After two years research fellowship in the Biomedical Research Center of Baxter Bioscience in Vienna, Austria, he headed the Pharmaceutical Development Center for parenteral colloids of B.Braun in Crissier, Switzerland from 1997-2007 before joining Novartis.

Niels Montano Frandsen

Master of Science degree in Chemical Engineering from the Technical University of Denmark
PhD in molecular biology from the University of Copenhagen

• 8 years of basic science (bacterial genetics) in Paris, France at the Institut Jacques Monod and the Institut de Biologie et Physico-Chimique studying bacterial physiology and cell division in E. coli and coupling between morphogenesis and gene expression during sporulation in Bacillus subtilis.
• 4 years as Senior Scientist at GlaxoSmithKline, Verona, Italy in antibacterial exploratory research
• 4 years in Pantheco A/S and Santaris Pharma, Hoersholm, Denmark – Head of molecular biology department – drug development based on antisense oligonucleotide technology
• 7 years as Product Manager in Exiqon – development of antisense life science tools for functional analysis of non-coding RNA
Coauther of 14 scientific publications

Heico Frima

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

Eleonore Fröhlich

Prof. Dr. med. Dipl. Biochem.

Eleonore Fröhlich obtained her degree in Medicine and her Diploma in Biochemistry at the Universities Tübingen and Heidelberg in Germany. She worked as physician, then returned to the University of Tübingen and specialized in Histology & Embryology and Anatomy. Eleonore Fröhlich worked as group leader in drug development at the biotech company Oridis-Biomed in Graz, Austria. Since 2007, she is employed at the Medical University of Graz as Director of the Core Facility Microscopy at the Center for Medical Research. She is also adjunct professor at the University of Tübingen, Key Researcher at the Research Center Pharmaceutical Engineering, Graz and acts as Scientific Advisor of the Animal Welfare Body of the Medical University of Graz.
Her current research focuses on the assessment of biological, mainly toxicological, effects of nanoparticles by in-vitro studies. She is particularly interested in uptake and nanoparticle effects on cell organelles such as lysosomes and nuclei, and chronic effects of nanoparticles. An additional focus of her work is the development of exposure systems as alternatives to in-vivo experimentation. Eleonore Fröhlich is responsible for toxicity testing in several cooperative projects; she acts as reviewer in 38 journals, and is member of the Editorial Board of 6 journals. As Member of the European Center for Nanotoxicology and official data provider for the OECD she is also involved in the standardization of nanoparticle testing.

Alberto Gabizon

Alberto (Abraham) Gabizon, received his M.D. at the School of Medicine in Granada, Spain (1975), and his Ph.D. in Cell Biology from the Weizmann Institute of Science in Rehovot, Israel (1979). He completed his residency in Oncology at the Hadassah Medical Center in Jerusalem, and received the Israeli board certification in Radiation and Medical Oncology in 1985. Between 1985-1988, he was a research associate fellow at the Cancer Research Institute of the University of California in San Francisco, USA, where he helped develop a new generation of long-circulating liposomes known as Stealth liposomes which have greatly improved stability and selective accumulation in tumors. In 1989, Dr. Gabizon returned to Jerusalem, Israel, and continued his research and clinical activity at the Hadassah Medical Center. 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 title.
Dr. Gabizon has received the Spanish National Prize of Medicine Graduation (1975), the Research Career 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 140 articles and specialized book chapters.
Dr. Gabizon's inventorship and research contribution played a key role in the development of DOXIL (pegylated liposomal doxorubicin, also known as Caelyx), a unique anticancer formulation extensively used in the clinic with important pharmacologic and safety advantages over conventional chemotherapy. His most recent invention currently in clinical studies is PROMITIL (pegylated liposomal mitomycin-C prodrug). He recently founded Lipomedix Pharmaceuticals Inc., a start-up company aimed at developing further PROMITIL and other inventions in the field of cancer nanomedicine.

Ruth Gabizon

EDUCATION

• 1971-74 - B.Sc in Chemistry, Hebrew University, Jerusalem.
• 1974-76 - Israel Defence Forces.
• 1976-78 - M.Sc. studies in the Department of Biophysics; Weizmann Institute of Science (Rehovot, Israel), under the supervision of Prof. Meir Wilchek.
• 1979-84 - Ph.D. student . Department of Molecular Biology; Hebrew University Medical School, under the supervision of Prof. Shimon Schuldiner.
• 1985-88 - Postdoctoral studies in the Department of Neurology, University of California at San Francisco, under the supervision of Prof. Stanley Prusiner.

ACADEMIC APPOINTMETNS

• 1.10. 1990 - Lecturer in the Hebrew University Medical School.
• November 1996-Senior lecturer in the Hebrew University medical School. (tenured)

• June 2002-Associate Professor in the Hebrew University Medical School.
• May 2010: Full Professor in the Hebrew University Medical School

RESEARCH GRANTS

• 2008-2010 : Chief Scientist Department of Health. "Interaction of plasma fractions with PrPSc and prion infectivity", 300000 Shekel
• 2009-2012: ISF Morasha Foundation: Identifying early biological markers of prion disease in TG mice carrying the E200K PrP mutation linked to familial Creutzfeldt-Jakob disease (CJD) in Libyan Jews". In collaboration with Dr Zeev Meiner. 300000 $

PUBLICATIONS

1. Genetic prion disease: no role for the immune system in disease pathogenesis?
Friedman-Levi Y1, Binyamin O, Frid K, Ovadia H, **Gabizon R**. Hum Mol Genet. 2014 Mar 25. [Epub ahead of print]
2. PrP(ST), a soluble, protease resistant and truncated PrP form features in the pathogenesis of a genetic prion disease. Friedman-Levi Y, Mizrahi M, Frid K, Binyamin O, **Gabizon R**. PLoS One. 2013 Jul 26;8(7):e69583.
3. Snord 3A: a molecular marker and modulator of prion disease progression.
Cohen E, Avrahami D, Frid K, Canello T, Levy Lahad E, Zeligson S, Perlberg S, Chapman J, Cohen OS, Kahana E, Lavon I, **Gabizon R**.
PLoS One. 2013;8(1):e54433.
4. Targeting of prion-infected lymphoid cells to the central nervous system accelerates prion infection. Friedman-Levi Y, Hoftberger R, Budka H, Mayer-Sonnenfeld T, Abramsky O, Ovadia H, **Gabizon R**.
Neuroinflammation. 2012 Mar 21;9:58.
5. Copper is toxic to PrP-ablated mice and exacerbates disease in a mouse model of E200K genetic prion disease. Canello T, Friedman-Levi Y, Mizrahi M, Binyamin O, Cohen E, Frid K, **Gabizon R**.
Neurobiol Dis. 2012 Mar;45(3):1010-7.
6. Fatal prion disease in a mouse model of genetic E200K Creutzfeldt-Jakob disease.
Friedman-Levi Y, Meiner Z, Canello T, Frid K, Kovacs GG, Budka H, Avrahami D, **Gabizon R**. PLoS Pathog. 2011 Nov;7(11):.
7. Tau and 14-3-3 of genetic and sporadic Creutzfeldt-Jakob disease patients in Israel.
Meiner Z, Kahana E, Baitcher F, Korczyn AD, Chapman J, Cohen OS, Milo R, Aharon-Perez J, Abramsky O, **Gabizon R**, Rosenmann H. J Neurol. 2011 Feb;258(2):255-62. doi: 10.1007/s00415-010-5738-6. Epub 2010 Sep 9.

Jacinthe Gagnon

Jacinthe Gagnon was born in QuŽbec (Canada) on July 10th 1982. She studied Biochemistry at Concordia University, MontrŽal (Canada), and obtained her B.Sc. in 2006. Afterwards she did a M.Sc. in Advanced Materials with specialization in Biomaterials at the University of Ulm (Germany) and obtained her degree in 2008. Miss Gagnon is currently working on her Ph.D. at the Chemistry Department of the University of Fribourg (Switzerland) under the supervision of Prof. Katharina M. Fromm. Her main research interest is the nanoencapsulation of silver-based antimicrobial drugs for biomaterials applications. She has developed cerium oxide nanocapsules containing silver nanoparticles with long-term antimicrobial action. During this Ph.D., she had the opportunity to do part of her research project under the supervision of Prof. Rachel A. Caruso at the University of Melbourne (Australia) where she developed titanium dioxide coatings and nanocontainers. Her hobbies include cycling, dancing, climbing, reading and traveling.

Marcos Garcia-Fuentes

Marcos Garcia-Fuentes is B.Pharm. (1998), M.Sci. (2000) and Ph.D. (2004) from the University of Santiago de Compostela, obtained under the supervision of Prof. Maria J. Alonso. He was visiting scientist at the NSF Program on Therapeutic and Diagnostic Devices, School of Chemical Engineering, Purdue University, US, under the supervision of Prof. Nicholas Peppas. Between 2005 and 2007, he was a Intraeuropean Marie-Curie Postdoc at ETH Zurich (supervisors Hans P. Merkle and Prof. Lorenz Meinel). From 2007, Marcos returns to the Universidad of Santiago de Compostela with a tenure-track contract. Since February 2013, Marcos Garcia-Fuentes is Associate Professor in Pharmacy and Pharmaceutical Technology at the School of Pharmacy, and Principal Investigator at the Center for Research in Molecular Medicine and Chronic Diseases (CIMUS) and the Health Research Institute, University of Santiago de Compostela.

Marcos Garcia-Fuentes had important contributions to the design of novel nanocarriers for the oral delivery of peptides, including lipid-chitosan nanoparticles and stimuli-sensitive nanogels. Many of these technologies continue to be investigated for translation towards clinical use. More recently he has focused on the development of new biomaterials for modulating stem cell behavior; an important topic in tissue and organ regeneration, that he has extended to applications in oncology through the link of cancer and tumor initiating cells.

Marcos Garcia-Fuentes is coauthor of more than 30 international, peer-reviewed papers, accumulating well over 1000 citations since 2009 (H-index=19). Many of them as first or senior author in the top journals in the areas of Pharmaceutics and Biomaterials. He has been a recipient of the "Most Cited Paper Award" for the Journal of Controlled Release in 2005. He is also co-inventor in three patents, one already approved in Spain and China, and in the process of extension in another six areas. He has participated in the obtention of funding in competitive calls (national, European) for value over 1.5 M€ (over 300K€ as Principal Investigator).

Further information
http://www.enthuse.me/marcosgf#/
http://webspersoais.usc.es/persoais/marcos.garcia/Research.html

Ehud Gazit

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

Prof. Ehud Gazit is is Professor and the incumbent of the Chair for Biotechnology of Degenerative Diseases at Tel Aviv University. From 2012-2014 he served as the Chief Scientist of the Israeli Ministry of Science and Technology (MOST). In the years 2008-2012 he served as Tel Aviv University Vice President for Research and Development and the Chairman of the board of directors of Ramot Ltd., the technology transfer company of Tel Aviv University. Prior to his appointment as Vice President, Gazit served in different academic and administrative positions at Tel Aviv University, including the Head of The Chemistry-Biology double major track, a member of the University Committee for Appointments and Promotions, the Head of the Academic Committee of the Ilona Rich Institute for Nano-Biology and Nano-Biotechnology, and a member of the managing board of the Center for Nanoscience and Nanotechnology. Gazit received his B.Sc. (summa cum laude) after completing his

studies at the Special Program for Outstanding Students of Tel Aviv University (Currently the Adi Lautman program), and his Ph.D. (with highest distinction) as a Clore Fellow at the Department of Membrane Research and Biophysics, Weizmann Institute of Science in 1997. For his Ph.D. work, he received the John F. Kennedy Award in 1996. He has been a faculty member at Tel Aviv University since 2000, after completing his postdoctoral studies as a European Molecular Biology Organization (EMBO) and Human Frontiers Science Program (HFSP) fellow at Massachusetts Institute of Technology (MIT) where he also had held a visiting appointment (2002–2011).

Gazit's research is directed toward the study of protein folding, misfolding, and self-assembly. His work has resulted in the identification of elements that facilitate the assembly of amyloid fibrils, associated with Alzheimer's disease, and he has identified novel ways to inhibit this process. His laboratory was the first to discover aromatic dipeptides that form nanotubes and nanospheres with unique mechanical and chemical properties. Applications of these nanoassemblies include ultra-sensitive biosensors, energy-storage devices, and metallic nanowires. His work has been published in prestigious journals such as Science, Nature Nanotech., Nature Chem. Biol., and Cell. He is or was on the editorial board of eight journals including Nanomedicine, PLoS ONE, Amyloid and Current Chemical Biology. Gazit had received numerous awards and honors including the Landau Research Award, Dan David Scholarship Award, and TAU Research Council Prize. His technology transfer achievements were acknowledged by inclusion in the 2008 list of "100 Innovations from Academic Research to Real-World Application" by the Association of University Technology Managers (AUTM).

Peter Gehr

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

Martina Giannaccini

EDUCATION

2012 PhD with honors, Scuola Superiore Sant'Anna, Pisa
2008 MSc with honors, University of Pisa

CURRENT POSITION

2012-2014 Researcher Assistant, Nanomedicine Lab, a jointed lab between Scuola Superiore, Sant'Anna, Pisa and University of Pisa

PUBLICATIONS

1. **Giannaccini M**, Cuschieri A, Dente L, Raffa V. Superparamagnetic Nanoparticles: A Biodistribution Study Using Xenopus laevis Embryos. JNDT, 2013, 1:11-18.
2. **Giannaccini M**, Giudetti G, Biasci D, Mariotti S, Barsacchi G, Andreazzoli M. Rx1 defines retinal precursor identity by repressing alternative fates through the activation of TLE2 and Hes4. Stem Cell, 2013, doi: 10.1002/stem.1530.
3. **Giannaccini M**, Cuschieri A, Dente L, Raffa V. Non-mammalian vertebrate embryos as models in Nanomedicine. Nanomedicine, 2013, doi: 10.1016/j.nano.2013.09.010.
4. Riggio C, Calatayud MP, **Giannaccini M**, Sanz B, Torres TE, Ibarra MR, Dente L, Goya GF, Cuschieri A, Raffa V. The growth of neuronal processes can be directed via magnetic nanoparticles and magnetic fields. Nanomedicine, 2014, doi: 10.1016/j.nano.2013.12.008.
5. **Giannaccini M**, Giannini M, Calatayud MP, Goya GF, Cuschieri A, Dente L, Raffa V. Magnetic nanoparticles as intraocular drug delivery system to target Retinal Pigmented Epithelium (RPE). Int. J.Mol. Sci. 2014, 15:1590-1605.
6. **Giannaccini M**, Giudetti G, Biasci D, Mariotti S, Degl'Innocenti A, Perrotta M, Barsacchi G, Andreazzoli M. Characterization of the Rx1 dependent transcriptome during early retinal development. Dev Dyn, 2014, accepted with major revision.

MAIN COMMUNICATIONS

1. Riggio C, Calatayud MP, **Giannaccini M**, Sanz B, Torres TE, Fernández-Pacheco R, Ripoli A, Ibarra MR, Dente L, Cuschieri A, Goya GF, Raffa V. The orientation of the neuronal growth process can be directed via magnetic nanoparticles under an applied magnetic field. International Conference Nanoparticles and nanotechnologies in medicine 2013 (NPMED13), Bresso, Milan, Italy June 19th – 21st 2013.
2. **Giannaccini M**, Giudetti G, Biasci D Mariotti S, Barsacchi G, Andreazzoli M. Rx1 specifies the properties of retinal progenitorsby controlling multiple gene programs, 14th International Xenopus Conference, Giens Peninsula, France, Sept 9th – 13th 2012.
3. **Giannaccini M**, Giudetti G, Biasci D., Mariotti S, Della Santina M, Degl'Innocenti A, Barsacchi A, Andreazzoli M. Evidenze di nuovi meccanismi molecolari controllati dal fattore di trascrizione Rx1 nello sviluppo retinico", 57th G.E.I. (Italian embryologic group) conference, Monteortone, Padova, Italy, June 5th – 8th 2011. (Oral Communication).
4. **Giannaccini M**, Giudetti G, Biasci D, Mariotti S, Della Santina M, Degl'Innocenti A, Barsacchi G, Andreazzoli M. "A multi-step transcriptional analysis indicates multiple roles of Xrx1 in retinal developmet", 13th International Xenopus Conference, Lake Louise, Canada Sept 12th – 16th 2010.

Peter Gimeson

Peter joined GE Healthcare Life sciences 2009 as senior application specialist for microcalorimetry covering Europe, Middle East and Africa. The function allows him to interact with users on a daily basis regarding all aspects of instrumentation, assay developments and data analysis in Isothermal Titration Calorimetry and Differential Scanning Calorimetry. He is based in Uppsala, Sweden, working closely with GE internal and Instrument user groups in both academic and industrial environments. A large part of his work is conducting training sessions, support visits and data analysis consultancy on/off site. Peter holds a Ba in Chemistry, Umeå University, Sweden.

Biana Godin (Vilentchouk)

Biana Godin (Vilentchouk) is an Assistant member at Houston Methodist Research Institute (HMRI) and an Adjunct Assistant Professor at University of Texas – Graduate School of Biomedical Sciences and at University of Houston Department of Electrical and Computer Engineering.
After completing her PhD in Pharmaceutical Sciences at the Hebrew University of Jerusalem under the supervision of Prof. Elka Touitou, Prof. Godin was recruited for her postdoctoral training in the field of Cancer Nanomedicine to the team of Prof. Mauro Ferrari, at the University of Texas Health Sciences Center. She was offered her current position in 2010.

During the past few years Prof. Godin have published peer-reviewed articles and invited reviews in top journals including *Nature Nanotechnology (IF 31,2), Accounts for Chemical Research (IF 20.8), Trends in Pharmacological Sciences (IF 9.3), Advanced Drug Delivery Reviews (IF 12.9) and Cancer Research (IF 8.7)* as well as numerous invited book chapters, and a number of submitted US patents. She has also received national and international awards for her work including recent feature on her research in the NIH director blog. She currently serves on the editorial boards and as a reviewer for more than twenty scientific journals.
Prof. Godin's research focuses primarily on innovative approaches for treatment and diagnosis of cancer as well as applications of nanomedicine to the field of obstetrics and infectious diseases. She is one of the project leaders on two multicenter multi-investigator NIH National Cancer Institute grants: Physical Science in Oncology and Center for Cancer Nanotechnology Excellence and a PI on TATRC/DoD grant and a number of NIH pilot projects.

Nicolas Gouze

Nicolas Gouze has an engineer's degree in optronics from the University Paris XI and studied Innovation Management at the University of Valenciennes (France). Since 2004 he is working with the Department Future Technologies and Europe of VDI/VDE-IT. From 2004-2010 he was involved in technology transfer and innovation issues within the Innovation Relay Centre (IRC) and Enterprise Europe Network (EEN). Since 2010 Nicolas is involved in the ETP Nanomedicine, and he took over the management of the platform's secretariat in 2013. Nicolas is also coordinator of NANOMED2020 (2012-2014), a Coordination and Support Action aiming at delivering concrete recommendations to the European Commission to push forward the field of nanomedicine under Horizon 2020.
The ETP Nanomedicine was established in 2005 as a joint venture of the European Commission and CEOs of large industrial companies, SMEs and academic research institutions to investigate and advance joint activities in the area of nanotechnology in medicine. Since 2005 the ETPN published a number of strategic documents outlining the needs and roadmaps for nanomedicine research in Europe. The ETPN contributed to set up numerous European funded projects providing a first impression of the conditions for a suitable social and economic environment and the structural requirements for an efficient translation of R&D results into innovative Nanomedicines. The ETPN supports its members in coordinating their joint research efforts and improving communication amongst the members as well as towards the EC and the European Member States.

May Griffith

Dr. May Griffith is Professor of Regenerative Medicine and Director of the Integrative Regenerative Medicine (IGEN) Centre at Linköping University, Sweden. Her research interests are in the area of Regenerative Medicine, with special interests in biomaterials enhanced or enabled cell-based regeneration. She has been focussing mainly on corneal regeneration. Most recently, she has completed a Phase I clinical study where cell-free biomimetic scaffolds implanted enabled regeneration of human corneas that normally on their own, do not regenerate. Dr. Griffith's biosynthetic - and also biomimetic - materials have also been successfully tested in collaboration with other researchers for use in cartilage and cardiovascular regeneration. In 2013, Dr. Griffith led her EU Nanomedicine team on the project, "I-CARE" to win the ETPN nano medicine award for regenerative medicine. One of the implants optimised in I-CARE will be entering clinical trials in early July 2014.

Christopher D. T. Guiffre

Christopher D. T. Guiffre, J.D. has served as our Senior Vice President and Chief Business Officer since
2012. Prior to that, Mr. Guiffre held a number of senior executive positions at various biopharmaceutical
companies. From 2010 to 2012, he served as President and Chief Executive Officer of Alvos Therapeutics, Inc., a private biotechnology company subsequently acquired by Arrowhead Research Corp.; from 2008 to 2009, he served as Chief Business Officer at Hydra Biosciences, Inc., a private biopharmaceutical company; and from 2001 to 2008, he served as a senior executive at Cubist Pharmaceuticals, Inc., most recently as Senior Vice President, General Counsel and Secretary. From 1997 to 2001, Mr. Guiffre held several positions at Renaissance Worldwide, Inc., including Vice President, General Counsel and Clerk. Prior to that, he was an Associate at Bingham McCutchen LLP. He received a B.S. degree from Babson College, a J.D. from Boston College Law School and an M.B.A. from Boston College Carroll School of Management.

Hans-Joachim Güntherodt

- 1963 Diploma in Physics at the Swiss Federal Institute of Technology (Eidgenössische Technische Hochschule – ETH) in Zurich
- 1967 PhD degree from ETH Zurich
- 1968 Several Visits at research centers in Jülich and Karlsruhe for neutron scattering experiments
- 1971 Visiting Professor at FU Berlin
- 1973 Habilitation at ETH Zurich
- 1974 Full Professor in physics at the Institute of Physics in Basel and department head
- 1974/1981 On sabbatical leave at the IBM Thomas J. Watson Research Center, Yorktown Hights, USA
- 1986-87 Dean of the Faculty of Natural Science of the University of Basel
- 1992 Rector designatus of the University of Basel
- 1993 Sabbatical leave at Tokyo Institute of Technology, Japan
- 1994-96 Rector of the University of Basel
- 1996 Member of the Board and Module Coordinator of the Swiss Priority Program Micro and Nanosystems Technology (MINAST)
- 1998 Director of the Swiss Priority Program Micro and Nanosystems Technology (MINAST) of the ETH Board
- 1999 Scientific Director of the Technology Oriented Program 'The Nanometer in Science and Technology of the 21st Century' 'TOP NANO 21'.
- 2001 Director of the National Center of Competence in Research (NCCR) 'Nanoscale Science – Impact on Life Sciences, Sustainability, Information and Communication Technologies'
- 2004 Member of the Executive Board of IUVSTA (International Union of Vacuum Science, Technology and Applications). Head of the division 'Nanometer Structures'.
- 2005 Honorary Doctor (Dr. h.c.) of the University of Neuchâtel
- 2006 Organization of ICN+T2006 – International Conference on Nanoscience and Technology 2006 in Basel; Alliance of Nano09 and STM06

Gregor Haefliger

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

Sonja Hartl

MSc.

Sonja Hartl obtained her degree in Science in Bioanalytical Sciences at FH JOANNEUM Graz in Austria. She started her professional career as scientific assistant at the BioNanoNet Forschungsgesellschaft mbH in Austria where she gained expertise in regulations and standardization of nanomaterials by working on the Central Europe project on nanotechnologies for chemical enterprises focusing on "How to foster the responsible use of nanotechnologies and manage associated risks". Furthermore Ms. Hartl is part of the EU FP7 project NANoREG, substitute member of the MODENA Cost Action (Modelling of Nanomaterials Toxicity), represented in national committees on nano-information and she is part of the Austrian Standardization Working Group "Nanotechnologies and Nanomaterials". Additionally she is supporting the network, communication and dissemination activities of BioNanoNet.
Her current research is focusing on the communication and support of nanotechnologies amongst the broad public within the EU FP7 project NanoDiode on Developing Innovative Outreach and Dialogue on responsible nanotechnologies in EU civil society.

Kostas Hatzixanthis

Address: Norwich BioIncubator, NRP,
Norwich, NR4 7UH, UK
Phone: 0044 + 1603450728
E-mail: khatzixanthis@procartabio.com

CAREER

June 2012 - present – Procarta Biosystems Ltd.
Director of Research
Supports CSO with all aspects of running the Company. Principle responsibilities include:
• Drug process development and scale up - budgets and timelines
• identifying key partners (CROs and academic collaborators) and managing and maintaining those relationships and networks
• Line management of 7 staff
• Identification of suitable grant funding opportunities and consortia build up
• Coordination and writing of all EU applications for grant funding and management of awarded EU grants (2 major grants awarded in 2013)
• Coordination & writing of UK grant applications and management of awarded grants Expertise in Nanomedicine

MAJOR ACHIEVEMENTS

Successful application and management of a TSB High Value Manufacture Feasibility Grant (4 months, £25K) and a TSB Formulation Feasibility Grant (Project Co Ordinator, 9 months, £75K, ongoing)
Successful application for an EU Marie Curie Industry Academia Partnership grant (project DNA – TRAP, Nanomedicine, 2013 - 2017, 4 partners, £2M). Appointed as Project Coordinator responsible for scientific and financial management and reporting to the EU.
Successful application (partner) for an FP7 Nanomedicine grant (project NAREB, 2014 - 2018, 15 partners, £9M). Principle Investigator for Procarta.
Secured grant and managing funds from the Growing Business Fund (UK).

June 2007- May 2012 – Phico Therapeutics Ltd.
• Clinical Trial Co-ordinator
Supported consultant Clinical Project Managers with all aspects of the Company's first Phase I clinical trial, with a novel biologic, including internal procedural set up, study set-up, protocol finalization, regulatory and ethics approvals, study conduct and completion.
• Senior Process Development Scientist
Development and successful scale up of a downstream process (DSP). Process successfully provided material for the completed Ph I trial.

Key presentations at:
• Knowledge Transfer Network (KTN) meeting on Innovation and Challenges for Next Generation Anti-Infective Therapeutics, Alderley Park, 3/2014; Department of Chemistry, University of Florence, 1/2014; 'Eurotides', Prague, 11/2013;
• Posters at: ECCMID, Berlin, 4/2013; ICAAC", Boston, 9/2010
Author in 15 scientific papers and member of the Institute of Clinical Research (UK), since 2008

James L. Hedrick

James L. Hedrick received his Ph.D. from James McGrath at Virginia Tech in Material Science and Engineering. He joined IBM Research in 1985 in the Advance Organic Materials Group. James has focused on the synthesis and basic structure property relationships on synthetic polymers for advance microelectronic and biomedicinal related applications. Areas of emphasis include organocatalytic methods to bio-compatible/degradable polymers, functional oligomers, copolymers and complex architectures. He is the recipient of the ACS, Division of Polymer Chemistry, Carl S. Marvel Award 2003, ACS, Division of Polymer Chemistry, Industrial Sponsors Award 2006, Belgian Polymer Chemistry Award 2008, 2009 Cooperative Research Award in polymer science and engineering with Robert Waymouth of Stanford (ACS PMSE Division), and ACS Fellow in Poly- mer Division. He has co-authored more than 390 papers and has more than 100 patents issued.

Michael Hehenberger

As a member of IBM's research team, Michael Hehenberger was focused on the creation of partnerships between IBM Research and global Life Sciences organizations, including the bio-pharmaceutical, diagnostics and food industries, academic (medical) research centers, and government sponsored research. The research collaborations were based on the joint desire to extend the frontiers and to improve the productivity of life sciences and medical research.
Dr. Hehenberger has published over 40 scientific papers and has organized / presented at conferences on high performance computing, computational chemistry and biology, cheminformatics, unstructured and structured data analytics, clinical genomics, biobanking , (imaging) biomarker informatics, DNA sequencing technology and clinical nanomedicine.

After his retirement from IBM, he will focus his efforts on the emerging field of "Translational Nanomedicine".
Michael Hehenberger holds advanced degrees in physics (Dipl.Ing., TU Vienna, Austria) and quantum chemistry (Ph.D. and Dr.Sc., Uppsala University, Sweden).

Inge Herrmann

Inge Herrmann (1985) studied Chemical and Bioengineering at the ETH Zurich with a stay at the TU Delft in 2007. During her PhD studies in the group of Professor Wendelin Stark at the ETH Zurich, she developed a nanomagnet-based blood purification technology in collaboration with the University Hospital Zurich. After her PhD in biomedical engineering and postgraduate studies in clinical trials management, she worked at the Center of Clinical Research at the University Hospital in Zurich and at the University of Illinois (visiting scientist). In spring 2013, Inge Herrmann started a research fellow with Professor Molly Stevens at the Imperial College in London. Her research interests include therapeutic applications of magnetic nanoparticles, organ-protective small molecules with applications in sepsis and ischemia reperfusion injuries and the development of point-of-care biosensors.

Heinrich Hofmann

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

Patrick Hunziker

Patrick Hunziker has studied Medicine the University of Zurich, Switzerland. He received a doctoral decree based on thesis work in experimental immunology from the University of Zurich and did further research in experimental haematology at University Hospital in Zurich, Switzerland. He earned specialist degrees in Internal Medicine, Cardiology and Intensive Care Medicine. As a fellow the Massachusetts General Hospital, Harvard Medical School, worked on cardiac imaging in a joint project with the Massachusetts Institute of Technology, Cambridge.
His professional activities in Europe, the U.S., Africa and China gave him a broad insight into the needs for the medicine of the future in a variety of settings. Hunziker became involved in medical applications of Nanoscience in the late nineties and has been the pioneer physician in Nanomedicine in Switzerland since then. With improved prevention, diagnosis and cure of cardiovascular disease as his main research topic, he worked in the nanoscience fields of atomic force microscopy, nanoptics, micro/nanofluidics, nanomechanical sensors and polymer nanocarriers for targeting.
He is the co-founder and president of the European Society of Nanomedicine, co-founder of the European Foundation for Clinical Nanomedicine and co-initiator of the European Conference for Clinical Nanomedicine and is clinically active as deputy head of the Clinic for Intensive Care Medicine at the University Hospital Basel, Switzerland. In November 2008 Patrick Hunziker became professor for Cardiology and Intensive Care Medicine at the University of Basel.

Qun Huo

Dr. Huo is a tenured associate professor in the NanoScience Technology Center at University of Central Florida (UCF), Orlando, FL, USA. She also holds joint appointments in the Department of Chemistry (College of Science), Department of Materials Science and Engineering (College of Engineering), and the Burnett School of Biomedical Science (College of Medicine) at UCF. Dr. Huo's primary research interest is gold nanoparticles for biomedical applications. Her group invented and developed a new platform analytical technique for chemical and biological detection and analysis by combining gold nanoparticle probes with dynamic light scattering detection. This technique is now used by other research laboratories and commercial entities to develop new sensors and bioassays for environmental and food safety monitoring, new drug discovery, biomolecular research, and nanomedicine development. Dr. Huo and her students are using this technique for cancer biomarker research and to develop new diagnostic tests for cancer detection. One molecular test developed from their research for aggressive prostate cancer detection has entered into clinical validation study stage. Dr. Huo's research has been supported by a number of major research funding from the United States National Science Foundation (NSF),

Florida Department of Health, State of Florida, and other miscellaneous funding agencies. She was a recipient of NSF CAREER award and Scholar Boost award from the State of Florida New Florida 2010 program. She was an invited speaker in the 2012 Gordon Research Conference on Noble Metal Particles (Mount Holyoke College, MA, USA). She has authorized and co-authorized more than 75 peer-reviewed articles in the field of gold nanoparticles, nanomedicine and other related research areas.

Qun Huo (Ph.D.) received her B.Sc. degree in polymer science from University of Science and Technology of China (1991), M.Sc. degree in chemistry from Sun Yatsen University (1994), and Ph.D. degree in Chemistry from University of Miami (1999).

REPRESENTATIVE PUBLICATIONS

1. Jans, H.; **Huo, Q**. Gold nanoparticle-enabled biological and chemical detection and analysis. Chem. Soc. Rev. 2012, 41, 2849-2866.
2. **Huo, Q**.; Litherland, S.A.; Sullivan, S.; Hallquist, H.; Decker, D.A.; Rivera-Ramirez, I. Developing a nanoparticle test for prostate cancer scoring. J. Translational Medicine, 2012, 10:44 (open access).
3. **Huo, Q**.; Cordero, A.; Bogdanovic, J.; Colon, J.; Baker, C.H.; Goodison, S.; Pensky, M. A facile nanoparticle immunoassay for cancer biomarker discovery. J. Nanobiotechnology, 2011, 9:20 (Open Access).
4. Jans, H.; Liu, X.; Austin, L.; Maes, G.; **Huo, Q**. Dynamic light scattering as a powerful tool for gold nanoparticle bioconjugation and biomolecular binding study. Anal. Chem. 2009, 81, 9425-9432.
5. Liu, X.; Dai, Q.; Austin, L.; Coutts, J.; Knowles, G.; Zou, J.; Chen, H.; **Huo, Q**. A One-step homogeneous immunoassay for cancer biomarker detection using gold nanoparticle probes coupled with dynamic light scattering. J. Am. Chem. Soc. 2008, 130, 2780-2782.
6. Dai, Q.; Liu, X.; Coutts, J.; Austin, L.; **Huo, Q**. A one-step highly sensitive method for DNA detection using dynamic light scattering. J. Am. Chem. Soc. 2008, 130, 8138-8139.

Larn Hwang

Ph.D., VP Regulatory & Clinical Operations
Sorrento Therapeutics Inc.
e-mail: lhwang@sorrentotherapeutics.com

EDUCATION

Ph.D., Molecular Microbiology
University of Texas Southwestern Medical Center at Dallas, Texas - 1994

AWARDS

Leukemia Society of North Central Texas Postdoctoral Fellowship 1994 – 1998

EMPLOYMENT

• Sorrento Therapeutics, Irvine, CA
2013-present VP Regulatory & Clinical Operations
• IGDRASOL, Fountain Valley, CA
2011-present Chief Operating Officer
IGDRASOL is the leader in next generation nanoparticle paclitaxel therapeutics.
• Abraxis Bioscience Inc, Marina Del Rey, CA
2005-2011 Head of Cell Biology
• Carlsbad Technology, Carlsbad, CA
2005 QC Microbiologist
• Salk Institute, La Jolla, CA
2003-2004 Postdoctoral Scientist
• Johnson & Johnson Pharmaceutical Research & Development, La Jolla, CA
1999 – 2002 Postdoctoral Research Scientist
• University of Texas Southwestern Medical Center, Dallas, TX
1994 – 1999 Postdoctoral Fellow

PUBLICATIONS

1. Sachdeva M, Wu H, Ru P, Hwang L, et al. (2011) Oncogene 30:822-31
2. Desai NP, Trieu V, Hwang LY, et al. (2008) Anticancer Drugs. 19:899-909.
3. Hwang, L.-Y., et al. (2001). Immuno. Res. 24, 245-272
4. Simmons, A. D., M.M. Musy, C. S. Lopes, L.-Y. Hwang, et al. (1999) Hum. Mol. Genet., 8, 2155-2164.
5. Ayi, T.-C., J. T. Tsan, L.-Y. Hwang, et al. (1998) Oncogene 16:2143-2148
6. Baer, R., L.-Y. Hwang, et al. (1997) Chromosomal Translocations and Oncogenic Transcription Factors, F.J. Rauscher III and P.K. Vogt, Editors. Springer P.55-65
7. Tsan, J. T., Z. Wang, Y. Jin, L.-Y. Hwang, et al. (1997) In The Yeast Two-hybrid System, P. L. Bartel, & S. Fields, Editors. Oxford University Press: Oxford. P. 217-232
8. Wu, L.C., Z.W. Wang, J. T. Tsan, M .A. Spillman, A. Phung, X. L. Xu, M.-C. W. Yang, L.-Y. Hwang, et al. (1996) Nature Genetics 14:430-440
9. Hwang, L.-Y., et al. (1995) Curr Opin in Immuno 7:659-664
10. Xia, Y., L.-Y. Hwang, et al. (1994) Oncogene 9:1437-46.
11. Hwang, L.-Y., et al. (1993) Oncogene 8: 3043-6
12. Cheng, J.-T., H.-L. Hsu, L.-Y. Hwang, et al. (1993) Oncogene 8: 677-83
13. Sanz, I., L.-Y. Hwang, et al. (1988) Ann. N. Y. Aca. Sci. 546:133-42

Helinor Jane Johnston

PhD BSc

QUALIFICATIONS

• PhD in Nanotoxicology, Edinburgh Napier University (2005-2008)
'Evaluating the uptake, intracellular fate and functional consequences of hepatocyte exposure, to a range of nanoparticles in vitro'
• Pharmacology and Toxicology with an extra mural year BSc (1st class honours), King's College London (2001 –2005).

EMPLOYMENT HISTORY

• Lecturer, Deputy Director of the NanoSafety Research Group, School of Life Sciences, Heriot-Watt University (Jan 2011 – Present).
• Higher Scientific Officer, Department for Environment, Food and Rural Affairs (Defra), Chemicals and Nanotechnologies (CN) Division (Oct 2009-Dec 2010).
• Postgraduate Research Fellow, Edinburgh Napier University (Oct 2008- Aug 2009).
• Industrial Trainee at Novartis Horsham Research Institute (June 2003-July 2004).

SELECTED PUBLICATIONS

• Stone V, Pozzi-Mucelli S, Tran L, Aschberger K, Sabella S, Vogel U, Poland C, Balharry D, Fernandes T, Gottardo S, Hankin S, Hartl MG, Hartmann N, Hristozov D, Hund-Rinke K, **Johnston H**, Marcomini A, Panzer O, Roncato D, Saber AT, Wallin H, Scott-Fordsmand JJ (2014) ITS-NANO - Prioritising nanosafety research to develop a stakeholder driven intelligent testing strategy Particle and Fibre Toxicology 11 :9
• Brown, D. M., **Johnston H**., Gubbins, E., and Stone V. (2013a). Cytotoxicity and cytokine release in hepatocytes and macrophages exposed to gold nanoparticles - effect of biological dispersants. (Journal of Biomedical Nanotechnology In press).
• Brown, D. M., Kanase N., Gaiser B., **Johnston H**., Stone V. (2013b). Inflammation and gene expression in the rat lung after instillation of silica nanoparticles: effect of size, dispersion medium and particle surface charge. (Toxicology Letters. In press).
• Brown, D. M., Varet J., **Johnston H**., Chrystie A., Stone V. (2013c). Silica nanoparticles and biological dispersants: genotoxic effects on A549 lung epithelial cells. (Toxicology in vitro In Press).
• Brown, D. M., Gubbins, E., **Johnston H**., and Stone V. (2013d). Serum enhanced cytokine responses of macrophages to silica and iron oxide particles and nanomaterials – a comparison of serum to lung lining fluid and albumin dispersants. (Journal of Applied Toxicology In Press).
• Kermanizdeh A, Gaiser B; **Johnston H**, Brown D; Stone V. Toxicological impact of engineered nanomaterials on the liver – a review. British Journal of Pharmacology
• **Johnston H** Pojana G, Zuin S, Jacobsen NR, Møller P, Loft S, Semmler-Behnke M, McGuiness C, Balharry D, Marcomini A, Wallin H, Kreyling W, Donaldson K, Tran L, Stone V. (2012). Engineered nanomaterial risk. Lessons learnt from completed nanotoxicology studies. Potential solutions to current and future challenges. Crit Rev Toxicol. 43; 1-20

• **Johnston H**, Brown D, Kermanizadeh A, Gubbins E, Stone V. (2012). Investigating the relationship between nanomaterial hazard and physicochemical properties: Informing the exploitation of nanomaterials within therapeutic and diagnostic applications. J Control Release. [Epub ahead of print]

CONFERENCE AND MEETING ATTENDANCE

• Nanotoxicology, Venice, 2007 (oral presentation)
• 7th World Congress on Alternatives and Animal Use in Life Sciences 2009, Rome (Oral Presentation)
• Pharm Sci Conference, Nottingham, September 2011 (invited speaker)
• European Environment Agency emerging contaminants workshop, Copenhagen, December 2011 (invited speaker)
• German Federal Institute for Risk Assessment, Nano Silver meeting, February 2012, Berlin (invited speaker)
• Medicines and Healthcare Regulatory Agency (MHRA) seminar, London, March 2012 (invited speaker)
• 12th European Symposium on Controlled Drug Delivery, Holland, April 2012 (invited speaker)
• 2012 Annual UK Review Meeting on Outdoor and Indoor Air Pollution, Cranfield University, May 2012 (invited speaker)
• Delft Discussions on Soft Matter, Delft University, Jan 2013 (invited speaker)
• Aerosol Society Meeting, University of Hertfordshire, March 2013 (invited speaker)

Lloyd Johnston

Ph.D., Chief Operating Officer and Senior Vice President R&D, Selecta Biosciences, Inc., Watertown, MA

Selecta Biosciences is developing an entirely new class of targeted immunotherapies and vaccines that induce antigen-specific immune activation or an antigen-specific immune tolerance for therapeutic and prophylactic applications using our proprietary nanoparticle technology. At Selecta Dr. Johnston is responsible for all pharmaceutical development activities including formulation, analytical development, process development, manufacturing, quality control, quality assurance and regulatory affairs. Dr. Johnston has over 15 years of pharmaceutical industry experience and has translated multiple particulate technologies from the early discovery phase to clinical trials as well as had significant involvement in commercialization activities. Prior to joining Selecta Biosciences, Dr. Johnston was Vice President of Operations for Alkermes having responsibilities for process development, scale-up, and manufacturing for pulmonary and sustained release injectable products as well as leadership of Alkermes 90,000 square foot manufacturing facility in Chelsea, MA. Dr. Johnston was also a project leader and member of Steering Committees for numerous products through various stages of development from Phase 1 through registration. Dr. Johnston was an original member of Advanced Inhalation Research (AIR®) Inc., a private company acquired by Alkermes in 1999. Prior to joining AIR, Dr. Johnston was a professor in the Department of Chemical Engineering at the University of New South Wales in Sydney, Australia. He received his B.S. in Chemical Engineering from Queen's University in Canada, and his M.S. and Ph.D. in Chemical Engineering from the Massachusetts Institute of Technology.

David A. Kerr

Director Corporate Strategy
IBM Corporation, Armonk, NY

David Kerr is a Director on the IBM Corporate Strategy team where he focuses on the Healthcare industry, Cloud Computing and emerging technologies. Mr. Kerr has over 25 years experience in the IT industry as a technology and business leader for software development and strategy. He led teams developing systems software and vertical industry solutions prior to assuming his current role. Most recently Mr. Kerr has been focusing on the application of IBM Watson-based analytic and cognitive technologies in healthcare and medicine with a particular focus on oncology working with the leading cancer care centers in the USA.

Hyun Koo Kim

MD., PhD., Associate Professor, Department of Thoracic and Cardiovascular Surgery, College of Medicine, Korea University Guro Hospital, 97, Gurodong-Kil, Guro-Ku, Seoul, 152-703, Korea
Office: 82-2-2626-1180, Fax: 82-2-866-6377
Mobile: 82-2-010-8752-5485
kimhyunkoo@korea.ac.kr

MEDICAL LICENSES AND BOARD CERTIFICATIONS

• Mar 1996; Korean License of Medical Doctor (License No.: 58305)
• Feb 2001; Korean Board Certification of Thoracic and Cardiovascular Surgery (License No.: 797)
• Mar 2009; Korean Board Certification of Critical Care Medicine (License No.: 764)

EDUCATIONAL BACKGROUNDS

•) 1988. 3-1996. 2; M.D. & Bachelor in Medical Science
College of Medicine, Korea University, Seoul, Korea
• 2000. 2; Master in Medical Science
Graduate School of Korea University, Seoul, Korea
• 2004. 2; Ph.D. in Medical Science
Graduate School of Korea University, Seoul, Korea

SCI ARTICLES (SELECTION)

1. **Kim HK**, Son HS, Fang YH, Park SY, Hwang CM, Sun K. The Effects of Pulsatile Flow Upon Renal Tissue Perfusion During Cardiopulmonary Bypass: A Comparative Study of Pulsatile and Nonpulsatile Flow. ASAIO Journal 2005;51(1):30-36.
2. **Kim HK**, Kim WH, Hwang SW, Lee JY, Song JY, Kim SJ, Jang KY. Predictive Value of Intraoperative Transesophageal Echocardiography in Complete Atrioventricular Septal Defect. Ann Thorac Surg 2005 80:56-9.
3. **Kim HK**, Choi YH, Ryu SM, Kim HK, Chae YS, Sohn YS, Kim HJ. Infected Infradiaphragmatic Retroperitoneal Extralobar Pulmonary Sequestration. Journal of Korean Medical Science J Korean Med Sci. 2005 Dec;20(6):1070-2.
4. **Kim HK**, Kim WH, Kim SC, Lim C, Lee CH, Kim SJ. Surgical strategy for pulmonary coarctation in the univentricular heart. Eur J Cardiothorac Surg. 2006 Jan;29(1):100-4.
5.**Kim HK**, Choi YH, Cho YH, Sohn YS, Kim HJ. Intercostal Neuralgia Caused by a Parosteal Lipoma of the Rib. Ann Thorac Surg. 2006 May;81(5):1901-3.
6. **Kim HK**, Kim HJ, Kim JW, Sohn YS, Choi YH. Changes in N-terminal pro B-type natriuretic peptide concentration: comparative study of percutaneous transluminal coronary angioplasty and off-pump coronary artery bypass graft. J Korean Med Sci. 2007 Feb;22(1):16-9.
7.**Kim HK**, Choi YH, Cho YH, Ryu SM, Sohn YS, Kim HJ. A comparative study of pericostal and submuscular bar fixation technique in the Nuss procedure. J Korean Med Sci. 2007 Apr;22(2):254-7.
8. **Kim HK**, Choi YH, Shim JH, Baek MJ, Sohn YS, Kim HJ. Modified Ravitch procedure: using a pectus bar for posttraumatic pectus excavatum. Ann Thorac Surg. 2007 Aug;84(2):647-8.
9. **Kim HK**, Choi YH, Shim JH, Cho YH, Baek MJ, Sohn YS, Kim HJ. Endoscopic Evaluation of the Anastomosis after Esophagectomy with Gastric Tube Reconstruction. World J Surg. 2008 Sep;32(9):2010-4.
10.**Kim HK**, Jo WM, Jung JH, Chung WJ, Shim JH, Choi YH, Lee IS. Needlescopic Lung Biopsy for Interstitial Lung Disease and Indeterminate Pulmonary Nodules: a report on 65 cases. Ann Thorac Surg. 2008. Oct, 86(4):1098-103
11. **Kim HK**, Kim HJ, Shim JH, Baek MJ, Sohn YS, Choi YH. Endovenous Lasering vs. Ambulatory Phlebectomy of Varicose Tributaries in Conjunction with Endovenous Laser Treatment of the Great or Small Saphenous Vein. Ann Vasc Surg. 2009 Mar;23(2):207-11.

Silke Krol

Silke Krol received her degree in Chemistry and Biochemistry at the University of Muenster, Germany. Since 2001 she works in Italy on nanotechnological solutions for medical problem.
Actually she is with Fondazione IRCCS Neurologic Institute "Carlo Besta" in Milan as Principal Investigator and assistant to the director. Her major research interests is the design, development and characterization of nanoparticles as multifunctional drug delivery or as selective drugs for different diseases such as diabetes, cancer, cardiovascular disease, or antiviral drug. Additionally she studies 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. She has 4 pending patents for possible future drugs for prion disease treatment, viral diseases, and cancer diagnostics.
She is still infrequently lecturing as contract professor for "Nanomedicine" at the University of Udine and as guest lecturer for "Nanotoxicology" at the University of Turin and for "Neurodegenerative disease and nanomedical approaches" at the Department of Medical Nanotechnology, School of Advanced Medical Technologies, Tehran University of Medical Sciences, Tehran, Iran. Moreover in 2009 she worked as an expert consultant for the United Nations. She is member of the advisory board of the CLINAM-Foundation of the journal "Euro-Nanotox-Letters", associate editor of "Frontiers in Nanobiotechnology" and adjunct faculty member at the Pakistan Institute of engineering and applied science. She serves as external expert reviewer for National projects in France, Italy, and Greece. She is frequently peer-reviewing for Nanoscale, Nanomedicine, Nanoletters, Journal of controlled release, and others.

Christine Kubiak

PharmD, PhD, ECRIN Executive Manager, Inserm, Paris- France

Christine Kubiak graduated in pharmacy and obtained a PhD in pharmaceutical sciences in drug targeting. She worked for 15 years in the pharmaceutical industry in clinical development and in the management of international clinical research projects in different therapeutic areas. From 2004 to 2006 she worked in a clinical research centre in a University Hospital and was in charge of the development of the quality system and of the projects management. In parallel, she obtained a master degree in quality management.
In 2006, she joined Inserm as European Correspondent and then Executive manager for the European Clinical Research Infrastructure Network (ECRIN) programmes. The objective of ECRIN, that is now an operational European infrastructure with a legal status, is to connect national networks of academic clinical research centres and to provide coordinated support to multinational clinical research in Europe. She is also involved in a broad spectrum of European projects including clinical trials or related to clinical trials legislation and management.

Ninh (Irene) M. La-Beck

Pharm.D.

Assistant Professor, Department of Immunotherapeutics & Biotechnology
School of Pharmacy, Texas Tech University Health Sciences Center
1718 Pine St, Abilene, TX 79601
325-696-0433; irene.la-beck@ttuhsc.edu

POSITIONS AND EMPLOYMENT

• 2008-2010 Fellow, Division of Pharmacotherapy and Experimental Therapeutics, University of North Carolina School of Pharmacy, Chapel Hill, NC
• 2010-2012 Assistant Professor, Department of Pharmacy Practice, Texas Tech University Health Sciences Center School of Pharmacy, Abilene, TX
• 2012- Assistant Professor, Department of Immunotherapeutics and Biotechnology, Texas Tech University Health Sciences Center School of Pharmacy, Abilene, TX
2013- Primary Member, Graduate School of Biomedical Sciences: Biotechnology Program, Texas Tech University Health Sciences Center, Abilene, TX
2013- Associate Member, Graduate School of Biomedical Sciences: Pharmaceutical Sciences Program, Texas Tech University Health Sciences Center, Amarillo, TX

GRANT AND JOURNAL REVIEWER

• 2012 Early Career Reviewer, NIH Center for Scientific Review, Developmental Therapeutics Study Section, ad hoc reviewer
• 2013 Drug Intoxication & Detoxification: Novel Approaches, ad hoc reviewer
• 2013 Anti-Cancer Drugs, ad hoc reviewer

PEER-REVIEWED PUBLICATIONS

1. Dumond JB, Vourvahis M, Rezk NL, Patterson KB, Tien H, White N, Jennings SH, Choi SO, Li J, Wagner MJ, **La-Beck NM**, Drulak M, Sabo JP, Castles MA, MacGregor TR, Kashuba ADM. (2010). A phenotype-genotype approach to predicting CYP450 and P-glycoprotein drug interactions with the mixed inhibitor/inducer tipranavir/ritonavir. Clinical Pharmacology & Therapeutics, 87:735-42. (Impact factor 6.846)
2. La-Beck NM, Zamboni BA, Gabizon A, Sidone BJ, Edwards RP, Tzemach D, Schmeeda H, Sapir R, Amantea M, Gehrig PA, Zamboni WC. (2012). Factors affecting the pharmacokinetics of pegylated liposomal doxorubicin in patients. Cancer Chemotherapy and Pharmacology, 69:43–50. (Impact Factor 2.795)
3. Gibson JM, Alzghari S, Ahn C, Trantham H, **La-Beck NM**. (2013). The role of pegylated liposomal doxorubicin in ovarian cancer: a meta-analysis of randomized clinical trials. The Oncologist, July 23, 2013, E-published ahead of print. (Impact factor 4.095). *Featured on F1000 Prime and MDLynx.*
4. Zamboni WC, Caron W, Lay JC, Fong A, **La-Beck NM**, Kumar P, Newman S, Zamboni BA, Crona D, Clarke-Pearson D, Brewster W, Van Le L, Bae-Jump V, and Gehrig P. Translational studies of phenotypic probes for the mononuclear phagocyte system and liposomal pharmacology. Journal of Pharmacology and Experimental Therapeutics. Accepted September 2013. (Impact factor 6.846)

INVITED CHAPTERS AND REVIEWS

1. La-Beck NM and Zamboni WC. (2009). Pharmacokinetics and pharmacodynamics of nanoparticle anticancer agents. National Cancer Institute Alliance for Nanotechnology in Cancer Bulletin; 3(1):3-6.
2. Walko CM, La-Beck NM, Walsh MD. (2011). Renal Cell Carcinoma. In: DiPiro JT, Talbert RL, Yee GC, Matzke GR, Wells BG, editors. Pharmacotherapy: A Pathophysiologic Approach, Eighth Edition. New York: McGraw-Hill Medical.
3. Zamboni WC and La-Beck NM. (2012) Carrier-Mediated and Targeted Cancer Drug Delivery. In: Spitz DR, Dornfield KJ, Krishnan K, Gius D, editors. Oxidative Stress in Cancer Biology and Therapy. First Edition. New York: Humana Press.

Twan Lammers

Twan Lammers studied Pharmacy at the University of Utrecht. He obtained a DSc degree in Radiation Oncology from Heidelberg University in 2008, and a PhD degree in Pharmaceutics from Utrecht University in 2009. From 2007 until 2010, he worked as a Postdoctoral Fellow at the Department of Pharmaceutics at Utrecht University. Since 2009, he has been appointed as a Group Leader at the Department of Experimental Molecular Imaging at the University Clinic and the Helmholtz Center for Biomedical Engineering at RWTH Aachen University. In 2012, he was awarded an ERC starting grant (NeoNaNo), and took up a part-time position as an Assistant Professor at the Department of Targeted Therapeutics at the University of Twente. He has published approximately 80 research articles and reviews, and is an Editorial Board Member of the American Journal of Nuclear Medicine and Molecular Imaging, Clinical and Translational Imaging, the Journal of Controlled Release, and Theranostics. His primary research interests include drug targeting to tumors, image-guided drug delivery and tumor-targeted combination therapies.

Katharina Landfester

Katharina Landfester joined the Max Planck Society in 2008 as one of the directors of the Max Planck Institute for Polymer Research. She studied Chemistry at the Technical University of Darmstadt and in Strasbourg. In 1995, she received her doctoral degree in Physical Chemistry after working with Prof. H.W. Spiess at the Max Planck Institute for Polymer Research. In 1996, she moved for a doctoral stay at the Lehigh University. She returned to Germany in 1998, working at the Max Planck Institute of Colloids and Interfaces in Golm. There, she led the miniemulsion group. In 2003, she accepted a chair (C4) of Macromolecular Chemistry at the University of Ulm.
In 1992 and 1994, and 1996 she obtained stipends for her research activities in Strasbourg and in the US. In 1998, she received the Liebig stipend of the Chemical Industry Fund (FCI). In 2001 she was awarded the Reimund Stadler prize of the German Chemical Society (GDCh) and the prize of the Dr. Hermann Schnell Foundation, followed by the Bruno Werdelmann Lecturer in 2012 and the BAYER Lecturer in 2014.
Since 2011, she is Member of the National Academy of Science and Engineering (ACATECH). She has published 400 peer-reviewed papers and holds 30 patents.

Karin Lason

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

Dong Soo Lee

M.D.Ph.D.

1. Dept of Nuclear Medicine,
Seoul National University Hospital,
101 Daehak-ro Jongnogu Seoul,
110-744 Korea
Tel: 82-2-2072-2501 Fax; 82-2-745-7690
Email: dsl@plaza.snu.ac.kr

CURRENT APPOINTMENT

- President of Korean Society for Nanomedicine (2012)
- 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
- Past-President of Korean Society of Nuclear Medicine
- Past-President of Korean Society of Human Brain Mapping
- Past-Vice-President of Korean Society of Cognitive Science
- Director, Innovation Cluster of Advanced Medical Imaging, (2005-11)

ACADEMIC DEGREE

M.D. 1982 Seoul National University
Ph.D. 1990 Seoul National University

Field of specialization:
Nuclear Medicine (Neurology and Cardiology) and Molecular Imaging.

SHORT SCIENTIFIC BIOGRAPHY

- Published 279 articles in SCI journal (1999-2012).
- Received Daiichi, Abbott, KOFST, SNUH, IBA, Pfizer, SNU Alumni Academic Awards in 1996, 1999, 2001, 2001, 2002, 2003, 2006.
- Editorial Board of Journal of Nuclear Medicine, European Journal of Nuclear Medicine and Molecular Imaging, Journal of Nuclear Cardiology, The Open Journal of Nuclear Medicine, Journal of European Journal of Nuclear Medicine Research, Nanomedicine: Nanotechnology, Biology, and Medicine, American Journal of Nuclear Medicine, Current Molecular Imaging
- Fellow of American College of Cardiology.

Lada Leyens

E-mail: lada.leyens@swissmedic.ch
Nationality: German
Country of birth: Spain
Languages: Spanish, English, French and German

After finishing the primary and secondary education and the Baccalaureate in Spain, I moved to London, UK, to do a degree in Human Genetics at University College London (UCL). Following the degree I relocated to Switzerland to work in the clinical trials department of a medical devices company during near three years, where I was involved in the planning, execution and analysis of clinical trials. I then decided to move back to London to do a degree in International Health Policy (Health Economics) at the London School of Economics and Political Sciences (LSE). Within the Masters I did a placement at the Strategy Unit of the Department of Health (London, UK). Since January 2013, I have been back in Switzerland and working at Swissmedic in the Clinical Trials Unit. My first role was as Clinical Study Reviewer and then I moved to the GCP inspections unit for clinical trials with medicinal products. At Swissmedic, I am also part of the Nanomedicine Working group, an interdisciplinary group formed by colleagues from different departments to discuss current issues in nanomedicine. I am also currently doing a part-time external PhD on Personalised Medicine at the Institute for Public Health Genomics, Maastricht University.

Julianna Lisziewicz

Dr. Julianna Lisziewicz is the President and Chief Scientific Officer of eMMUNITY, a US-Hungarian company. eMMUNITY has an enabling technology of personalizing immunotherapy that will fundamentally change the prevention and treatment of cancer and infectious diseases. eMMUNITY's team has developed immunogenetic tests and an associated algorithm for selecting the most effective treatment for cancer patients and designed DNA-based nanomedicines that are 10 times more immunogenic than any vaccine or immunotherapy tested to date.

Dr. Lisziewicz has previously founded two successful companies in the US: Research Institute for Genetic and Human Therapy to discovery and clinical investigation of new treatment approaches for HIV, and Genetic Immunity, Inc for clinical development and commercialization of a HIV therapeutic vaccine product developed at RIGHT. She has been directing all the vaccine-related R&D from discovery to preclinical and through clinical trials. She raised over $40 million funding from grants, contracts, investment and loans. She sold Genetic Immunity to Power of the Dream Ventures, Inc. in 2012. Dr Lisziewicz has >100 peer reviewed publications in leading scientific journals including New England Journal of Medicine, Science, AIDS, JID.

Beat Löffler

Beat Löffler studied Communication Sciences, Philosophy and Political Science. He received an MA at Freie Universität Berlin and improved himself in Life Sciences / Biology for further 2 years. In 1983 he started his first company for concepts and new media. Six years later he became Director of the International Hightech-Forum of the Swiss Industries Fair. After working for further 6 years in the new technology sector as developer and conference organiser, creating concepts for emerging technology events, he started in1994 his present company "L&A Concept Engineering" and specialised in the fields of the development of innovation concepts and of science and knowledge promotion initiatives as well as in leadership-training and interdisciplinary bridging events. Fields of work are · Computational Fluid Dynamics · Materials Science · Energy Technology and · Life Sciences. Beat Löffler held numerous mandates for projects developed by his company, e.g.: He wrote and developed the BioValley Upper Rhine Network-Initiative and coached it as Secretary General for 6 years, signed responsible for the Trade Fair for Simulation and Visualisation SIMPAT and developed the Leadership Training EUROPRENEUR together with INSEAD, Fontainebleau and HSG, St. Gallen. He had a mandate during 4 years of the Centre Européen de Management in Colmar and spent 4 years as life science business development-consultant with lead EMEA for the Japanese company NEC High Performance Computing in Düsseldorf, Germany. In 2005 he conceived and realised the European Summit for New Materials in Energy and Mobility in Essen, Germany for "Initiativkreis Ruhrgebiet". He coconceived and realised the 1st World Summit for New Materials in Energy Technology in Lisbon, 2006. In the same year he started the development of a concept for a conference for applied Nanomedicine. In 2007, he founded together with Patrick Hunziker, MD, the European Foundation for Clinical Nanomedicine, started up the European Society for Nanomedicine and co-founded the International Society for Nanomedicine. Since 2007, he is CEO of the CLINAM Foundation and Secretary General of the European Society for Nanomedicine and the International Society for Nanomedicine. His own company has since then developed towards new fields and includes Nanotechnology in Health. Beat Löffler signs responsible for the European Annual Summit for Clinical Nanomedicine and Targeted Medicine which is in Europe the largest platform for this discipline. He is leading the Dissemination Package within 3 FP7 Projects.

Witold Łojkowski

Institute of High Pressure Physics, Polish Academy of Sciences, Sokolowska 29/37, 01-142 Warsaw, Poland
Tel.: +48 22 8880006
Fax: +48 22 6324302,
e-mail: wl@unipress.waw.pl

STUDY & DEGREES

- 1975 MsC in physics at the Warsaw University, Warsaw
- 1980 PhD in Materials Science, Warsaw University of Technology
- 1991 Habilitation, "Structure of Interfaces between Crystals", Kraków Academy of Mining and Metallurgy
- 2012 Full Professor

POSITIONS

- Head of Laboratory of Nanostructures, Institute of High Pressure Physics, Polish Academy of Sciences.
- Prof. at the Bialystok Univeristy of Technology, Faculty of Management and Production Engineering. Teaching Nanotechnology and Materials Science

RESEARCH AND TRAVELS

- 1981 -1982 Alexander von Humboldt Scholarship, University of Saarbrucken, Germany. Research on the structure of interfaces between crystals under supervision of Prof. Herbert Gleiter
- 1986 – 2008: Research visits to Max-Planck Institute for Metal Research, Stuttgart, Osaka University, St. Jerome University in Marseilles, University of Ulm, Germany.

AWARDS

- 2007 Distinguished with the Award "Golden Medal for Merits" by the President of Poland for achievements in science and education
- 2006 Distinguished with the Award "Ambassador of Polish Congresses" by the Polish Tourism Association, for the achievements in organizing international congresses in Poland

R&D INTERESTS

- Solvothermal synthesis of nanoparticles and their applications
- Development of microwave reactors for nanoparticle synthesis
- Bio-nano materials
- Optical nano-sensors for gases
- Mechanisms of plastic deformation of metals, role of nanostructure
- Structure of grain boundaries and interfaces
- Foresight and roadmapping in the field of nanotechnology
- Project management with application of the Theory of Constraints

PARTNER OR COORDINATOR IN RUNNING EUROPEAN PROJECTS

- 2009 – 2014 FP7 Nanofate. Nanoparticle Fate Assessment and Toxicity in the Environment 2009 – 2011 OXYNANOSEN: Oxygen sensor based on luminescence of nanozirconia.
- Eranet project 2010 – 2014 Bioimplant. Bioimplants for bone regrowth in oncologic patients 2011 -2014 SONOSCA: Sonochemical technology for bone regrowth.
- Eranet project 2012 -2015 FP7 SHYMAN: Solvothermal synthesis of nanoparticles

MAJOR COORDINATED PROJECTS

- 2000 – 2003 High Pressure– Center of Excellence Project granted by the EC to the Institute of High Pressure Physics
- 2006 -2008 Research Network WITNANO: Virtual Institute for Nanoparticles Technology
- 2004 -2009 COST actions, Coordinator of Working Group "High Pressure Microwave Synthesis of Nanoparticles".
- 2008 -2011 DONANO – Doped Nanoparticles for Innovative Industry 2011 – 2013 Nanoforce: Nanotechnology for Chemical Enterprise

MEMBERSHIPS AND FUNCTIONS

• 2010 – 2014 Polish speaker in the European NMP – Nanotechnology, Materials and Production Technology Programme Committee in the 7th Framework Research Programme of the European Commission.
• Polish Speaker in the COST domain committee "Materials, Physics and Nanosciences". Selection of projects to be funded.
• Polish speaker at the OECD Working Party on Nanotechnology. Participation in the projects "Business environment of nanotechnology" and "Nanomedicine"
• Executive Committee of European Materials Research Society
• Clinam – Clinical Nanomedicine Association

OTHER ACTIVITIES

• Co-organiser of the European Materials Research Society Fall Meeting held in September in Warsaw every year, which gathers about 800 participants and about 10 symposia.
• Co-organiser of over 10 national and international conferences

PUBLICATIONS AND PATENTS

190 papers, cited over 2274 times, H-index 23 (according to google scholar) Co author of 14 chapters in books and Editor of 8 conference proceedings and books, 10 patents

Patrick Y. Lu

Patrick Y. Lu, Ph.D., is the founder and President/CEO of Sirnaomics, Inc., USA (since 2007) and Chairman/CSO of Suzhou Sirnaomics Pharmaceuticals, Ltd., in Biobay, SIP, China. He also serves as "1000 Talents" Experts, and Adjunct Professor of Nanjing University and South China University of Technology. Dr. Lu started his biopharmaceutical industry career in 1993 and severed as a lab head and senior scientist in Novartis and Digene (Until 2000). Dr. Lu was the co-founder and Executive Vice President of Intradigm Corporation (2001-2006). Patrick has authored more than 50 scientific papers, review articles and book chapters, and holds 35 issued and pending international patents. He has been an invited speaker in many international conferences throughout the world. Dr. Lu has been awarded a number of grants from US NIH, the State and County governments. Under his leadership, Sirnaomics has developed novel siRNA therapeutic programs and established partnerships with Chinese Pharmaceutical companies and US biotech companies. In China, Patrick has received a number of awards for his entrepreneurial successes.

Karsten Mäder

Karsten Mäder is the head of the Department of Pharmaceutics and Biopharmaceutics at the Institute of Pharmacy at the Martin-Luther-University Halle-Wittenberg in Germany. He studied Pharmacy at the Humboldt-University in Berlin from 1986-90. After completion of his Diploma and PhD at the same place he was a DAAD Postdoc scholar at the Dartmouth Medical School (NH, USA) in 1994/95. In 1996/97 Karsten returned to the Humboldt University Berlin and received a Habilitation scholarship from the German Research Foundation DFG. After his Habilitation he worked as a scientist at the Philipps-University Marburg (1997/98) and at the Free University Berlin (1998/2000). Karsten joined the Pharmaceutical Industry in Basle, Switzerland from 2000-2003. Since 2003 he is Full Professor of Pharmaceutics at the Martin-Luther-University Halle-Wittenberg.
His main research areas include polymer- and lipid based nanodrug delivery systems, in vitro and in vivo characterization of DDS by non-invasive ESR- NMR- spectroscopy and Imaging and multispectral Optical Imaging. Tight interdisciplinary research cooperations with polymer chemists and medical scientists exist to improve drug delivery to tumours by stimulus-sensitive Nano-DDS.
He published around 140 papers, several book chapters and patents. He works as an editor for the European Journal of Pharmaceutics and Biopharmaceutics and also as a member of the editorial board for the Journal of Controlled Release. He received several awards, including the Scheele-award of the German Pharmaceutical Society, the CRS - Capsugel award for innovative aspects in controlled drug release, the Young Investigator Award of the International EPR society, the 1. Prize "Pharma Technik"- award of the Bundesverband der Arzneimittelhersteller, the APV Research Award for Outstanding Achievements in Pharmaceutical Sciences, and the Phoenix award in Pharmaceutics.

Alessandro Maiocchi

Alessandro Maiocchi graduated in Industrial Chemistry in 1989 at the Science Faculty of the University of Milan. He is working in the Bracco Group companies since 20 years as a senior scientist covering several roles in the R&D organization. Currently he is the Research Projects Manager at the Bracco Research Centre in Italy. From 2004-2010 he served as contract professor at the Dept. of Biotechnology and Molecular Sciences at the University of Varese in Italy. His current research activity is focused on the design and development of small and nanosized probes for molecular imaging applications in Magnetic Resonance, Ultrasound and Optical Imaging. He is member of several societies and author of more than 150 scientific publication on international journals and conference proceedings in the field of drug design, contrast agents characterization, pharmaceutical product development and imaging methods.

Harald Mangge

Univ.Prof.Dr.med.Harald Mangge
Head of the Research Unit on Lifestyle and Inflammation-associated Risk Biomarkers
Vicespeaker of the Cardiovascular Research Field ; MUG Coordinator BioTechMed Initiative

Clinical Institute for Medical and Chemical Laboratory Diagnosis, Medical University of Graz, A-8036 Graz, Auenbruggerplatz 36
harald.mangge@klinikum-graz.at; harald.mangge@medunigraz.at
http://www.medunigraz.at/styjobs

SHORT PROFILE

Harald Mangge is a Medical Doctor and Associate Professor at the department of laboratory medicine of the medical university of Graz, Austria. He conducts the research unit on lifestyle and inflammation-associated biomarkers. His research focuses on the role of immune-mediated inflammation and adipokines in cardiovascular and metabolic diseases. Concerning Nanomedicine, "functionalized" nanocarriers for an improved diagnosis and treatment of vulnerable atherosclerotic plaques are investigated.

MAIN RESEARCH INTERESTS

Atherosclerosis, obesity, inflammation, nanomedicine, biomarker research

SELECTED MAIN PUBLICATIONS

1. Mangge H, Almer G, et al. Inflammation, adiponectin, obesity and cardiovascular risk. Curr Med Chem 2010;17(36):4511-20. PMID: 21062254
2. Mangge H, Almer G, et al. Nuchal thickness of subcutaneous adipose tissue is tightly associated with an increased LMW/total adiponectin ratio in obese juveniles. Atherosclersis, 2009 Mar;203(1):277-83. doi: 10.1016/j. PMID: 18656877
3. Almer G, Mangge H et al. Interleukin-10: an anti-inflammatory marker to target atherosclerotic lesions via PEGylated liposomes. Mol Pharm. 2013 Jan 7;10(1):175-86. doi: 10.1021/mp300316n. Epub 2012 Dec 4. PMID: 23176185

Mira Marcus-Kalish

miram@post.tau.ac.il

Dr. Mira Marcus-Kalish is currently the director for international research affairs at Tel Aviv University and a Senior Research Fellow at ICTAF – Interdisciplinary Center for Technological Analysis and Forecasting. Her main areas of interest are mathematical modelling, data Analysis, converging technologies and data mining (including a patented rule discovery tool for Bio-Medicine).

Mira Marcus-Kalish holds a Ph.D in operations research from the Technion, Haifa, where she developed a computerized system for E.C.G. diagnosis. Her B.Sc. is in Statistics and Biology from the Hebrew University in Jerusalem. Her post doctorate training was at Harvard University, at the MBCRR laboratory (Molecular Biology Computer Research and Resource) and the Dana Farber Cancer Institute. Based on her background she was part of the "matrix of biological knowledge" Initiative in Santa Fe and was deeply involved in converging technologies thereafter, including the recent EU-US Wtec-NBIC2 initiative-"converging of knowledge, technology and society".

Coming back to Israel, she joined the Weizmann Institute working with Prof. Ephraim Katzir mainly on protein interactions. She was involved at the private business enterprise, before joining Tel Aviv University, as the scientific advisor and then the enterprise marketing director of IBM Israel.

Dr. Kalish was and is involved in many EU framework projects, such as: the Nano2Life NoE (Network of Excellence), SkinTreat, ReNaChip, EpoCan and recently the NanoAthero project and the Human Brain Project (HBP) flagship.

Recent projects focused on personalized medicine, skin treatments, rehabilitation of the discrete sensory motor learning function, cerebellar motor learning, protein- protein interactions, drug toxicity analysis, learning machine systems, multilevel multisource data mining applied to neurology, dialysis patients, biomarkers, genetics, etc.

Massimo Masserini

Massimo Masserini is full Professor of Biochemistry and Molecular Biology at the Medical School, University of Milano-Bicocca , Italy, where he is Head of the Nanomedicine Center (NANOMIB), an interdepartmental Consortium devoted to the bio-medical applications of nanotechnology . His research interests have always been focused on neurochemistry and molecular mechanisms of neurodegeneration, biochemistry of cell membranes and on physicochemical studies on membrane models, in particular liposomes. He has authored or co-authored over 130 papers in national and international journals. He has Coordinated the NMP Project "Nanoparticles for therapy and diagnosis of Alzheimer Disease (NAD)", involving 18 Partners of 13 European Countries, that has recently been awarded among the best completed FP7 projects. Until now, the activities of the Project led to file 4 patents and to the publication of 53 papers on the major journals of nanomedicine.

Eric Mayes

Dr Eric Mayes is CEO of Endomagnetics Ltd and has spent nearly two decades developing and leading nanomaterials companies. Eric has led Endomagnetics from an early academic spinout to an international market presence with revenues growing at 100% CAGR. He is a versatile and entrepreneurial general manager with international business development experience in commercialising nanomaterials in consumer electronics and healthcare applications.

Eric joined Endomagnetics in 2010 from Cambridge Display Technology (CDT) where he most recently served as Director of Commercial Development. CDT was acquired by Sumitomo Chemical Company in 2007 for $285m, and Eric remained to support coordination and integration of their commercial activities. Prior to joining CDT in 2006, Eric founded and served as CEO of NanoMagnetics that developed materials for the data storage industry. For his role in NanoMagnetics, he was named the Royal Society of Chemistry's "Entrepreneur of the Year 2003". Given his entrepreneurial background, Eric is regularly invited to present on topics of technology entrepreneurship and nanomaterials commercialisation in downstream applications.

Mayes' technical background spans bioinorganic chemistry, materials science and physics, and he obtained a BSc in Physics from Arkansas State University and a PhD in Chemistry from the University of Bath. Despite his commercial focus, he remains engaged in technology development and is an author on over a dozen patents, ten peer-reviewed journal articles and two book chapters on nanomaterials and their applications. A dual UK/US citizen, Eric was a researcher in structural biology at the US Department of Energy's Lawrence Livermore National Laboratory (LLNL) before moving to the UK to undertake his PhD in 1996. He is a Fellow of the Royal Society of Chemistry.

Joshua McCarrol

Dr Joshua McCarroll is a Cancer Institute NSW Research Fellow and Project Leader within the Tumour Biology & Targeting Program at the Children's Cancer Institute and Australian Centre for NanoMedicine, UNSW Australia. He received his PhD at UNSW Australia in 2005. After his PhD he completed postdoctoral training at the University of Massachusetts Medical School USA and the University of Minnesota, USA. During this time he designed and utilised non-viral nanoparticles to deliver therapeutic doses of siRNA and miRNA inhibitors to silence genes and miRNAs involved in regulating hypercholesterolemia in mice as well as identify novel drug targets for the treatment of pancreatic cancer. In 2007, he returned to Australia to continue his work on nanoparticle research as well as identification of therapeutic gene targets for adult and childhood cancers. His research interests include, understanding the role of the microtubule cytoskeleton in regulating chemotherapy drug resistance and metastases in lung and pancreatic cancers and the use of nanotechnology to develop RNAi therapeutics to target genes involved in promoting drug resistance and tumour growth.

Scott E. McNeil

Dr. McNeil serves as the Director of the Nanotechnology Characterization Laboratory (NCL) for Leidos Biomedical Research Inc. and the Frederick National Laboratory for Cancer Research, where he coordinates preclinical characterization of nanotech cancer therapeutics and diagnostics. At the NCL, Dr. McNeil leads a team of scientists responsible for testing candidate nanotech drugs and diagnostics, evaluating safety and efficacy, and assisting with product development -- from discovery-level, through scale-up and into clinical trials. NCL has assisted in characterization and evaluation of nearly 300 nanotechnology products, several of which are now in human clinical trials. Dr. McNeil is a member of several working groups on nanomedicine, environmental health and safety, and other nanotechnology issues. He is an invited speaker to numerous nanotechnology-related conferences and has several patents pending related to nanotechnology and biotechnology. He is also a Vice President of Leidos Biomedical Research Inc.

Prior to establishing the NCL, he served as a Senior Scientist in the Nanotech Initiatives Division at SAIC where he transitioned basic

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

Paula Melariri

Dr. Paula Melariri completed her PhD in the division of Pharmacology in 2010 at the University of Cape Town, South Africa. My doctoral degree research exposed me to a wide range of research methodology and disease control using natural products. She is currently employed by the Council for Scientific and Industrial Research, as a Senior Researcher, in the Material Sciences and Manufacturing department, Polymers and Composites, Encapsulation and Drug Delivery group. This group has been recognized as the African Centre of Excellence (CoE) in Nanomedicine, by the African Network for Drugs and Diagnostics Innovations (ANDI). Dr Melariri is leading the research activities in investigating novel nanomedicine treatments for the combat of malaria at the CoE. She is passionate about neglected tropical diseases and infectious diseases of poverty. Her goal is to make positive and innovative contributions to analytical work, research publications and development in her chosen field. Dr. Melariri has published several articles and is a reviewer to several journals. She has attended several scientific meetings, initiated collaborations locally and internationally and contributes meaningfully to human capital development.

PATENT 2013 - Co-inventor in a provisional patent application entitled microemulsion formulation and method of manufacture (with respect to the antimalarial tafenoquine)

SELECTED PUBLICATIONS

• Etusim P.E., Kalu C., Nduka F.O., Kalu E.C., **Melariri P.E.**, Nwoke M., and Aduaka A.C., (2013), Research Studies on the Prevalence of Malaria Parasite among Children with Splenomegaly in Aba, Metropolis, Abia State, Nigeria, Journal of Medical and Applied Biosciences, Vol.5, No.1, Pp. 56-66

• Nanomedicine in the development of drugs for poverty related diseases. Rose Hayeshi, Boitumelo Semete, Lonji Kalombo, Lebogang Katata, Yolandy Lemmer, **Paula Melariri**, Belle Nyamboli and Hulda Swai. In Drug Discovery for Africa, Kelly Chibale, Mike Davies-Coleman, Collen Masimirembwa, 2012.

• **Melariri P.E**., Campbell,W.E., Etusim, P.E., Smith, P.J. 2012. In vitro and in vivo antimalarial activity of linolenic and linoleic acids and their methyl esters. Advanced studies in Biology, vol 4, no. 7, 333-349

• **Melariri P.E.,** Campbell,W.E., Etusim, P.E., Smith, P.J. Antiplasmodial properties and bio-assay guided fractionation of ethyl acetate extracts from Carica papaya leaves . Journal of Parasitology Research, doi:10.1155/2011/104954.

REFEREES

• Prof. Hulda Swai, Encapsulation and Drug Delivery CSIR, Council for Scientific and Industrial Research), (hswai@csir.co.za)

• Prof. Peter Smith, Pharmacology, University of Cape Town, (peter.smith@uct.ac.za)

Konstantinos Mitsakakis

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

Moein Moghimi

BSc (Man.), PhD (Lond.), FIoN
Department of Pharmacy
University of Copenhagen
Universitetsparken 2
DK-2100 Copenhagen Ø, Denmark
Tel. +45 35 33 65 28
E-mails: moien.moghimi@sund.ku.dk
imedicine.moghimi@outlook.com
http://nano.ku.dk/english/research/cpnn/

Moein Moghimi is Professor of Nanomedicine and Biopharmacy, Head of Nanomedicine Research Group, and Director of the Centre for Pharmaceutical Nanotechnology and Nanotoxiocology at the Department of Pharmacy (Faculty of Health and Medical Science), University of Copenhagen, Denmark. As an Affiliate Professor, he further leads the Pharmaceutical Nanotechnology Group at the NanoScience Center (Faculty of Science), University of Copenhagen. Moein is also a Full Member/Professor at Houston Methodist Research Institute (Methodist Hospital, Texas Medical Center), Houston (USA), Honorary Professor of Nanomedicine at the Multidisciplinary Research Center, Shantou University (China), and the elected Fellow of the Institute of Nanotechnology (FIoN), UK. Before joining Copenhagen, 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 pharmaceutical nanoscience, and fundamental nanomedicine/nanotoxicology. He has pioneered research in design and surface engineering of nanoparticles and functional nanosystems for parenteral site-specific targeting/drug delivery and imaging modalities (e.g., splenotropic entities, lymphotropic agents, 'phagocyte-resistant' nanoparticles and cancer nanomedicine) as well as the molecular basis of nanomaterial immune toxicity and polymer cytotoxicity.

Moein has over 170 peer-reviewed publications/patents to his credit. He has served as invited Theme Editor for several Theme Issues of the prestigious *Advanced Drug Delivery Reviews (Elsevier), Maturitas (Elsevier), Journal of Biomedical Nanotechnology (American Scientific Publishers)* and *Current Drug Delivery (Bentham).* He acts in the capacity of Associate Editor for *Nanomedicine: Nanotechnology, Biology and Medicine (Elsevier)* and *Journal of Biomedical Nanotechnology* and a member of the editorial/advisory board of over 20 peer-reviewed international journals, including *Advanced Drug Delivery Reviews, Nanomedicine-UK (Future Medicine), Journal of Liposome Research (Informa Healthcare), Drug Delivery (Informa Healthcare) and Molecular and Cellular Therapies (BioMed Central).*

Over the past two decades, Moein has been practicing 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. Furthermore, he is a regular invited assessor and expert evaluator in nanomedicine/nanotoxicology for governmental bodies, research councils and private organizations world-wide (over 50 establishments in 25 countries). As a frequent speaker and chair at many international conferences and organizations, Moein has given over 300 invited speeches, keynotes and plenaries.

Nassajian Mohamadreza

PhD student
Address:Chemin de Bonne-esperance 14, 1006, Lausanne, Switzerland
Phone: +41 78-930.90.98
Email: Nassajian@gmail.com

I started my PhD at 2010 at EPFL Lausanne Switzerland. Managing a NRP62 Suisse national fond project at Laboratory of biomechanical orthopaedics (LBO), under the supervision of Prof. Dominique Pioletti. My thesis subject was developing a self-heating hydrogels for a smart mechanically-controlled drug delivery system, a novel high efficient system to deliver growth factors to knee cartilage and nucleus pulposus focal defects. The result of the work was published in top journals of field and received many feedbacks from Suisse and worldwide medias. During my work at LBO I obtained many material science and bioengineering skills including: tissue engineering (cell culture and treatment, cell encapsulation, biocompatibility study of biomaterial, RNA extraction, gene expression, histology of biomaterial and biological tissues), advanced microscopy skills (confocal microscopy, super-resolution microscopy STED, electron microscopy SEM,TEG, and Micro-CT scan), material science skills(synthetic organic techniques and characterization of biomaterials (NMR, FTIR, DSC), polymerization techniques). I had Cooperation with Hopital Orthopédique Lausanne CHUV and material science and polymer laboratories.
Form 2007 to 2010 I did my master studies in biomechanics at Sharif University of Technology (SUT) in Tehran, Iran. My thesis topic was developing a biomechanical model for evaluation of shoulder function: detecting the relationship between muscles activation patterns and shoulder joint torques using muscle synergies. Meantime, I had internship at Research Center for Science and Technology In Medicine (RCSTIM), Tehran. My work was the design and production of a new laparoscopic trocar for improving dialysis catheter placement in laparoscopy surgery. The new designed tool reduced significantly the surgery time and improved the efficiency of surgery. During my master I obtained some skills at musculoskeletal modeling and nervous system signaling pass-way, EMG signal processing, bio-sensors and bio-instrument design.
From 2007 to 2009 I was also faculty member at Feiz Institute of Higher Education, Isfahan, Iran. I held university courses in Applied Mathematics and Differential Equations for 200 students each semester, a remarkable managing and organization experience.
From 2002 to 2007 I did my bachelor studies in mechanical engineering at Sharif University of Technology (SUT). The thesis topic was developing a finite element model for analysis of undersea pipeline with S-lay method. I obtained various skills in mechanical engineering skills including mechanical characterization of soft and non-linear materials, viscoelastic and poroelastic, finite element modelling, hydraulics and pneumatics, micro fluid modelling, machine design and development.
Publications: Over 12 scientific papers in most prominent journals and conferences of the field.
www.researchgate.net/profile/Mohamadreza_Nassajian/?ev=hdr_xprf

Sitaramaiah Mokkapati

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

Jan Mollenhauer

Prof. Dr.

Jan Mollenhauer was born in Kiel, Germany, in 1968, studied biology from 1989-1994 at the University of Cologne, Germany, and received his PhD in 1998 from the University of Heidelberg, Germany. In 2003 he received his habilitation in Molecular Medicine from the University Heidelberg, which was mentored by the Nobel laureate in Medicine or Physiology 2008, Prof. Harald zur Hausen. Until 2008 he worked as group leader in the Division of Molecular Genome Analyses (Head: Prof. Annemarie Poustka) at the German Cancer Research Center, Heidelberg. In 2008 he joined the University of Southern Denmark, Odense, as Professor for Molecular Oncology. Since 2010, he is director of the Lundbeckfonden Center of Excellence NanoCAN (Nanomedicine Research Center for Cancer Stem Cell Targeting Therapeutics). Jan Mollenhauer received the Future Award in Health Sciences 2005 and was listed in the 2007 edition of the Who Is Who of Emerging Leaders. In 2009 he was awarded with the Leo og Ingeborg Dannins Fondens Legat, and received the Fyens Stiftstidende Researcher Award in 2010. Research focuses on the role of epithelial protection factors in cancer, on the development and application of novel functional genomics techniques for cancer drug target discovery, and the design of tailored nanodrugs for personalized cancer therapy.

Chrit Moonen

Chrit Moonen did his Masters in Molecular Sciences and his Ph.D. in biophysics (Wageningen University). He did part of his studies with Nobel Laureate Wüthrich in Zürich, Switzerland. He went for a postdoctoral period to the University of Oxford (Sir Georg Radda). He then worked at the University of California at Davis as a Visiting Research Scientist before becoming head of the NIH In Vivo NMR Research Center from

1987-1996. He moved back to Europe (Bordeaux, France) in 1996 where he has been director of the laboratory "Molecular and Functional Imaging: from Physiology to Therapy" until 2011. He is currently professor at the Division of Imaging at the University Medical Center in Utrecht, the Netherlands. He coauthored over 200 scientific papers. H-index is 55. He was President of the "International Society of Magnetic Resonance in Medicine" (2006), and of the "Society for Molecular Imaging" (2009). He received the European Magnetic Resonance Award 2000, is a Fellow of the International Society of Magnetic Resonance in Medicine, of the European Society of Magnetic Resonance in Medicine and Biology, and of the World Molecular Imaging Society. His recent work is mainly in MRI guided Focused Ultrasound, image guided drug delivery, and molecular and cellular imaging.

Kouros Motamed

Ph.D.
kmotamed@sorrentotherapeutics.com
http://www.linkedin.com/in/kourosmotamed

Experienced translational medicine investigator with proven record of success in directing scientific productivity of multidisciplinary research teams in academic and industry settings.

WORK EXPERIENCE

• 2013-Present SORRENTO THERAPEUTICS, Irvine, CA (www.sorrentotherapeutics.com)
(A Public Next Generation Targeted & Personalized Cancer Therapeutics Company)
VP of Clinical Development & Nanomedicine
- Clinical development of 2 licensed nanoparticle paclitaxel formulations for US/EU approval
- PK optimization of nanoparticle paclitaxel formulations

• 2012-2013 IGDRASOL, Inc., Fountain Valley, CA (www.igdrasol.com)
(A Start-Up Personalized Nanomedicine Therapeutic Company)
Chief Scientific Officer
- Licensed 2 Phase 3-ready nanoparticle paclitaxel formulations for US FDA approval
- Directed the design & formulation of mAb-targeted paclitaxel nanoparticles

• 2012-2013 BIOMIGA DIAGNOSTICS, Fountain Valley, CA (www.biomigadiagnostics.com)
(A Start-Up Personalized Medicine Diagnostics Company)
Chief Technical Officer
- Directed assay design, sales and marketing of Point-of-Care, Therapeutic Drug Monitoring (TDM) medical tests.
- Directed outreach programs to company clients, device manufacturers, key opinion leaders, clinician collaborators and investigator-initiated clinical trials.
- Formulated long-term visions and strategies for personalized medicine product lines.

• 2007-2012 ABRAXIS BIOSCIENC, LLC (acquired by CELGENE in 2011), Los Angeles, CA (A $10 billion pharmaceutical company)
Group Head, MOA and Molecular Group
Generation and evaluation of novel in-house and licensed drug delivery systems
- Extensive experience in design and execution of MOA studies for FDA filings
- Establishment and validation of IHC biomarkers in support of FDA filings.

• 2002-2007 GEORGIA HEALTH SCIENCES UNIVERSITY Augusta, GA
Assistant Professor, Dept. of Pathology & Vascular Biology Center
- Established novel in vitro co-culture systems of ovarian cancer cells, primary mesothelial cells and macrophages to test the inhibitory function of novel proteins and small molecules.
- Directed multi-disciplinary in-house and outside collaborations to evaluate the role of extracellular matrix proteins in mouse models of Diabetes (STZ, ob/ob), Flow-induced Vascular Remodeling, and Stroke (transient middle cerebral artery occlusion, MCAO).
- Established mouse models of early and late stage ovarian and prostate cancer to study the molecular signaling of tumor growth and metastasis.

PUBLICATIONS

• Over 30 peer-reviewed publications in high impact journals
• Over 50 published abstracts

PATENTS

• 9 patents/patent applications

Jan Mous

PD Dr.

CEO of PharMida AG, Basel, and COO of Midatech Ltd., Oxford, UK.

EDUCATION

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

PROFESSIONAL CAREER

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

Bert Müller

Bert Müller received a diploma in mechanical engineering, Berlin 1982, followed by M.Sc. degrees in Physics and English both from the Dresden University of Technology in 1989. In 1994, he obtained a Ph.D. in experimental physics from the University of Hannover, Germany. For his achievements he was granted with the Morton M. Traum Award of the American Vacuum Society in 1994. From 1994 to 2001, he worked as a researcher at the Paderborn University, Germany, as Feodor Lynen Fellow and research associate at the EPF Lausanne and as team leader at the Physics Department, Materials Department and Department of Information Technology and Electrical Engineering at ETH Zurich. He became a faculty member of the Physics Department at ETH Zurich in April 2001. After his election as Thomas Straumann-Chair for Materials Science in Medicine at the University of Basel, Switzerland and his appointment at the Surgery Department of the University Hospital Basel in September 2006, he founded the Biomaterials Science Center in March 2007. He teaches physics and materials science at the ETH Zurich and the University of Basel and currently supervises more than a dozen doctoral students from medicine, dentistry, physics, nanosciences, chemistry and biomedical engineering. 2014 he was elected as Fellow of SPIE.

Matilu Mwau

Associate Professor
College of Health Sciences, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000-00200, Nairobi, Kenya. Email: matilu.mwau@gmail.com

Chief Research Officer
Centre for Infectious & Parasitic Diseases Control Research (CIPDCR), Kenya Medical Research Institute (KEMRI), P.O. Box 3-50400 Busia
Telephone: +254728073633, Email: mmwau@kemri.org, matilu.mwau@gmail.com. Skype: the-king-of-scotland

Date and Place of birth: 1st July 1972, Machakos District, Eastern Province, Kenya; Marital status: Married. 2 children.

CAREER SUMMARY

Health systems strengthening specialist, infectious diseases specialist, laboratory specialist, administrator.
CAREER OBJECTIVE: "To create a Brand of Myself in all that I do"

EDUCATION

• 2010: Certificate in Strategic Leadership, Kenya Institute of Administration
• 2006-7: Master of Tropical Medicine (MTM), Nagasaki University, Japan
Thesis: Development of a rapid serological screening assay for Yellow fever virus infection in Kenya.
• 2000-3: Doctor of Philosophy (DPhil) in Clinical Medicine (Immunology), Oxford University, England. Thesis: Clinical Evaluation of a Candidate HIV-1 Clade A DNA/MVA Vaccine Designed for Kenya.
• 1992-8: Bachelor's Degree in Medicine and Surgery (MB, Ch.B.), University of Nairobi, Kenya
• 1987-90: Kenya Certificate of Secondary Education, Alliance High School, Kenya. National #19
• 1979-86: Kenya Certificate of Primary Education, Cianda Primary School, Kenya. National #101

OTHER TRAINING

Certificates-Strategic leadership, Good Laboratory Practise, Good Clinical Practise, Ethical Research, Biosafety, Project Cycle Management, Advanced Life Support, International Health, Flow Cytometry, Commodity Supply Chain Management, Laboratory Animal Handling, Research Methodologies, Clinical Trials Monitoring, HIV Drug Resistance, WHO Assessor for Lab Accreditation, Financial management.

AWARDS AND HONOURS

• 2013: Ambassador, Africa Society for Laboratory Medicine
• 2010: IAS Scholar, XVIII International AIDS Conference Scholarship, Vienna, Austria
• 2007: Distinction. Master of Tropical Medicine, Nagasaki University, Japan
• 2006: JICA Scholar, Nagasaki University Institute of Tropical Medicine, Japan
• 2000: IAVI Scholar, Trinity College, Oxford University, UK
• 2001: MRC Max Perutz Essay Competition, 1st Prize, UK
• 2001: Outstanding Scholar, Trinity College, Oxford University, UK
• 2001: Young Scientist Award, 2nd International Conference on Vaccine Development and Immunotherapy in HIV, San Juan, Puerto Rico
• 2001: Young Scientist Award, Nobel Symposium No. 119, Karolinska Institutet, Sweden
• 2001: Queen's Anniversary Prize for Higher and Further Education
• 1998: Tokai Elective Term Scholarship, Tokai University, Japan

KEY COMPETENCIES

Strategic Leadership: Conversant with the theory and practise of strategic leadership.
Professional: Health Systems Strengthening Specialist, Infectious Diseases Researcher, Laboratory specialist, virologist, immunologist, medical doctor.
Regulatory Affairs: Wide knowledge of the local regulatory environment for medical devices, medicinal products and technologies. Biases. Health systems Strengthening, Administration. Teaching and the pursuit of knowledge. Tropical Diseases Research (HIV, Malaria, Arboviruses, Neglected Tropical Diseases), ART Programme Development. HIV Diagnostics, Early Infant Diagnosis of HIV. Institutional Systems strengthening, Laboratory Systems Strengthening, Development of Laboratory Standard Operating Procedures. Development of diagnostic tests. Point of care diagnostics. Commodity supply chain management. Disease Surveillance and Response. mHealth. Point of care technologies. Knowledge brokerage: Getting the right mix of people and information together to tackle the right issue at the right time is the essence of brokering knowledge. I am proficient in assembling winning teams.
Laboratory: Building of new laboratories. Proficient in the performance and interpretation of routine clinical laboratory tests. Proficient in Elispot assays, ELISA and BED ELISA, Flow Cytometry, Plaque Reduction Neutralisation Tests, Loop Amplification, Cell Culture Techniques, Molecular Biology Techniques, Tissue Typing, Cytolytic Assays, Viral Loads, Sequencing etc. Computing: Proficient in most Mac and PC Applications in common usage (Strong preference for the Mac OS environment). Microsoft Office Applications (Excel, Access, Power Point, Word), OpenOffice Applications, Mac OS X Applications (Office, Numbers, Keynote, Pages etc), Adobe Illustrator, Photoshop, Endnote, Stata. Data management: Advanced level skills in statistics. Stata. Language skills Proficient in English, Swahili, Kikuyu, Kikamba, Working knowledge of Embu, Meru. Other skills: Oratory and Writing. Quantitative and qualitative research methods 2010-2013: Secretary, and founder member, KEMRI Annual Scientific and Health (KASH) Committee. In July 2010, KEMRI decided to mainstream and institutionalize its scientific and health agenda. The KASH Committee was established in August 2010 to spearhead this paradigm shift. It plays a unique role within KEMRI, and is not tied to a specific operational mission: The KASH Committee supplies scientific options for the entire KEMRI, and is designed to be the "scientific engine" for transforming KEMRI.

Noha Nafee

PhD
Helmholtz Institute for Pharmaceutical Research Saarland (HIPS)
Department of Drug Delivery (DDEL)
Saarland University , Campus A4-1
D-66123-Saarbrücken , Germany
Phone: +49 681 302 4764
Fax: +49 681 302 4677
Email: noha.nafee@mx.uni-saarland.de, n.nafe3@gmail.com

Noha Nafee studied Pharmacy in the Faculty of Pharmacy, Alexandria University, Alexandria, Egypt and worked as demonstrator in the Department of Pharmaceutics, where she got her Master degree in Pharmaceutical Sciences in the field of buccal mucoadhesive drug delivery systems. In 2004, she was awarded a 2-years DAAD doctoral scholarship in the Department of Biopharmaceutics and Pharmaceutical Technology, Saarland University, SaarbrŸcken, Germany, where she continued her PhD entitled 'Cationically-modified nanoparticles for the pulmonary delivery of telomerase inhibitor 2'O-Methyl RNA for the treatment of lung cancer' (Tutor: Prof. Dr. Claus-Michael Lehr). Noha received her PhD degree in Natural Sciences in 2008, and then continued as a Post Doc in the same institute contributing to two projects: Saarbridge project entitled 'Polymers for the time-controlled drug release in immunotherapy' and NanoStarch project entitled 'Nanoscale carriers for targeted transport of antineoplastic drugs'. In 2009, she returned to Egypt and worked as lecturer in the Department of Pharmaceutics, Faculty of Pharmacy, Alexandria University and was the PI of research project entitled 'Nanotechnological approach to the enhancement of drug delivery systems for photodynamic therapy'. Currently, Noha is awarded an Alexander von Humboldt post-doctoral fellowship in Department of Pharmaceutical Technology and Biopharmacy, Philipps-University Marburg, Marburg, Germany (host: Prof. Dr. Marc Schneider), where she is engaged in a project focusing on 'Nanocarrier-mediated delivery of novel quorum sensing inhibitors' in collaboration with Helmholtz Institute for Pharmaceutical

Research Saarland (HIPS).
Her research interests deal with novel and targeted delivery systems for the treatment of cancer, cystic fibrosis and wound healing with particular insights on polymer- and lipid-based nanocarriers for pulmonary drug and gene delivery. Special focus relates to the interaction of these nanocarriers with biological barriers such as mucosal and epithelial barriers.
The scientific output of Noha Nafee can be elaborated as 14 research articles in peer reviewed journals (h index = 9), Nį of Citations (702, Google Scholar and 484, Scopus), 3 book chapters, one patent, 5 podium presentations and 21 posters in international conferences.

Nobuhiro Nishiyama

Professor
Polymer Chemistry Division, Chemical Resources Laboratory, Tokyo Institute of Technology ; R1-11, 4259 Nagatsuta, Midori-ku, Yokohama 226-8503, Japan
tel: +81- 45-924-5240/ fax: +81-45-924-5275
email: nishiyama@res.titech.ac.jp
lab URL: http://www.bmw.res.titech.ac.jp

EDUCATION

- 1998–2001 Ph.D. Materials Science, The University of Tokyo
- 1996–1998 M.S. Materials Science, Tokyo University of Science
- 1992–1996 B.S. Materials Science, Tokyo University of Scienc

PROFESSIONAL CAREER

- 2012–present Professor, Polymer Chemistry Division, Chemical Resources Laboratory, Tokyo Institute of Technology
- 2006–2012 Associate Professor, Center for Disease Biology and Integrative Medicine, Graduate School of Medicine, University of Tokyo
- 2004–2006 Assistant Professor, Center for Disease Biology and Integrative Medicine, Graduate School of Medicine, University of Tokyo
- 2003–2004 Assistant Professor, Division of Tissue Engineering, The University of Tokyo Hospital
- 2001–2003 Postdoctoral Fellow, Faculty of Pharmaceutics, University of Utah (Supervisor: Prof. Jindrich Kopecek)

AWARDS AND HONORS

- 2012 The Young Investigator Award, the Japanese Cancer Association
- 2009 The Young Investigator Award, The Japan Society of Drug Delivery System
- 2007 Award for Encouragement of Research in Polymer Science, The Society of Polymer Science, Japan

RECENT PUBLICATIONS

1. H. Chen, L. Xiao, Y. Anraku, P. Mi, X. Liu, H. Cabral, A. Inoue, T. Nomoto, A. Kishimura*, **N. Nishiyama***, K. Kataoka*, Polyion complex vesicles for photo-induced intracellular delivery of amphiphilic photosensitizer. J. Am. Chem. Soc. 136 (1) 157-163 (2014)
2. Y. Miura, T. Takenaka, K. Toh, S. Wu, H. Nishihara, M. R. Kano, Y. Ino, T. Nomoto, Y. Matsumoto, H. Koyama, H. Cabral, **N. Nishiyama***, K. Kataoka*, Cyclic RGD-linked polymeric micelles for targeted delivery of platinum anticancer drugs to glioblastoma through the blood-brain tumor barrier. ACS Nano 7 (10) 8583-8592 (2013)
3. H. Cabral, M. Murakami, H. Hojo, Y. Terada, M. R. Kano, U. -I. Chung, **N. Nishiyama***, K. Kataoka*, Targeted therapy of spontaneous murine pancreatic tumors by polymeric micelles prolongs survival and prevents peritoneal metastasis. Proc. Natl. Acad. Sci. USA. 110 (28) 11397-11402 (2013)
4. H. Takemoto, K. Miyata, S. Hattori, T. Ishii, T. Suma, S. Uchida, **N. Nishiyama**, K. Kataoka*, Acidic pH-responsive siRNA conjugate for reversible carrier stability and accelerated endosomal escape with reduced IFNα-associated immune response. Angew. Chem. Int. Ed. 52 (24) 6218-6221 (2013)
5. S. Wu*, V. Kasim, M. R Kano, S. Tanaka, S. Ohba, Y. Miura, K. Miyata, X. Liu, A. Matsuhashi, U.-I Chung, K. Kataoka, **N. Nishiyama***, M. Miyagishi*, Transcription factor YY1 contributes to tumor growth by stabilizing hypoxia factor HIF-1α in a p53-independent manner. Cancer Res. 73 (6) 1787-1799 (2013)
6. H. Cabral, Y. Matsumoto, K. Mizuno, Q. Chen, M. Murakami, M. Kimura, Y. Terada, M.R. Kano, K. Miyazono, M. Uesaka, **N. Nishiyama***, K. Kataoka*, Accumulation of sub-100 nm polymeric micelles in poorly permeable tumours depends on size. Nature Nanotech. 6 (12) 815-823 (2011)
7. M. Murakami, H. Cabral, Y. Matsumoto, S. Wu, M. R. Kano, T. Yamori, N. **N. Nishiyama***, K. Kataoka*, Improving drug potency and efficacy by nanocarrier-mediated subcellular targeting. Science Transl. Med. 3 (64) 64ra2 (2011)

Yanay Ofran

PH.D.

Bar Ilan University
Goodman Faculty of Life Sciences
Molecular and Computational Biology Program
Institute of Nanotechnology and Advanced Materials, Nanotechnology Building
Ramat Gan 52900, Israel; Tel: +972(3) 531-9972
E-mail: yanay@ofranlab.org, Ofran Lab: http://www.ofranlab.org

PROFESSIONAL PREPARATION

- 1999 B.Sc., Physics and Biology, Hebrew University, Jerusalem, Israel
- 2003 Ph.D., Molecular Biophysics and Biochemistry, Columbia University, New York USA
- 2006 Postdoctoral, Computational Biology, Columbia University, New York, USA
- 2007 Postdoctoral, Biochemistry, Tel Aviv University, Tel Aviv, Israel

APPOINTMENTS AND POSITION

- 11/2007-present Member, Institute of Nanotechnology and Advanced Materials, Bar Ilan University, Israel
- 11/2007-present Senior Lecturer, Faculty of Life Sciences, Bar Ilan University, Israel
- 11/2007-present Program for Computational Biology, Bar Ilan University, Israel
- 11/2007-present Program for Biophysics, Bar Ilan University, Israel
- 08/2003-01/2006 Research Fellow, Center for Computational Biology and Bioinformatics, Columbia Univeristy, NY, USA
- 02/2006-8/2007 Safra Fellow, Tel Aviv University
- 1/1/2010-present Founder, Biolojic Design Ltd.

Andrew Owen

Andrew Owen, Ph.D. FSB holds a personal Chair in the Department of Molecular and Clinical Pharmacology at the University of Liverpool, UK. He is also affiliated to the MRC Centre for Drug Safety Science and the Wolfson Centre for Personalised Medicine. Professor Owen has contributed to over 110 original research and review publications, book chapters and patent applications. In recent years, research funding has been secured from the Medical Research Council, Engineering and Physical Sciences Research Council, US National Institutes of Health and the British Society for Antimicrobial Chemotherapy. He is Chair of the British Society for Nanomedicine (www.BritishSocietyNanomedicine.org), a fellow of the Society of Biology and a member of the Nanomed Focus Group Steering Committee and British Pharmacological Society. Professor Owen is also a member of the editorial board for Nanomedicine: nanotechnology, biology and medicine.

Rick Panicucci

Novartis Institute for Biomedical Research
250 Massachusetts Ave.
Cambridge, MA 02139
857-205-0109(Mobile); 617-871-3165 (Office)
riccardo.panicucci@novartis.com

PROFESSIONAL EXPERIENCE

• 2007-Present: Adjunct Professor at Massachusetts College of Pharmacy
• 2004-Present: Novartis Institute for Biomedical Research, Development Site Head for Cambridge and Global Head of Chemical & Pharmaceutical Profiling (CPP)
Responsible for the operation of the Development organization in Cambridge. Also responsible for early Chemical & Pharmaceutical Development in the US, Europe and Asia, including selecting the best molecules for clinical development. Lead the creation and implementation of innovative technologies to assess the "developability" of molecules during drug discovery, the development of novel drug delivery and device technologies and the management of a global team of 75 scientists. Responsible for managing an internal budget of >$20M USD and an external technology budget of $6M USD.
• 1998-2003: Vertex Pharmaceuticals, Director of Formulation Development
Responsible for compound selection, preformulation and formulation development. Dosage form strategies for Phase I- 3 clinical trials. Developed high-throughput technologies for assessing the "developability" of NCE's. Evaluation of external pharmaceutical technologies to enable the formulation development of our clinical candidates. Manage an outsourcing budget of $8M. Responsible for writing CMC sections for drug product sections of IND's and NDA's.
• 1997-1998: Biogen, Senior Scientist, Pharmaceutical Development
Leadership role in small molecule programs. Range of responsibilities includes preformulation studies, formulation development for phase I-III clinical studies and technology transfer. Coordinate with preclinical departments to develop and execute protocols for toxicity, PK and PD studies. Interface, including contract negotiations, with CRO's to accomplish R&D goals.
• 1994-1996: Symbollon Pharmaceuticals, Director of Research and Development
Developed a R&D program for Symbollon's iodine technology. The focus of the research was to use this technology to develop treatments for Helicobacter pylori (collaboration with AstraMerck) and diseases of the skin (Watson Pharmaceuticals) and the oral cavity (Unilever). This required the formulation of proteins and small molecules into various dosage forms.
• 1990-1993: Bausch & Lomb: Senior Scientist, Formulation Research and Process Development
Responsible for the discovery, formulation and stability studies of novel antimicrobial compounds. Additional responsibilities included the evaluation of dosage forms and pharmaceutical technologies for scale-up and clinical supplies. Responsibility applies to both domestic and international products. Served as project team leader responsible for validating the bulk synthesis of PHMB.

EDUCATION

• 1988-1989: Postdoctoral Fellow, University of California, Santa Barbara
• 1987-1988: Postdoctoral Fellow, Ontario Cancer Institute at University of Toronto
• 1983-1987: Ph.D. in Physical Organic Chemistry, University of Toronto
• 1979-1983: B.S. in Chemistry, summa cum laude, McMaster University

HONORS AND AWARDS

• 1988: Young Investigator Award from the American College of Radiology, University
• 1985 : Open Scholarship, University of Toronto
• 1983 : Natural Science & Engineering Research Council Undergraduate Student Research Award
• 1982: M.J. Morton and F.P. Olson Prize for the most promising young scientist in chemistry

Marisa Papaluca Amati

Internal Medicine specialists, Marisa joined the EMA in late 1994 and occupied scientific and managerial positions in the EMA Unit for Human Medicines Development and Evaluation.
Deputy Head of Quality up to 2002 and of the Efficacy and Safety Sectors up to 2009, Marisa is currently Head of the Scientific Support office providing scientific support to the Agency core activities in transversal and multidisciplinary areas such as clinical trials statistical methodology, raw data analysis, non-clinical drug development, pharmacogenomics and nanotechnology.
The office is also in charge of the EMA the Innovation Task Force, reference group at EU and international level for innovative pharmaceuticals developments with current increasing activities on novel clinical trials designs, genomic biomarkers, combined products, synthetic biology and nanomedicines.

Renato Paro

Renato Paro studied and received his Ph.D. at the Biozentrum of the University of Basel. After post-doctoral stays at the Department of Molecular Biology of the University of Edinburgh and the Department of Biochemistry of Stanford University, he continued his career at the Center for Molecular Biology of the University of Heidelberg (ZMBH) in Germany as an independent group leader. He became a Professor of Molecular Biology at the Faculty of Medicine and Faculty of Biosciences of the University of Heidelberg and between 2001 und 2004 was the Acting Director of the ZMBH. In 2006 he was appointed Founding Director of the new Department of Biosystems Science and Engineering (D-BSSE) of the ETH Zurich in Basel and Professor of Biosystems at the University of Basel.
The major research areas of his laboratory focus on mechanisms of epigenetic gene control and cellular signaling. Key contributions concern the role of the Polycomb and Trithorax chromatin proteins in epigenetic regulation, including their parts in development and disease. Chromatin controls the activity of genes in a eukaryotic cell and maintains gene expression patterns epigenetically stable and heritable during cell division. In the past the laboratory was the first to identify and describe at the molecular level the transgenerational inheritance of epigenetic traits in a complex organism. His group generates systems-level comprehensive descriptions of chromatin structures to provide tissue specific epigenetic typing of cells. The aim is to be able to alter specifically the fate of cells towards the needs required for tissue engineering and regenerative medicine. Additionally, the laboratory has continuously developed new technologies, the best known being chromatin immunoprecipitation (ChIP) now used worldwide to detect and map the in vivo distribution of chromatin and DNA-associated proteins.

Dan Peer

Prof. Dan Peer is an associate professor that leads an NIH-funded lab in the Faculty of Life Science and the faculty of Engineering at Tel Aviv University (TAU). He is also the director of the Focal Technology Area (FTA) on Nanomedicines for Personalized Theranostics, a national nanotechnology initiative supported also by the Leona M. and Harry B. Helmsley Nanotechnology Research Fund.
He was recruited to Tel Aviv University from Harvard Medical School in 2008.
Prof. Dan Peer's work was among the first to demonstrate systemic

delivery of RNA molecules using targeted nanocarriers to the immune system and he pioneered the use of RNA interference (RNAi) for in vivo validation of new drug targets within the immune system. He generated an international recognition and collaboration in inflammatory bowel diseases (IBD) and oncology area. He received numerous awards; among them he was recognized by the AAAS excellence in Science program for young investigators and was recently awarded the innovator (2010) and the breakthrough (2011, 2013) awards from the Kenneth Rainin Foundation on his pioneering work in inflammatory bowel diseases (IBD).
His current interests include the generation of novel platforms for delivery of therapeutics and imaging payloads into specific cell types and the utilization of these nanocarriers also for in vivo discovery and validation of new drug targets in leukocytes implicated diseases. He is an editor of several books in the field of nanomedicine, Editor of Molecular and Cellular Therapies (Springer), an associate editor of the Journal of Biomedical Nanotechnology, and of Biochemistry, and on the editorial boards of the Journal of Controlled Release (Elsevier), Nanotechnology (IOP), Biomedical Microdevises (Springer), Cancer Letters (Elsevier) and Bioconjugate Chemistry (ACS).
He has more than 45 pending and granted patents. Some of them have been licensed to several pharmaceutical companies and one is under a phase II clinical evaluation. In addition, based on his work, 2 spin-off companies were generated LeukoBiosciences in the US and Quiet Therapeutics in Israel, aiming to bring nanomedicine into clinical practice.

Giacomo Pongiglione

Born in Lucca (Italy) onl 3/2/1949
Address Genova, Via S. Bartolomeo degli Armeni 21/12
Phone +39 010-886282
e-mail giacomo.pongiglione@opbg.net

TRAINING

• 15 July 1973 Graduated cum laude in Medicin and Surgery at University of Genova Medical School
• 15 July 1976 Specialty cum laude in Pediatrics at the University of Genova Medical School
• 21 April 1980 E.C.F.M.G.
• 16 July 1980 Speciality cum laude in Cardiology at the University of Ferrara.
• 1981-1982 Fellow, Dept. of Cardiology. The Hospital for Sick Children, Toronto, Ontario, Canada.
• September 1983 Visiting fellow in Echocardiography. The Hospital for Sick Children. Toronto, Ontario
• June 1985 Visiting fellow in Interventional Cardiology. The Hospital for Sick Children. Toronto, Ontario
• March-June 1987 Visiting fellow in Electrophysiology. The Children's Memorial Hospital. Chicago, Illinois.
• September-December 1987 Visiting fellow in Electrophysiology. Texas Children's Hospital. Houston, Texas
• May- August 1989 Visiting Research Associate Professor. The Children's Memorial Hospital. Chicago, Illinois
• October-December 1990 Visiting Research Associate Professor in Electrophysiology. The Children's Memorial Hospital. Chicago, Illinois

David Pozo Perez

David Pozo Perez pursued his graduate degree in Biology (Biochemistry and Cell Molecular Biology profile) at the University of Seville Faculty of Biology (1992), and received his Ph.D. in Cellular and Molecular Biology (1998) from the University of Seville, where he studied several aspects related to signal transduction and the role of vasoactive intestinal peptide (VIP) in inflammatory/autoimmune diseases. He subsequently joined the Division of Immunology at Cambridge University as an EMBO postdoctoral fellow and Marie Curie Fellow from 2000-2003. He did a second post-doctoral stint at the Department of Immunology of Weizmann Institute of Science (2004) in Israel before joining the Department of Biochemistry and Molecular Biology as assistant professor (2005). In 2006, he got the Award for Recognition of Outstanding Neuropeptide Research in the Winter Neuropeptide Conference (Breckenridge, Colorado, USA), a joint conference between the American and European Peptide societies.
He obtained his national "habilitation" (2007, University of Salamanca) and he was appointed associate professor at the University of Seville Medical School since March 2008. Full professor since 2014. In 2008, his laboratory was moved to CABIMER, the Andalusian Centre for Molecular Biology and Regenerative Medicine. His interest in nanomedicine is related to the potential applications of neuropeptide-based nanoparticles as nanodiagnostic and smart-delivery tools in immune diseases. Other research interests are related to the potential applications of neuropeptide-based nanoparticles as nanodiagnostic and smart-delivery tools in immune disease; the role of adipose tissue-derived mesenchymal stem cells (AD-MSCs) have in vivo immunosuppressive properties applicable for the control of the multiple sclerosis, in particular the immunomodulatory roles of neuropeptides and its cellular and molecular mechanisms of action; and the generation of antigen specific T regulatory cells as an approach to cell therapy. In 2009, he was appointed as Director of BIONAND, the Andalusian Center for Nanomedicine and Biotechnology in Malaga. From 2009 to 2013 he set the scientific core facilities and the kick-off the only centre specifically devoted to nanomedicine and nanobiotechnology for health in Spain. He has more than 75 scientific papers, several book chapters, and 4 patents. As author, he has an h index of 30. He published in top-reviewed journals in his field as Journal of Immunology, FASEB J or PNAS. He serves as coordinating editor of Journal Nanoparticle Research (Springer) and as member of the editorial board of Nanomedicine: NBM (Elsevier).

http://www.cabimer.es/web/en/dept/cs/advanced-therapies-in-neuroprotection/

Jai Prakash

Dr. Prakash obtained his PhD (cum laude) from the University of Groningen on the topic "Renal-specific delivery of anti-fibrotic agents". Thereafter, he worked as a postdoctoral fellow in the group of Prof. Klaas Poelstra at the University of Groningen with a joint position at BiOrion Technologies, Groningen as Vice President, Preclinical Research. After that, he joined Karolinska Institutet in Stockholm as Assistant Professor in the Department of Oncology-Pathology. There he obtained several research grants from Swedish Cancer Foundation, Marie Curie program and Swedish Research Council. In 2012, he joined University of Twente as tenure-track Assistant Professor at the Department of Targeted Therapeutics of MIRA institute for Biomedical Technology. His research is focused on the design of new cell-specific drug targeting strategies against myofibroblasts and macrophages in tumor stroma and fibrotic diseases. He is currently supervising 4 PhD students and a postdoc. His team is developing novel targeting ligands against these cells. Combining these ligands with nanocarriers, he aims to develop novel nanomedicine for the delivery of therapeutic agents such as small drug molecules, therapeutic proteins, and microRNA. These approaches will also be employed for the development of in vivo diagnostics of tumor and fibrotic diseases.

Ruth Prassl

Ruth Prassl received her PhD degree in Chemistry and Physics at the Karl-Franzens University of Graz, Austria, in 1986 and her habilitation for Physical Chemistry from the University of Graz in 1999. Since 2012 she holds a position as Associate Professor at the Institute of Biophysics of the Medical University of Graz. Before she was senior researcher and leader of the research group "Lipoproteins and Nanomedicine" at the Institute of Biophysics and Nanosystems Research of the Austrian Academy of Sciences. Apart from being lecturer at the University of Applied Sciences she acts as key researcher in the Research Center of Pharmaceutical Engineering and as scientific partner in the Ludwig Boltzmann Institute for Lung Vascular Research in Graz, Austria. Since 2009 she is Vice president of the Erwin Schrödinger Society of Nanosciences in Austria. She is a member of several professional societies and editorial boards. She was awarded the IRPC Award in Biophysics and Molecular Biology in 2006, the specific Award for Nanosciences and Nanotechnologies sponsored by the country of Styria in 2007, and the Science Park Graz Ideas Competition Award in 2013. She is author or coauthor of more than 50 publications and one patent. She is referee for numerous international journals and expert reviewer for funding organizations.

Her research interests are in molecular biophysics with special focus on structure analysis of lipoproteins and membrane associated proteins. A second major thrust of her research addresses the design and development of self-assembling nanostructures including amphiphilic designer peptides and liposomal nanocarriers for diagnosis and therapy. At present a research focus exists in the development of targeted surface modified liposomes for oral and pulmonary administration, the delivery of micro RNA to adipocytes and the imaging of atherosclerotic plaques using targeted liposomes as functionalized nanocarriers.

Adriele Prina-Mello

Trinity College Dublin, Dublin Ireland
AMBER (Advanced Materials and BioEngineering Research Centre) and CRANN Nanotech Principal Investigator, Senior Research Fellow of the School of Medicine and lecturer at Trinity College Dublin.

ETP-Nanomedicine executive board and chair of the Characterization and Toxicology working group.

Main research interest is on nanomedicine, advanced nanomaterials for biomedical applications, nanodiagnostics, drug delivery and biosensors.

Dr Prina-Mello is involved in developing and advancing several multidisciplinary research projects between University, Research Hospital and Industry partners for future applications in medicine and nanotechnology industry.

Dr. Prina-Mello is involved in several EU FP7 funded projects: Deputy Coordinator of NAMDIATREAM (NMP), dissemination coordinator of MULTIFUN (NMP), and TCD partner of Celtic Alliance for NanoHealth (INTERREG), project partner of AMCARE (SME), and Irish coordinator of NANoREG project on regulatory testing of nanomaterials.

Dr. Prina-Mello has extensively published and contributed to the dissemination of topical peer-review scientific works, lead opinions and perspectives.

Uri Raviv

APPOINTMENTS

• 1/2013 – present. Associate professor. The Institute of Chemistry, The Hebrew University of Jerusalem
• 10/2006 – 12/2012. Senior Lecturer. The Institute of Chemistry, The Hebrew University of Jerusalem.

EDUCATION

• 1991 to 1994 B.Sc. Degree in chemistry, Summa cum Laude, The Hebrew University of Jerusalem, Givat Ram, Jerusalem, Israel.
• 1995 to 1997 M.Sc. Degree in physical chemistry (June 1997), The Hebrew University, Department of Physical Chemistry in the laboratory of Prof. Sanford Ruhman. Thesis topic: 'Femtosecond Photolysis of Phenyllithium'.
• 1997 to 2002 Ph.D. Degree in Chemistry, Feinberg Graduate School of the Weizmann Institute of Science, Rehovot, Israel. Department of Materials and Interfaces, in the laboratory of Prof. Jacob Klein. Thesis topic: 'Monomeric and Polymeric Fluids Under Confinement'.

PROFESSIONAL EXPERIENCE

• 11/2002 – 10/2006, Post-doc (EMBO and Human Frontiers fellow) – Departments of Materials and Physics, Material Research Laboratory, University of California at Santa Barbara, in the laboratory of Prof. Cyrus R. Safinya.

AWARDS AND HONORS

• 1993 Rector award - Hebrew University.
• 1993 Intel - Dean Award - Hebrew University and Intel Ltd.
• 1994 Dean award - Hebrew University.
• 1995 Rector award - Hebrew University.
• 2000/1 Eshkol fellowship for Ph.D. students - The Israeli Ministry of Science.
• 2002 The Elchanan E. Bondi Memorial Prize – The Feinberg Graduate School of the Weizmann Institute of Science
• 2002/3 European Molecular Biology Oraganization Long-Term Fellowship for post-doc.
• 2003-5 The International Human Frontier Science Program Organization (HFSPO) - Long-Term Fellowship for post-doc.
• 2003/4 Materials Research Laboratory Executive Vice Chancellor fellowship.
• 2006 HFSPO - Career Development Award.
• 2007 Golda Meir Lecturer Fellowship, The Hebrew University of Jerusalem.
• 2007 Alon Fellowship for young scholars.

Oren Regev

www.bgu.ac.il/~oregev

Oren Regev is a professor in the department of Chemical Engineering at Ben-Gurion University of the Negev in Beer-Sheva, Israel. He received his PhD in 1992 from the Technion, IIT and was a visiting scientist in Lund, Eindhoven and Texas A$M universities. His research focuses on self-aggregation systems, nanotube dispersion and composite materials using electron microscopy of liquids and x-ray scattering. He has published approximately 100 papers (>3000 citations) and two patents.

Bernd Riebesehl

Dr. Bernd Riebesehl is Principal Fellow & Technical Project Leader in the Pharmaceutical Development Parenteral & Topical Dosage Forms of Novartis Pharma AG, Basel, Switzerland since 2008.
At Novartis he is leading the Parenteral Technology Platform Nanomedicine and the technical development of several parenteral drug products.
He completed his thesis in Pharmaceutical Technology at the Technical University of Braunschweig.
1992 he started his industrial career at Lilly Forschung GmbH in Hamburg leading several teams for preformulation, early phase development and formulation development. In his role as Research Advisor in Pharmaceutical R&D he led several initiatives enabling the formulation of poorly soluble drugs. In 2007 he became Director of Pharmaceutical Development at Speedel Experimenta AG, Basel.

Cristianne Rijcken

Cristianne Rijcken is CEO of Cristal Therapeutics, a pharmaceutical company developing first-in-class nanomedicinal products for the treatment of various diseases on the base of its proprietary polymeric technologies (CriPec®). Cristianne studied Pharmacy and obtained her PhD at the Department of Pharmaceutics (both Utrecht University). During and after her PhD project, she laid the basis of Cristal Therapeutics, by the development of the CriPec® platform and obtaining preclinical validation with various drugs. Cristianne founded Cristal Therapeutics in Spring 2011 and has setup a lean and mean organisation with its headquarter in Maastricht (The Netherlands). Her ambition is the translation of innovative technologies into products with clear competitive advantage for current medical needs. Cristianne was awarded multiple grants and prizes including the Simon Stevin Gezel Award in 2008 and the Inspiring Young Scientist Award in 2014. She is (co-)author of approximately 25 scientific publications and co-inventor of 6 patent applications.

Douglas Robinson

PhD, MSS, MPhys
Managing Director of TEQNODE Limited
Research fellow at University Paris-Est, Paris (F);
Research fellow at the University of Utrecht - NanoNextNL, Utrecht (NL)
Research fellow at the Science Policy Research Unit (SPRU), University of Sussex, Brighton (UK)
Dr. Douglas K. R. Robinson is a consultant and contract researcher on innovation and emerging markets based on potentially breakthrough technologies. Originally trained as a Physicist, and earning a Doctorate in the Sociology of Innovation and two post Doctorate positions in Innovation management and innovation policy, Douglas leverage's his experience of more than 10 years as an analyst in through his consultancy TEQNODE, and through a number of continuing academic projects through three University affiliations in the UK, France and The Netherlands.
Sectors of interest to Douglas include (but are not limited to), Nanomedicine, additive Manufacturing, marine biotechnology, sensor technologies and novel materials.
Alongside these activities, Douglas has been involved characterising and assessing the socio-economic impacts of new technologies and understanding how to operationalise notions around responsible research and innovation, it is on these topics for which he has been invited to speak at this meeting.

Wendi V. Rodrigueza

Chief Scientific Officer

EDUCATION/TRAINING

• 04/88 University of British Columbia, Vancouver, BC B.S., Biochemistry
• 04/94 University of British Columbia, Vancouver, BC Ph.D., Biochemistry
• 07/97 Medical College of Pennsylvania, Philadelphia, Postdoctoral Biophysical Chemistry
• 07/98 Thomas Jefferson University, Philadelphia, PA, Postdoctoral Cellular Biology

PERSONAL STATEMENT

My passion is discovering and developing biopharmaceutical product candidates forward from discovery through Proof-of-Concept in Phase II clinical development. My expertise lies in drug delivery and formulation of peptides, proteins and oligonucleotides with lipids/liposomes into product candidates for cardiovascular disease or cancer. I have co-invented several drug product candidates while at Esperion and now at ProNAi (e.g. ETC-216, ETC-588 and PNT2258). At ProNAi, I am responsible for research and overseeing manufacture of PNT2258 and other DNAi candidates.

POSITIONS AND EMPLOYMENT

• 1984-1987 Teaching Assistant, University of British Columbia, Vancouver, BC, Canada
• 1994-1997 Postdoctoral Fellow, Medical College of Pennsylvania, Philadelphia, PA
• 1997-1998 Postdoctoral Fellow, Thomas Jefferson University, Philadelphia, PA
• 1998-2003 Project Team Leader, Development of biopharmaceutical drug candidate, Esperion Therapeutics, Ann Arbor, MI
• 2000-2003 Director, Product Development, Esperion Therapeutics, a Division of Pfizer Global Research & Development, Ann Arbor, MI
• 2004 Sr. Program Manager, CuraGen Corporation, Branford, CT
• 2004-2005 Assistant Director, Clinical Planning and Operations, CuraGen Corporation, Branford, CT
• 2005-2006 Director, Project Management, Cardiovascular Disease Area, Novartis Institute of Biomedical Research, Cambridge, MA
• 2006-2008 Consultant, Project Leader, Novartis Institute of Biomedical Research, Cambridge, MA
• 2006-Present Vice President, Product Development, ProNAi Therapeutics, Kalamazoo, MI
• 2011-Present Chief Scientific Officer, ProNAi Therapeutics

OTHER EXPERIENCE AND PROFESSIONAL MEMBERSHIPS

• Fellow of the American Heart Association
• Board Member, Pacific Therapeutics, Inc.

HONORS

• 2007 Speaker, Global Drug Delivery Summit, London, England
• 2008 Speaker, RNAi World Congress, Boston, MA
• 2009 Speaker, Drug Delivery Partnerships, Las Vegas, NV
• 2009 Speaker, Biologics Drug Delivery and Beyond, Boston, MA
• 2010 Keynote Address, Michigan Society for Medical Research, Ann Arbor, MI
• 2011 Speaker, TIDES, Boston, MA

C. SELECTED PEER-REVIEWED PUBLICATIONS

1. Goodwin, NC., McGovren, JP., **Rodrigueza, WV.**, Al-Katib, A., Kameel, A., and Mohammad, RM. Efficacy in non-Hodgkin's lymphoma xenograft models of PNT225X, a chemically unmodified DNA oligonucleotide directed directed against the Bcl2 locus. 2007. Abstract #4889. AACR Annual Meeting
2. Goodwin, NC., Endert, G., Herzog, N., Kerwitz, Y., Panzner, S., **Rodrigueza, W**. Amphoteric Liposome Formulation. 2009. PCT/US06/45955

3. US Patent Nos. 6,312,719, 6,139,871 and 7,435,717. Liposome compositions and methods for the treatment of atherosclerosis
4. US Patent No. 7,435,717 Pharmaceutical Formulations, Methods, and Dosing Regimens for the Treatment and Prevention of Acute Coronary Syndromes
5. Williams KJ, Scalia R, Mazany KD, **Rodrigueza WV**, Lefer AM. (2000) Rapid restoration of normal endothelial functions in genetically hyperlipidemic mice by a synthetic mediator of reverse lipid transport. Arterioscler. Thromb. Vasc. Biol. 20(4):1033-9.
6. **Rodrigueza, W. V.**, Temel, R.E, Lund-Katz, S, Rothblat, G.H., Phillips, M.C. and Williams, D.L. (1999) Mechanism of scavenger receptor class B type I-mediated selective uptake of cholesteryl esters from high density lipoprotein to adrenal cells." J. Biol. Chem. 16;274(29):20344-50
7. Williams, KJ, Phillips MC, and **Rodrigueza, WV**. (1998) Structural and metabolic consequences of liposome-lipoprotein interactions" Adv. Drug Deliv. Rev. 8;32(1-2):31-43.
8. Williams, KJ and **Rodrigueza, W. V**. (1998) Curr Opin Lipidol. 9(5):511-3
9. Phillips, J.E., **Rodrigueza, W. V.**, Johnson, W.J. (1998) Basis for the rapid efflux of desmosterol from cells. J Lipid Res. 39(12):2459-70.
10. **Rodrigueza, W. V.**, Klimuk, S. K., Pritchard, P. H., and Hope, M. J. (1998) Cholesterol mobilization and regression of atheroma induced by large unilamellar vesicles. Biochim. Biophys. Acta 19;1368(2):306-20.
11. **Rodrigueza, W. V.**, Mason, P.E., Ireland, K.L., Lund-Katz, S, Phillips, M.C. and Mason, R. P. (1997) Interaction of apolipoprotein A-I with phospholipid-water interfaces. (Presented at CLC, St. Adele, Quebec)
12.**Rodrigueza, W.V.**, Mazany, K.E., Essenburg, A.E., Pape, M.E., Bisgaier, C.L. and Williams, K.J. (1997) Large versus small unilamellar vesicles mediate reverse cholesterol transport in vivo into two distinct hepatic metabolic pools: implications for the treatment of atherosclerosis. Arterioscler. Thromb. Vasc. Biol. 17, 2132-2139.

Steve R. Roffler

Institute of Biomedical Sciences
Academia Sinica
Taipei 11529, Taiwan
(886) 2-2652-3079
sroff@ibms.sinica.edu.tw

EDUCATION & TRAINING

- B.S. summa cum laude, Chemical Engineering University of Washington, Seattle, WA (1981)
- Ph.D. Chemical Engineering, University of California, Berkeley, CA (1986)
- Advanced Chinese, Stanford Center, Taipei, Taiwan (1987)
- Post-doctoral Fellow, Microbiology and Immunology, NDMC, Taipei, Taiwan (1987-91)

PROFESSIONAL EXPERIENCE

- 2004-present Research Fellow, Academia Sinica, Institute of Biomedical Sciences
- 2003-present Adjunct Associate Professor, National Yang-Ming University
- 1998-2004 Associate Research Fellow, Academia Sinica, Institute of Biomedical Sciences
- 1992-present Adjunct Associate Professor, National Defense Medical School
- 1991-1998 Assistant Research Fellow, Academia Sinica, Institute of Biomedical Sciences

RESEARCH IMPACT

Author of over 100 peer-reviewed scientific publications

TECHNOLOGY IMPACT

Co-inventor on several US and Taiwan patents
Over 300 licensing and commercial technology transfers

EDITORIAL BOARDS

Bioconjugate Chemistry (2008-present)
Current Drug Delivery (2009-present)
The Open Drug Delivery Journal (2009-present)

STUDENT TRAINING

23 M.S. and Ph.D. students and 6 post-doctoral fellows

SELECTED PUBLICATIONS

- S Tuve, BM Chen, Y Liu, TL Cheng, P Toure, PS Sow, Q Feng, N Kiviat, S Ni, ZY Li, **SR Roffler,** A Lieber. Combination of tumor-site located CTLA-4 blockade and systemic regulatory T cell depletion induces anti-tumor immunity but not autoimmunity. Cancer Res. 67:5929-39, 2007.
- TL Cheng and SR Roffler. Membrane-tethered proteins for basic research, imaging and therapy. Med Res Rev., 28: 885-928, 2008.
- KC Chen, CH Wu, CY Chang, WC Lu, Q Tseng, ZM Prijovich, W Schechinger, YC Liaw, YL Leu, **SR Roffler,**. Directed evolution of a lysosomal enzyme with enhanced activity at neutral pH by mammalian cell-surface display. Chem Biol. 15:1277-86, 2008.
- CH Lee, YH Chiang, SE Chang, CL Chong, BM Chen and **SR Roffler**. Tumor-localized ligation of CD3 and CD28 with systemic regulatory T cell depletion induces potent innate and adaptive antitumor responses. Clin Cancer Res., 15:2756-2766, 2009.
- YC Li, BM Chen, PC Wu, TL Cheng, LS Kao, MH Tao, A Lieber and **SR Roffler**. Mechanical forces acting on T cells immobilized via the TCR complex can trigger TCR signaling. J. Immunol. (Cutting Edge) 184: 5959-5963, 2010.
- YC Su, BM Chen, KH Chuang, TL Cheng and **SR Roffler**. Sensitive quantification of pegylated proteins and nanoparticles by second generation anti-polyethylene glycol monoclonal antibodies. Bioconjugate Chem. 21: 1264-1270, 2010.
- KC Chen, SY Wu, YL Leu, ZM Prijovich, BM Chen, HE Wang, TL Cheng and **SR Roffler**. A humanized immunoenzyme with enhanced activity for glucuronide prodrug activation in the tumor microenvironment. Bioconjugate Chem., 22: 938-948, 2011.
- TL Cheng, KH Chuan, BM Chen and **SR Roffler**. Analytical measurement of PEGylated molecules. Bioconjugate Chem., 23: 881-899, 2012.
- KC Chen, K Schmuck, LF Tietze and **SR Roffler**. Selective cancer therapy by extracellular activation of a highly potent glycosidic duocarmycin analog. Mol. Pharm., 10:1773-1782, 2013.

Eder Lilia Romero

Eder Lilia Romero was educated at University of La Plata, Argentina where she obtained her M.A. in Biochemistry and PhD in Exact Sciences (1996). Following a postdoctoral research in Groningen University, The Netherlands under the supervision of Prof. Gerrit Sherphof, she returned to Argentina in 1998. She is a Researcher at the National Council of Scientific and Technological Research (CONICET), Associate Professor of Chemistry at the Department of Science and Technology, National University of Quilmes (UNQ, 2008), and full Professor at the Villa Mercedes National University in San Luis, Argentina (UNViMe,2013), where she is responsible for the first Bioengineering career incorporating Nanotechnology. From 2007 she leads the Nanomedicine Research Program (NRP) in the UNQ. Since 2008 is a member of the Advisory Committee of the Argentinean Foundation for Nanotechnology (FAN). Since 2010, she is funding member and president of the Argentinean Association for Nanomedicines (Nanomed-ar). She has been responsible for the first second and third Nanomedicine Schools in Latino America (2008, 2010, 2012) and Editorial Board Member of ISRN Pharmaceutics (ISRN), Biosafety (OMICS Group) and Nanomedicine: Nanotechnology, Biology and Medicine (Elsevier), and is a consulting expert for local pharmaceutical companies.
Our research interest is focused in developing passively targeted nanomedicines for topical or mucosal delivery of siRNA, anti-parasitic and anti-mycotic agents, anti-inflammatory agents, and non parenteral vaccination strategies.

Michael Rossbach

Michael Rossbach, Dr. (*1978), is currently the Director of Business Development & ScientificProgramme Management of the Genome Institute of Singapore (GIS), A/Prof. at the NationalUniversity of Singapore (NUS) in bioanalytical chemistry, senior lecturer at the German Institute ofScience and Technology (TUM Asia) in Singapore in Biochemistry, Biomedical Imaging, BioorganicChemistry and Project Management in Life Sciences, lecturer at the Bonn-Aachen International Centerfor Information Technology (b-it) (Germany), in Life Science Informatics, and Prof. and a member ofthe Faculty of the Witten School of Management (Germany).
Prior to joing the GIS, he was a group leader at the Institute of Reconstructive Neurobiology,University of Bonn (Germany), and the Business Development Manager of LIFE&BRAIN.
Michael is the Principal of km2e & Company (Advisors in Health and Life Sciences), Entrepreneur-in-Residence at INSEAD Management School, Mentor at the Singapore Management University, VenturePartner of Triangle Venture Capital Group (Germany), Director of Business Development of A9CCapital (Bahrain), partner of BIOCLADES (Germany) and partner in three biotech and life sciencescompanies.
Michael studied biochemistry (PhD in immunology) at the University of New South Wales (Sydney,Australia), at the University of Witten/Herdecke (Witten, Germany) and at Harvard Medical School(Boston, USA).

Eckart Rühl

Eckart Rühl received his doctoral degree (1987) in physical chemistry from Freie Universität Berlin (Germany). After several post-doctoral stays in Orsay and Meudon (France), Oxford (UK), Hamilton (Ont., Canada), and Boulder (CO, USA) he received his habilitation in physical chemistry from Freie Universität Berlin (1993). He became a professor of physics in 1995 at the University of Mainz (Germany) and moved in 1996 to the University of Osnabrück (Germany), where he had a Chair for Experimental Physics with the emphasis on Environmental Physics. 2002-2006 he had the Chair for Physical Chemistry I at the University of Würzburg (Germany) and works since 2006 as a professor of physical chemistry at Freie Universität Berlin. His current research interests cover the following research areas: size dependent properties of matter; interactions of nanoparticles with biological matter; uptake of drugs and drug carriers into skin; ultrafast dynamical processes; development and use of novel methods for spectroscopy and spectromicroscopy. He is currently the speaker of the Collaborative Research Center 1112: “Nanocarriers: Architecture, Transport, and Topical Application of Drugs for Therapeutic Use”.

Kumiko Sakai-Kato

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

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

Kirsten Sandvig

Prof. Kirsten Sandvig is associated with Dept. of Biosciences, Universtity of Oslo, Norway and she is heading a research group at the centre of excellence “Centre for Cancer Biomedicine”, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo University Hospital. The Norwegian Radium Hospital is the main cancer hospital in Norway. Sandvig’s group, counting 23 members from eigth different countries, is interested in the mechanisms of endocytosis, intracellular transport and secretion. In some of our studies we are using protein toxins such as ricin and Shiga toxin, which are well established as markers for studies of membrane traffic, and which can be used as agents in cancer diagnosis and therapy. Our expertice is also applied to investigate uptake of nanoparticles, and in 2013 we obtained a large 5-year grant (Biodegradable nanoparticles in cancer diagnosis and therapy) from the Norwegian Research Council to build national competence in nanomedicine. This project involves collaboration between 10 Norwegian research groups covering synthesis of nanoparticles, in vitro and in vivo biology studies, in vivo imaging and clinical studies. In addition, international collaboration is included. We also characterize exosomes from prostate cancer cells with the goal of detecting lipid and protein biomarkers. Our research spans all the way from basic to translational medicine, including innovation. We aim at providing a rational basis for diagnosis, treatment and prevention of disease. The group has extensive national and international collaboration.
EDUCATION: M.Sci. for The technical University of Norway, Trondheim; Ph.D. from the Medical Faculty, Univeristy of Oslo, Norway. Research visits abroad at University of Michigan and at the biological laboratories, Harvard Cambridge, Mass. USA.
SCIENTIFIC ACTIVITY: Published around 290 articles and supervised a large number of Ph.D. students and master students. Sandvig has been invited as plenary speaker at more than 100 international meetings, and the work is heavily cited, Hirsch index is 67.
AWARDS AND HONOURS: Anders Jahres Medical Prize for young researchers, 1989 (first woman to receive this prize); The Norwegian Research Councils research prize, 1990; Member of the Norwegian Academy of Science and Letters, 1993; Stiansens Biomedical Research Prize, 1995; King Olav V´s Cancer Research Prize, 1998; Member of EMBO (European Molecular Biology Organization), 1998; Member of Academia Europea from 2002; Honorary Doctor at the University of Copenhagen, Denmark, 2007.
Member of the American Academy of Microbiology, 2010; The Fridjof Nansen Award for outstanding research in science and medicine, 2014.

Gottfried Schatz

Gottfried Schatz was born on August 18, 1936. After receiving his Ph.D. in Chemistry from the University of Graz in 1961, he joined the Biochemistry Department of the University of Vienna where he began his studies on the biogenesis of mitochondria and, together with others, discovered mitochondrial DNA. From 1964 to 1966 he worked as a postdoctoral fellow with Efraim Racker at the Public Health Research Institute of the City of New York and, after a brief interlude back in Vienna, emigrated to the USA in 1968 to assume

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 which he chaired from 1985 to 1987. His research dealt mostly with the mechanism of mitochondrial biogenesis and led to many key discoveries in this field. He served as Secretary General of the European Molecular Biology Organization (EMBO) and as member of many scientific advisory bodies around the world. His achievements were honored by numerous prestigious national and international prizes, honorary doctorates, and admission to scientific academies, including the US National Academy, the Royal Swedish Academy of Sciences, the American Academy of Arts and Sciences, and the Koninklijke Nederlandse Akademie van Wetenschappen. After his retirement in 2000, the Swiss Federal Government appointed him as president of the Swiss Science and Technology Council. After stepping down from this position in 2004, he became known to a wider public as lecturer, essayist and book author. During his years in Austria he also worked as a violinist with the Graz Philharmonic Orchestra and various opera houses in Graz and Vienna. He and his Danish wife have three children.

Raymond Schiffelers

Raymond Schiffelers studied Bio-Pharmaceutical Sciences at Leiden University (1990-1995). After an industrial traineeship at SmithKline Beecham Pharmaceuticals (UK) he did his PhD in medical microbiology at Erasmus University Rotterdam on liposomal targeting of antimicrobial agents (1996-2001). Subsequently he became post-doc at Utrecht University working on liposomes targeting tumor vasculature. In 2002-2003, at Intradigm Co (USA) he expanded his tumor vasculature-targeting work with polymers for delivery of siRNA. After his return to Utrecht University he became assistant and then associate professor. In 2011, he moved to the Laboratory for Clinical Chemistry & Hematology of the University Medical Center Utrecht to work on nanomedicine, both for diagnosis and therapy. In particularly, he focuses on extracellular vesicles in the circulation as inspiration for new drug delivery systems and diagnostic readouts. He is founding member of the International Society for Extracellular Vesicles (ISEV) founding member and member organizing committee for the ISEV2014-annual meeting, Associate Editor of the Journal Extracellular Vesicles, and Founder of EXCYTEX-an extracellular vesicle-based company.

Lukas Schlagenhauf

• 2003 – 2007Bachelor of Science in Materials Science, Swiss Federal Institute of Technology (ETHZ), Zurich, Switzerland

• 2007 Internship in the Mechanical Systems Engineering Department, Swiss Federal Laboratories for Materials Science and Technology (EMPA), Dübendorf, CH

• 2007 – 2010 Master of Science in Materials Science, Swiss Federal Institute of Technology (ETHZ), Zurich, Switzerland

• 2010 – 2011 Research assistant in the Laboratory for Nonmetallic Inorganic Materials, Swiss Federal Institute of Technology (ETHZ), Zurich, Switzerland

• 2011 – PhD student in the Laboratory for Functional Polymers, Swiss Federal Laboratories for Materials Science and Technology (EMPA), Dübendorf, Switzerland

Louis Schlapbach

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

Louis Schlapbach, born 1944 (Belp-Berne), Swiss, was Director of Empa (Swiss Federal Lab for Materials Science and Technology) and Full Professor of Physics at ETH 2001-2009. He graduated from the Swiss Federal Institute of Technology Zurich (ETH) in Experimental Physics and got his PhD in Solid State Physics – Magnetism also at ETH. As a postdoc at a CNRS laboratory in Paris, he studied hydrogen storage in intermetallic compounds. Back at ETH, he developed the surface science aspects of the hydrogen interaction with metals and alloys.

From 1988 to 2001, Louis Schlapbach was Full Professor for Experimental Physics at the University of Fribourg. As such he built up a research team of 20-25 people working on the topic „New Materials and their Surfaces“ resulting in about 40 PhD, 280 scientific papers and several patents. A strong collaboration with industry was established.

From 2001-2009, he was Director of Empa, a Materials Science and Technology Institution of the ETH domain with 800 coworkers in Dübendorf-Zurich, St. Gallen and Thun. He successfully transformed the former materials testing institution into a modern materials research and technology laboratory.

His research interests concern mainly nanoscopic properties of new materials and surfaces/interfaces, hydrogen interaction with solids, functional surfaces and coatings, materials for energy technology as well as new analytical tools. His NATURE-paper “Hydrogen-storage materials for mobile applications“ (414, p. 353, 2001) was cited more than 1900 times, and the Springer books “Hydrogen in Intermetallic Compounds I, II” were quickly sold out. He is co-editor of “Hydrogen as a future energy carrier”, Wiley 2008. (ISI Web of Knowledge 2013: 380 publications, average citations 30, h-Index above 50)

Louis Schlapbach was member oft he Research Council of the Swiss National Science Foundation SNF from 1997 to 2004; 2009-2015 he presides the steering committee of the National Research Programme NRP „Smart Materials“. He works as an expert of the Swiss Innovation Promotion Agency (KTI/CTI) and of the Swiss Academy for Technical Sciences. He is honorary member oft he Swiss Physical Society.

In 2009/10 he joins the new „Global Research Center for Environment & Energy based on Nanomaterials Science“ (GREEN) of the National Institute for Materials Science (NIMS), Tsukuba,Japan, part time, and participates at the hydrogen technology work of I2CNER at Kyushu University. He is on the Scientific Boards of NIMS and its nanoscience programme MANA, of the Hasler Foundation, of the Fonds National de la Recherche Luxembourg FNR, of the Agence Nationale de la Recherche ANR, France, and of the Dr. h.c. Robert Mathys Foundation (RMS) and became member of the Helmholtz Senat in 2010.

He spent sabbaticals at NIMS Tsukuba, at IBM Research Center San José, at Hebrew University in Jerusalem, at Stanford University, at Osaka National Laboratory, at CNRS Paris, and attended an INSEAD Executive Management Training.

Ruth Baumberger Schmid

Year of birth 1952
Nationality Swiss
Position Vice President Marketing
Institute SINTEF Materials and Chemistry / Biochemistry and Nanomedicine / Polymer Particles and Surface Chemistry

EDUCATION

Diplomierter Naturwissenschafter (Dipl. Natw.), Eidgenössische Technische Hochschule (ETH) Zürich, 1975; Doktor der Naturwissenschaften (Dr. Sc. Nat.), Eidgenössische Technische Hochschule (ETH) Zürich, 1979; Highschool teacher in chemistry (Befähigungsausweis für das höhere Lehramt, Fach Chemie), ETH Zürich, 1977

EXPERIENCE

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

MAIN FIELDS OF COMPETENCE

Scientific competence: Particle technology, encapsulation of solids and liquids, surface modification of polymers and composites, interactions between polymer surfaces and biological materials, targeted and controlled release, biodegradable polymers, biomaterials, nanomedicine, medical technology, organic chemistry
Business Development: Development of SINTEF's strategy in Life Sciences including Biotechnology, SINTEF's strategy in Medical Technology, a technology platform to prepare nano- and microparticles and -capsules based on the miniemulsion process for a broad variety of applications
Management: Research Management, project management

PROFESSIONAL MEMBERSHIPS

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

PUBLICATIONS

39 scientific publications, 17 patent and patent applications, 50 oral presentations, 17 poster presentations, 16 webinar, mass media and popular science publications.

SELECTED PUBLICATIONS

1. H. Johnsen & **R. Schmid**, J. Microencapsulation 24, 731-742 (2007). "Preparation of polyurethane nanocapsules by miniemulsion polyaddition."
2. **R. Schmid** & H. Johnsen, 34th Annual Meeting & Exposition of the Controlled Release Society, 7.-11.7.2007, Long Beach, CA, USA. "Biodegradable and biocompatible nanocapsules and particles prepared by the miniemulsion polymerisasjon."
3. A. Dessy, S. Kubowicz, M. Alderighi, C. Baroli, A. Piras, **R. Schmid** & F. Chiellini, Colloids and Surfaces B: Biointerfaces 87 (2), 236-242 (2011). "Dead Sea Minerals Loaded Polymeric Nanoparticles."
4. S. Kubowicz, P. Stenstad, H. Johnsen &**R. Schmid**, 38th Annual Meeting & Exposition of the Controlled Release Society, 31.7.-3.8.2011, National Harbour, Maryland, USA. "Protein-Coated Biodegradable Poly(butyl-2-cyanoacrylate) Nanoparticles with Stealth Surface Properties."
5. **R. Schmid**, P.M. Stenstad & Y. Mørch, "Ultrasound bubbles stabilized with multifunctional nanoparticles for combined diagnostics and therapy", International Workshop COST Action TD1004, 17.-18.2.2012, Torino, Italy.
6. S. Armada, **R. Schmid**, W. Equey, I. Fogoaga & N. Espallargas, J Thermal Spray Techn. 22, 10-17 (2013). "Liquid-Solid Self-Lubricated Coatings."
7. **R. Schmid**, Y.A. Mørch, P. Stenstad, R. Hansen, Y. Hansen, M. Afaczi, S. Eggen & C. de Lange Davies, BioNanoMed 2013, 13.-15.3.2013, Krems, Austria (invited speaker). "Multifunctional Nanoparticles for Ultrasound-mediated Diagnosis and Therapy."
8. Y.A. Mørch, P.M. Stenstad, **R. Schmid**, C. de Lange Davies, S. Eggen, A. Åslund & S. Snipstad, Nanobiotechnology Int. Workshop, EC JRC, 3.-5.12.2013, Ispra, Italy. "Characterization of nanoparticles and microbubbles."
9. **R. Schmid**, Y.A. Mørch, P. Stenstad, R. Hansen, S. Berg, Y. Hansen, M. Afaczi, S. Eggen, H. Blom & C. de Lange Davies, 40th Annual Meeting & Exposition of the Controlled Release Society, 21.-24.7.2013, Honolulu, Hawaii, USA. "Gas Bubbles Stabilized by Multifunctional Nanoparticles for Ultrasound-Mediated Durg-Delivery."

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 in the molecular pathways involved in the development of colorectal cancer. In 2004, Dr Schwartz start several 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 12 patents transfered to leading companies of the biotech and pharma sectors. In 2010 he was appointed as Director of Nanomedicine at the CIBBIM-Nanomedicine of the Vall d'Hebron Hospital Research institute (VHIR) 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 is also member of the Science Committee of the Vall d'Hebron Research Institute. and member of the Science Advisory Board of Oryzon Genomics, and Co-founder of ARGON Pharma a Spanish leading biotech company. He also leads several national and international projects involving SME's in which animal models are being used for preclinical validation of new therapies directed against cancer. Since 2011, Dr Schwartz Jr acts as the Associate Director 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.

Pablo David Scodeller

Date of Birth: 3rd of July 1980
Place of Birth: Buenos Aires, Argentina
E--mail: pabloscodeller@gmail.com
Working address: Pablo Scodeller, PhD
Vascular Mapping Laboratory of Dr. Erkki Ruoslahti
Sanford-Burnham Medical Research Institute
10901 North Torrey Pines Rd, Bldg 10, #1500
La Jolla, CA 92037, Phone: 858 646 3100 Ext. 4015

EDUCATION

• 2005 Five-year professional degree:
Biomedical Engineer at University of Mendoza, School of Enginnering.
Thesis: Fabrication of a Brainwave biofeedback monitor
www.um.edu.ar/biblio/biblioteca/titulo/1|14199|REALIZACION–DE–UN–MONITOR–DE–REALIMENTACION–DE–ONDAS–CEREBRALES)
• PhD in Chemistry obtained at University of Buenos Aires under the supervision of Dr. Ernesto J. Calvo (calvo@q1.fcen.uba.ar).
Thesis: Applications of enzymatic multilayers and enzymatic electrodes in biosensors and biofuell cell cathodes. Grade: outstanding.
The thesis is published online at:
http://digital.bl.fcen.uba.ar/Download/Tesis/Tesis_4831_Scodeller.pdf

PUBLICATIONS

1. Hyaluronan degrading Silica Nanoparticles for skin cancer therapy. **P. Scodeller***, N Salguero, P. Catalano, H. Duran, A Wolosiuk, G Soler–Illia. Nanoscale. 2013 Oct 21;5(20):9690–8
2. Wired Enzyme Core–shell Au Nanoparticle Biosensor. **P. Scodeller**, V. Flexer, R. Szamocki, E.J. Calvo, N.
Tognalli, H. Troiani, A. Fainstein. J. Am. Chem. Soc. 2008 Sep 24; 130(38):12690–7.
This Publication appeared in JACS Select, issue 5: Chemistry at the Nano Bio interface
3. Layer–by–layer self assembled osmium polymer mediated laccase oxygen cathodes for biofuel cells: The role of hydrogen peroxide. **P. Scodeller**, R. Carballo, R. Szamocki, L. Levin, F. Forchiassin, E. J. Calvo. J. Am. Chem. Soc. 2010; Aug 18;132(32):11132–40.
4. Effects of Nature and Charge of the Topmost Layer in Layer by Layer Self assembled Amperometric Enzyme Electrodes. E.J. Calvo, V. Flexer, M. Tagliazucchi, **P. Scodeller**. Phys. Chem. Chem. Phys. Volume 12, Issue 34, 14 September 2010, Pages 10033–10039
5. Redox molecule based SERS sensors. Nicolás G. Tognalli, **Pablo Scodeller**, Victoria Flexer, Rafael Szamocki, Alejandra Ricci, Mario Tagliazucchi, Ernesto Calvo, Alejandro Fainstein. Phys. Chem. Chem. Phys., 2009, 11, 7412 – 7423
6. Potential of Trametes trogii culture fluids and its purified laccase for the decolorization of different types of recalcitrant dyes. Grassi, E.M., **Scodeller, P**, Filiel, N.J, Carballo, R, Levin, L. International Biodeterioration & Biodegradation. Volume 65, Issue 4, July 2011, Pages 635–643
7. Hyaluronidase and other extracellular matrix degrading for cancer therapy: new uses and nano–formulations.**P. Scodeller***. Journal of carcinogenesis and mutagenesis. In press

Giacinto Scoles

Adjunct professor – University of Udine
Distinguished scientist at ELETTRA Synchrotron Light Source Laboratory in Triest
P.I. of an Advanced Grant from the ERC
Donner Professor of Science, Emeritus, Princeton University
Distinguished Adjunct Prof. of Biology, Temple University - Philadelphia

PERSONAL STATEMENT

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

- late '50s Mass spectroscopy
- '60s Transport properties and intermolecular forces
- '70s Intermolecular forces, Crossed molecular beam scattering
- '80s Noble gas clusters
- '90s Surface science, Fluid Helium clusters
- 2000s Manipulation of biomolecules, Biomolecular interaction at the nanoscale
- 2010s - Nanomedicine

POSITIONS AND HONORS

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

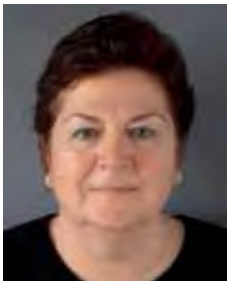
HONORS AND AWARDS

• 2006 Benjamin Franklin Medal in Physics (with J.P.Toennies) from the Franklin Institute.
• 2006 Research Prize of the Chemistry Faculty of the University of Bochum
• 2003 Creativity Award from the NSF 2003-5
• 2004 Texas A&M University, Frontiers in Chemical Research Lecturer
• 2004 Moscowitz Lecturer at the University of Minnesota
• 2003 Distinguished Visiting Professor, University of Florida, Gainesville.
• 2003 Earle K. Plyler Prize for Molecular Spectroscopy from the American Physical Society (with Kevin K. Lehmann).
• 2002 Peter Debye Award in Physical Chemistry from the American Chemical Society
• 2001 H. E. Gunning Lecturer, Dept. of Chem., University of Alberta
• 2000 Elected Foreign Member of The Royal Netherlands Academy of Arts and Sciences

• 2000 Honorary Science Doctorate from the University of Waterloo
• 1999 Samuel M. McElvain Lecturer, University of Wisconsin–Madison
1997 Elected Fellow of The Royal Society (United Kingdom)
• 1996 Recipient of an Honorary Doctorate in Physics from the University of Genoa
• 1995 Recipient of a Senior Fellowship of the Alexander von Humboldt Foundation
• 1995 Recipient of the 1995 Lippincott Award of the Optical Society of America, the Coblentz Society, and the Society for Applied Spectroscopy
• 1986 Senior Killam Fellowship.

RESEARCH SUPPORT

Giacinto Scoles has been recently granted an advanced ERC grant (2011, MONALISA QUIDPROQUO, MOlecular NAnotechnology for LIfe Science Applications: QUantitative Interactomics for Diagnostics, PROteomics and QUantitative Oncology) of nearly 3M€ over 5 years for an ambitious collaborative research project. The focus and the goal of his research is to introduce innovative devices and protocols (based on micro/nano-fluidics and on the nano-mechanical response of bio-molecular nano-strucures) to carry out precise, quantitative and low cost measurements on large, predetermined diagnostically relevant, subsets of the proteome obtained from very small samples in samples produced by a very small number of cells or within single cells with potential capability of measuring its interactions (Interactomics). By means of these measurements he hopes to make new inroads into quantitative diagnostics and disease monitoring.
Prof. Scoles is partner of an italian project recently granted by AIRC (Italian Association for Cancer Research) which aims at generating an innovative nanotechnology-based intrinsically high throughput platform to carry out real-time, highly sensitive, and inexpensive quantification of circulating biomarkers for cancer risk assessment (project title: "Application of Advanced Nanotechnology in the Development of Innovative Cancer Diagnostics Tools). Within this project, Scoles will introduce novel strategies to improve sensitivity and specificity of protein-protein (such as the antigen/antibody) recognition in all the diagnostic systems adopted by the rest of the team.
During his tenure at Princeton Scoles had, at steady state, three major grants: one from the NSF, one from DOI, and one from AFOSR for a total of about half a million dollar per year from 1993 to 2003. Furthermore Scoles was one of the main PIs who obtained from the NSF the original MRSEC grant (15 M$ over 5 years). In Italy, Scoles could count on a starting package to equip SENIL of about 200,000 euros from Elettra and an operating budget of 30,000 euros each per year from SISSA and ELETTRA. Finally, SENIL also enjoyed support from the Italian Institute of Technology for about 50,000 euros per year from 2006 to 2010. Finally, at CBM during the last three years has administered two major grants one CIPE grant from the Ministry of Italian Research for 2 million euros over 4 years to support the work of Dr. Krol at CBM. This grant was originally obtained by Prof. E. Di Fabrizio before he moved to University of Catanzaro. Finally, from 2006 until 2009 Scoles also administered a group grant from the European commission of 2 million euros for Nanotechnological Infrastructure (BINASP).



Hripsime Shahbazian

Mrs. Hripsime Shahbazian holds a MSc. in Medical Physics and a BSc in Molecular Physics. She worked as a Research Assistant in Physics Department at Carleton University while working towards her Master of Science degree. She joined Health Canada in 1988 as a Technology Assessor at the Medical Devices Bureau (MDB) and from 1991 to 1998 she was acting in different managerial roles within the Bureau. While at the MDB she contributed to the development and finalization of the new Medical Devices Regulations. Mrs. Shahbazian chaired and participated in various working groups involved in planning and implementation of the new regulations.
In 1998 Mrs. Shahbazian joined the Office of Science within the Therapeutic Products Directorate (TPD), at the Health Products and Food Branch (HPFB) as an Associate Manager. She is currently a Senior Science Advisor in the Office of Science. Her duties include management of the activities of Scientific/Expert Advisory Committees Committees, Scientific/Expert Advisory Panels, Reconsiderations and Second Level of Appeals. Mrs. Shahbazian is one of the key members working on the development and implementation of nanotechnology related activities at Health Canada. She chairs the Directorate (TPD) and Branch (HPFB) Working Groups on Nanotechnology and coordinates Nanotechnology related International activities for regulated health products for the Branch. She is a member of the Health Portfolio Nanotechnology Working Group composed of key officials across the department, coordinating departmental approach to science, policy and research needs for nanotechnology. Mrs. Shahbazian was a member of the Health Portfolio team that developed the Policy Statement on Health Canada's Working Definition for Nanomaterial.

Amotz Shemi

PhD
CEO Silenseed LTD, Israel

Dr. Amotz Shemi is the Chief Executive Officer and a co-founder in Silenseed. Prior to Silenseed, Dr. Shemi served as a Senior VP Technologies in Medinol LTD, a bare-metal and drug-eluting stents company. Beforehand, Dr. Shemi served as the CEO of ColorChip, a leader in Ion-exchange based Planar Lightwave Circuits (PLC); Dr. Shemi brings with him 25 years of experience in end-to-end management from concept level via development, regulatory approvals to actual sales. Shemi received his PhD degree in Physics and Astrophysics from the Tel Aviv University in Israel. Dr. Shemi is an inventor of a few patents, and a single author and co-author of about 35 scientific papers.

Marco Siccardi

Department of Molecular and Clinical Pharmacology, University of Liverpool, Liverpool, United Kingdom
siccardi@liverpool.ac.uk

Marco Siccardi graduated with an MSc in Clinical Biology (2006) at the University of Turin, Italy. He obtained his PhD at the University of Liverpool, Liverpool, UK (2011) focusing his research on molecular mechanisms influencing drug distribution and predictors of exposure in patients. During his post-doctoral research he developed physiologically-based mathematical models to investigate the pharmacokinetics of traditional formulation and nanoparticles in virtual patients and simulate the outcome of various clinical scenarios. He was recently appointed as a Lecturer in Nanomedicine across the faculties of Health & Life Sciences and Science & Engineering at the University of Liverpool. He has authored >50 peer reviewed publications, presenting his findings at several national and international congresses. In 2013, he was awarded with the "Most Informative Scientific Report 2013" at the Simcyp Academic in vitro – in vivo extrapolation (IVIVE) Awards, which recognise innovative teaching methods and cutting-edge published research in the fields of IVIVE, pharmaceutics, modelling and simulation. His research interests focus on the optimization of novel nanomedicine and traditional formulation for drug delivery based on experimental pharmacological data from in vitro and in vivo models, aiming to improve pharmacokinetics, efficacy and side effects. Moreover he is interested in the clarification of the ADME processes involved in drug disposition and the identification of nanoformulation characteristics influencing drug exposure, through the application of physiologically based pharmacokinetic models.

Tore Skotland

Tore Skotland is a biochemist by training and received his PhD from the University of Bergen, Norway in 1980. After 11 years at the university studying protein chemistry and enzymology, he moved to pharmaceutical R&D (Nycomed AS, Oslo, Norway) in 1983. He stayed within the same field of research for 26 years in one of the world leading companies developing contrast agents for medical imaging; Nycomed was bought by Amersham in 1997 and Amersham was bought by GE Healthcare in 2003. During the last 20 years in pharmaceutical R&D he was heading work to describe the biodistribution, metabolism and excretion of all types of contrast agents (water soluble as well as particle based) for CT, MRI, ultrasound, SPECT, PET and optical imaging. He has been involved in bringing 5 products to the marked (including 2 particle-based) and another 5 products into clinical trials (also including 2 particle-based). Skotland is the first or last author of publications related to all these 10 products. He is co-author of approximately 90 publications and is used as referee for many journals in the field of bioanalysis, metabolism, biochemistry, nanomedicine and contrast agents for medical imaging.

Skotland is since 2009 a senior researcher at the Centre for Cancer Biomedicine (one out of three Centres of Excellence in biomedicine in Norway) at The Norwegian Radium Hospital, the main cancer hospital in Norway, being part of Oslo University Hospital. He is there a member of a group studying exosomes and endocytosis and intracellular transport of protein toxins and nanoparticles. This group is now heading a new 5-year national competence building project in Norway. The project title is "Biodegradable NPs for cancer diagnosis and therapy". Skotland is co-ordinating the in vivo studies in this project, which has members from academia, university hospitals, research institutes and pharmaceutical industry. The groups involved have expertise in NP syntheses and characterization, in vitro studies of cellular uptake and intracellular transport, immunology studies, and studies using small animals with xenograft models (including different in vivo imaging modalities); clinicians are also involved.

MOST IMPORTANT PUBLICATIONS IN THE FIELD OF NANOPARTICLE RESEARCH

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

Bård Smedsrød

EMPLOYMENT

- 2011 - : Founder and CSO, D'Liver (www.dliver.com), a service and development providing company specializing on the biodistribution of biopharmaceuticals.

- 1993 - : Professor in Cell Biology; Head of Vascular Biology Research Group, Department of Medical Biology, University of Tromsø (uit.no/research/vbrg).

EDUCATION

1984: Ph.D. in Biochemistry/Cell biology at University of Uppsala, Department of Medical Chemistry. Thesis title: "Endocytosis of connective tissue macromolecules in liver endothelial cells".

SUPERVISION

17 PhD; 6 postdocs; 9 masters

PUBLICATION

155 scientific papers; h-index (web of science, May 2014): 33

CURRENT RESEARCH

centers around the biology and physiology/pathophysiology of the vertebrate scavenger endothelial cell (SEC) (= the liver sinusoidal cell, LSEC), and includes national and international collaboration, both with colleagues within our own field, with groups representing translational research, and with groups representing quite different fields (interdisciplinary research) such as nano materials physics and optical physics.

For many years we have performed pioneering work developing methods to isolate and cultivate LSECs, enabling basic studies of these cells. We have established methods for isolation and culture of LSECs from mouse, rat and pig, and as partner of the EU FP7 project HeMiBio we prepare early primary cultures of human LSECs. Moreover we study the phylogeny of SEC.

Another line of our research includes interaction of nanoparticles, or colloids, with LSECs, which are remarkably active in the clearance of blood borne colloids.

Alejandro Sosnik

Prof. Alejandro Sosnik received his Pharmacy degree from the University of Buenos Aires in 1994 and M.Sc. (equivalency) and Ph.D. degrees in applied chemistry from the Casali Institute of Applied Chemistry (The Hebrew University of Jerusalem, Israel, 2003) under the supervision of Prof. Daniel Cohn. Between 2003 and 2006, Prof. Sosnik spent a postdoctoral in the laboratory of University Professor Michael Sefton (Institute of Chemical Engineering and Applied Chemistry/Institute of Biomaterials and Biomedical Engineering, University of Toronto, Canada). In 2006, he was appointed Assistant Professor (tenure) of Pharmaceutical Technology at the Faculty of Pharmacy and Biochemistry (University of Buenos Aires) and Investigator of the National Science Research Council (Argentina). His research interests are focused at the interface of biomaterials science, nanotechnology, and therapeutics and oriented to the exploration of technologies for the encapsulation, delivery, and targeting of drugs involved in the pharmacotherapy of HIV, tuberculosis, cancer and other diseases with special interest in non-parenteral administration routes and the pediatric sub-population. He has directed several competitive research grants and supervised 3 junior staff scientists (CONICET), 5 postdocs (CONICET), 4 Ph.D. theses and several extra-mural undergraduate and graduate students. Prof. Sosnik is co-author of over 80 peer-reviewed articles, reviews, editorials and book chapters and co-inventor in 6 patents and patent applications. He is a Visiting Professor and Scientist at the National University of Colombia (Colombia), the University of Santiago de Compostela (Spain), Council for Scientific and Industrial Research (South Africa), the National Autonomous University of Mexico (Mexico), and the Fundació Sant Joan de Déu (Spain) where he taught graduate courses and presented invited conferences. Prof. Sosnik has established the "Iberoamerican Network of New Materials for the Design of Advanced Drug Delivery Systems in Diseases of High Socioeconomic Impact" (RIMADEL) of the CYTED Program that gathers eleven research groups and companies of Spain, Portugal, Mexico, Cuba, Colombia, Brazil and Argentina and coordinated it in the period 2011-2013. He recently joined the Department of Materials Science and Engineering of Technion as Associate Professor and was awarded the Marie Reintegration Grant of the European Commission for the period 2014-2018.

Ewa Stępień

Associate Professor in Medicinal Biology, Collegium Medicum of the Jagiellonian University, Faculty of Medicine, Chair of Clinical Biochemistry, Laboratory Diagnostics and Nutrigenomics Unit, Krakow, Poland

Formerly, I was involved in clinical project concerning the role of infection diseases in the cell stress and vascular function. Between 1997 and 2011, I organized and administered a routine laboratory for molecular diagnostics in John Paul II Hospital, Clinical Laboratory in Krakow, Poland. The prospects for collaboration with clinician were established, mostly with cardiac surgeons, cardiologists and clinical radiologists from Institute of Cardiology, Collegium Medicum of the Jagiellonian University. In those times (1997-2011), my research activity has been mostly related to the investigation of cardiac biomarkers, but it has also been connected with thrombosis and hemostasis regulation in cardiovascular disease. Since 2011 I have been employed in the Chair of Clinical Biochemistry of the Jagiellonian University Medical College, where I have intended to develop my scientific ambitions and education skills with students of medicine. Now, my research interests include a role of intrinsic and environmental factors influencing endothelial cell activation: compartmentalization, cytoskeleton reorganization and membrane shedding especially micropaticles formation (MPs). I am particularly interested in the regulation of these processes by ischemic shock (clinical and experimental hypoxia) and hyperglycemia and in their contribution to thrombosis and inflammatory processes. To investigate these aspects of the MPs, I am using a combination of cell biology and molecular biology techniques: immunofloorescence and scanning-electron microcopy (SEM), atomic force microscopy (AFM), flow cytometry, EIA, microarray and molecular methods. I am a grant holder for the project dedicated to the role of specific cell signaling (MPs and microRNA) in regulation of vascular cell senescence in diabetes. To reach such formulated goal I collaborate with an interdisciplinary team of clinicians, laboratory diagnosticians, molecular biologists and physicists.
I have been a member of European Society for Clinical Investigation, International Society on Thrombosis and Haemostasis and European Society of Cardiology (ESC Working Group on Thrombosis and ESC Working Group on Atherosclerosis and Vascular Biology). Additionally, I have been a member of the Polish Society of Cardiology, the Polish Society of Laboratory Chemistry. I am a reviewer in the international and national journals in medicine and clinical chemistry. I published more than 70 papers, as the first author: 12 experimental papers and 10 reviews (including 3 chapters in a textbook); as a co-author I published 32 experimental papers, 4 reviews, case reports, letters to the editor and reports from clinical trials. My total impact reaches 165 points, Hirsh index is 11, I have more than 2000 downloads and 400 citations.

Gert Storm

Professor Gert Storm studied biology at the Utrecht University, The Netherlands. He graduated in 1983. He obtained his Ph.D. degree in 1987 at the Dept. of Pharmaceutics of the same university. His research interests are in the fields of biopharmaceutics and drug targeting. In 1988-1989 he was a visiting scientist at Liposome Technology Inc. in Menlo Park, USA, and visiting assistant professor at the School of Pharmacy, UCSF, San Francisco. In 1990-1991 he was senior research scientist at Pharma Bio-Research Consultancy B.V. in Zuidlaren, The Netherlands. During this period he contributed to the design, co-ordination and evaluation of clinical pharmacological studies. In September 1991 he took up his position at the Utrecht University. In 1999, he was appointed adjunct professor at the Royal School of Pharmacy, Copenhagen. From July 2009 on, he is Honorary Professor in Biomacromolecular Drug Delivery at the University of Copenhagen. In 2000, he was appointed as professor (Targeted Drug Delivery) at Utrecht University. From 2012 on, he is also professor (Targeted Therapeutics) at the MIRA institute of the University of Twente. Furthermore, he also keeps a position at the University Medical Center Utrecht (UMCU) within the CBOI institute (Center for Image-Guided Oncological Interventions).
He is author/co-author of more than 400 original articles, reviews and book chapters, in the field of advanced drug delivery/drug targeting, and theme (co-)editor of Advanced Drug Delivery Reviews and the book 'Long Circulating Liposomes. Old Drug, New Therapeutics'. He was co-ordinator of an Integrated Project (FP6) on targeted nanomedicines (MediTrans) based on the collaboration of 30 european partners and funded by the EC and industry. He is program director of the program Drug Delivery embedded within the recently approved New Nano Initiative (NanoNextNL) strongly sponsored by the dutch government and industry. He is also principal investigator of a national industry-academia partnership (HIFU-CHEM) studying the clinical application of MRI-guided high-intensity focused ultrasound (HIFU) to improve cancer chemotherapy with temperature-sensitive targeted nanomedicines. He is course director of the GUIDE/UIPS/LACDR Course on Advanced Drug Delivery & Drug Targeting, co-sponsored and accredited by EUFEPS and the GALENOS Network, and held in The Netherlands. He is on the Board of Scientific Advisors (BSA) of the Controlled Release Society (CRS). He is on the Scientific Board of the spin-off company Enceladus Pharmaceuticals BV (Amsterdam). He is involved in organizing conferences in the field of advanced drug delivery, e.g. chairman of the ESF-UB Conference "Nanomedicine: Reality Now and Soon", held 23-28 October 2010 in San Feliu de Guixols, Spain. He is member of the editorial (advisory) board of a variety of scientific journals. He was involved in the foundation and is currently on the board of the European Society for Nanomedicine (ESNAM/CLINAM) and The Netherlands Platform for Targeted Nanomedicine (NPTN). He has received awards for his activities as translational pharmaceutical scientist.
contact: g.storm@uu.nl

Erik S.G. Stroes

Professor MD, PhD.
Internist Vascular Medicine
Dept. of Vascular Medicine
Academic Medical Center,
Amsterdam, The Netherlands

Erik Stroes received his medical degree from the University of Rotterdam in 1991, and subsequently was trained in internal medicine at the University Medical Center of Utrecht. He subsequently held several clinical and research fellowships/grants.
In 2001 he completed his Vascular Medicine specialization and continued his career as staff member of the Vascular Medicine department at the Academic Medical Center in Amsterdam, currently serving as a tertiary referral center for over 1500 patients each year. At present, he is chairman of the department of vascular medicine, with 8 staff members and 25 PhD students.
Prof Stroes is a member of the Council for Basic Science, American Heart Association, the International Atherosclerosis Society, board member of the Dutch Atherosclerosis society, member of the vascular biology working group.
He is the author or co-author of more than 260 scientific publications associated with his research interests.

Rudolf W. Strohmeier

Deputy Director-General of the European Commission's Directorate-General for Research and Innovation since 2010. In this capacity, he oversees the collaborative research activities of the Directorate-General. He acts as European Co-chair of the Executive Committee of the International Group on Earth Observations (GEO), alternating Chairman of the GB of the Innovative Medicines Initiative (IMI) and Member of the GBs of the Joint Undertakings Fuel Cells and Hydrogen (FCH) and BioBased Industries (BBI, in formation). He also chairs the European Research Area and Innovation Committee.
After studies of Law and Economics at the University of his home town Würzburg and that of Bonn, he started his career as teaching assistant at the Department of Civil and Commercial Law at the University of Würzburg in 1979. In 1980, he joined the Bavarian Liaison Office to the Federal Govern-ment in Bonn. Following a 2-years detachment to the Development Policy department of the European Commission, he established the Bavarian Information Office in Brussels and became its first Director.
From 1987, he served in the three Cabinets of Commissioner Peter Schmidhuber (Macroeconomics and Regional Policies, later Budget and Budget Control). In 1995, Agriculture and Rural Development Commissioner Franz Fischler appointed him Deputy Head of his Cabinet.
Nominated Head of Division in DG Agriculture and Rural Development in 2000, he joined the Cabinet of Commission President Prof. Romano Prodi in 2003 as Adviser i.a. for industrial, agricultural and environmental policies. From 2005-2010, he served as Head of Commissioner Viviane Reding's 2nd Cabinet (Telecommunication and Media).
He is alumni of the German Marshall Fund of the US, founding member of the Belgian-Bavarian Society, member of the Scientific Society for Middle Classes (Munich), editor of two books and author of various articles dealing with European policies.

Triantafyllos Stylianopoulos

Dr. Triantafyllos Stylianopoulos is a lecturer of Mechanical Engineering and Head of the Cancer Biophysics laboratory at the University of Cyprus.
He received a diploma in Chemical Engineering from National Technical University of Athens, Greece (2003) and a PhD also in Chemical Engineering from the University of Minnesota, USA (2008). He performed his post-doctoral training at the Department of Radiation Oncology at Harvard Medical School and Massachusetts General Hospital.
Dr. Stylianopoulos research work involves the combined use of computational methods and experimental techniques to dissect the tumor pathophysiology in order to optimize the delivery of drugs. He has co-authored 30 peer-reviewed articles in the fields of tumor pathophysiology, drug delivery, cancer nanomedicine and biomechanics, including 4 articles in Nature journals (Nature Nanotechnology, Nature Medicine, Nature Reviews Clinical Oncology and Nature Communications), 5 articles in PNAS and 2 in Cancer Research (h-index=17, hetero-citations>1,000, source Web-of-Science).
Dr. Stylianopoulos is the Principal Investigator of 2 research projects funded by the European Commission with total budget exceeding €1.6 M. This includes a highly selective Starting Grant by the European Research Council (ERC) with which Dr. Stylianopoulos has established the Cancer Biophysics Laboratory at the University of Cyprus.
Dr. Stylianopoulos is a member of the American Society of Mechanical Engineers, the European Society for Clinical Nanomedicine, the European Society of Biomechanics and the Greek Association of Computational Mechanics.

SELECTED PUBLICATIONS

• **T. Stylianopoulos**, J. D. Martin and R. K. Jain, "The role of mechanical forces in tumor progression and therapy", Annual Reviews of Biomedical Engineering, accepted for publication, 2014.
• **T. Stylianopoulos** and R.K. Jain, "Combining two strategies to improve perfusion and drug delivery in solid tumors", PNAS, 110 (46) 18632-18637, 2013.
• **T. Stylianopoulos**, J. D. Martin, M. Snuderl, F. Mpekris, S. Jain and R. K. Jain, 'Co-evolution of solid stress and interstitial fluid pressure in tumors during progression: Implications for vascular collapse', Cancer Research, 73(13), pp. 3833-3841, 2013.
• **T. Stylianopoulos**, J. D. Martin et al., 'Causes, consequences and remedies for growth-induced solid stress in murine and human tumors', PNAS, 109(38), pp. 15101-15108, 2012.
• V. P. Chauhan*, **T. Stylianopoulos***, J. D. Martin, Z. Popovic, W. S. Kamoun, M. G. Bawendi, D. Fukumura, and R. K. Jain, 'Normalization of tumour blood vessels improves the delivery of nanomedicines in a size-dependent manner', Nature Nanotechnology, 7, 383-388, 2012. *Equal contribution
• C. R. Wong, **T. Stylianopoulos**, J. Cui, J. Martin, V. P. Chauhan, W. Jiang, Z. Popovic, R. K. Jain, M. G. Bawendi and D. Fukumura 'Multistage nanoparticle delivery system for deep penetration into tumor tissue', PNAS, 108(6), pp. 2426-2431, 2011.
• J. W. Baish, **T. Stylianopoulos**, R. M. Lanning, W. Kamoun, L. L. Munn, D. Fukumura, R. K. Jain, "Scaling rules for diffusive drug delivery in tumor and normal tissues", PNAS, 108(5), pp.1799-1803, 2011.
• R. K. Jain and **T. Stylianopoulos**, 'Delivering Nanomedicine to Solid Tumors', Nature Reviews Clinical Oncology, 7, pp. 653-664, 2010.T.
• **T. Stylianopoulos**, B. Diop-Frimpong, L. L. Munn, and R. K. Jain, 'Diffusion Anisotropy in Collagen Gels and Tumors: The Effect of Fiber Network Orientation', Biophysical J., 99(10), pp. 3119-3128, 2010.
• **T. Stylianopoulos**, M. Z. Poh, N. Insin, L. L. Munn, D. Fukumura, M. Bawendi, R. K. Jain, "Diffusion of Particles in the Extracellular Matrix:The Effect of Repulsive Electrostatic Interactions", Biophysical J., 99(5), pp. 1342-1349, 2010.
• E. A. Sander, **T. Stylianopoulos**, R. T. Tranquillo, and V. H. Barocas, "Image-based Multi-scale Modeling Predicts Tissue-level and Network-level Fiber Reorganization in Stretched Cell-compacted Collagen Gels", PNAS, 106(42), pp. 17675-17680, 2009.

Janos Szebeni

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

numerous social commitments in Hungary and abroad, he is a founder and scientific director of an immune toxicity CRO in Hungary (SeroScience Ltd).

Shima Tavakol

Sh_tavakol@razi.tums.ac.ir

Ph.D of Medical Nanotechnology
Tehran University of Medical Sciences,
School of Advanced Technologies in Medicine, Tehran, Iran (April 2014).

Thesis: 'Investigation on neural differentiation potential of puramatrix as a self- assembling peptide nanofiber along with laminin and bone marrowhoming peptide motifs on endometrial stem cells towards neuron' Academic Supervisor: Prof. S. M. Rezayat and Prof. J. Ai

ACADEMICAL PROJECTS

• Investigation on repair of spinal cord injury in rat using self-assembling nanofiber of Puramatrix and Matrigel. Supervisor: Shima Tavakol, 2013, Student's Scientific Research Center, Tehran University of Medical Sciences (Finished).
• Investigation on polymerization of tubulin derived from brain of rat using self-assembling peptide nanofiber. Supervisor: Shima Tavakol, 2013, Student's Scientific Research Center, Tehran University of Medical Sciences (In progress).
• Investigation the recovery effect of self-assembling nanofiber containing bone homing peptide on a spinal cord injury model in rat. Supervisor: Seyed Mahdi Rezayat, 2013, Student's Scientific Research Center, Tehran University of Medical Sciences (In progress).
• Investigation on neural differentiation potential of PC12 cells on electrospun nanofibrous PLA/chitosan scaffold. Supervisor: Shima Tavakol, 2013, Student's Scientific Research Center, Tehran University of Medical Sciences (Finished).
• Study of antimicrobial and cytotoxicity effect of silver nano particle and silver nano particle citrate coated on protein structure, an in vitro study. Supervisor: Shima Tavakol, 2013, Student's Scientific Research Center, Tehran University of Medical Sciences (Finished).
• Chemical Synthesis, characterization and antimicrobial activity of Hydroxyapatite/Chitosan and Chitosan/nano-hydroxyapatite/nano-Silicon composite. Supervisor: Shima Tavakol, 2013, Student's Scientific Research Center, Tehran University of Medical Sciences (Finished).
• In vitro and In vivo study of bone regeneration of nanohydroxyapatite- chitosan composite powder. Supervisor: Shima Tavakol, 2011, Tehran University of Medical Sciences (Finished).
• Investigation on neural differentiation effect of puramatrix along with laminin and bone marrow homing peptide motifs on endometrial stem cells towards neuron. Supervisor: Prof. S. M. Rezayat and Prof. J. Ai, 2009, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences. (Finished)
• Osteogenesis study of peptides derived milk in rat, supervisor: Prof.S.M.Rezayat, 2011, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences. (Finished).
• Synthesis of biodegradable & biocompatible nano hydroxyapatie-PLGA T- plate by casting method, supervisor: Prof. J. Ai, 2009, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences. (Finished)
• Comparative study for bone regeneration ability between demineralized bone matrix (DBM) over nano composite hydroxyapatite-gelatin with mesenchymal stem cell (MSC) in Rat, supervisor: Prof.S.M.Rezayat, 2007, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences. (Finished)

PUBLICATIONS AND PRESENTATIONS

• Thermogel nanofiber induces neural-like cells from human Endometrial-Derived Stromal Cells; an in-vitro and in-vivo study in Rat. **Shima Tavakol**, Hadi Aligholi, Ali Gorji, Aresou Eshagh Abadi, Elham Hoveizi, Behnaz Tavakol, Seyed Mahdi Rezayat, Jafar Ai. Journal of Biomedical Materials Research: Part A. (2014) DOI: 10.1002/jbm.a.35117.
• The effect of Noggin supplementation in Matrigel nanofiber-based cell culture system for derivation of neural-like cells from human Endometrial-Derived Stromal Cells. **Shima Tavakol**, Mohammad Masummi, Seyed Mostafa Modarres Mousavi, Amir Amani, Seyed Mahdi Rezayat, Jafar Ai. Journal of Biomedical Materials Research: Part A. (2014) doi: 10.1002/jbm.a.35079.
• Functionalisation and surface modification of electrospun polylactic acid scaffold for tissue engineering. Elham hoveizi, Mohammad Nabiuni, Kazem Parivar, Sareh rajabizelati, **Shima Tavakol**. Cell Biology International. (2014) 38(1):41-9.
• The Effect of Laminated Hydroxyapatite/Gelatin Nanocomposite Scaffold Structure on Osteogenesis using Unrestricted Somatic Stem Cells and in Rat. **Shima Tavakol**, Mahmoud Azami, Ahad Khoshzaban, Iraj Ragerdi Kashani, Behnaz Tavakol, Elham Hoveizi, Seyed Mahdi Rezayat Sorkhabadi. Cell Biology International. (2013) 37: 1181.
• The Effect of Carrier Type on Bone Regeneration of Demineralized Bone Matrix in Rat. **Shima Tavakol**, Ahad Khoshzaban, Mahmoud Azami, Iraj Ragerdi Kashani, Hani Tavakol, Seyed Mahdi Rezayat. Craniofacial Surgery. (2013) 24(6):2135-40.
• Programming of human endometrial-derived stromal cells (EnSCs) into preoligodendrocyte cells by overexpression of miR-219. Somaye Ebrahimibarough, Hamid Reza Kouchesfehani, Jafar Ai, Mahmoodinia M, **Shima Tavakol**, mohammad Massumi.Neuroscience Letters. (2013) 537: 65.
• Bone regeneration based on nano-hydroxyapatite and hydroxyapatite/chitosan nanocomposites: an in vitro and in vivo comparative study. **Shima Tavakol**, M.R Nikpour , A.Amani, M. Soltani, S. M. Rabiee, S. M. Rezayat, P. Chen, M. Jahanshahi. Journal of Nanoparticle Research (2013) 15:1373
• In vitro and In vivo Investigations on Bone Regeneration Potential of Laminated Hydroxyapatite/Gelatin Nanocomposite Scaffold along with DBM. **Shima Tavakol** , Iraj Ragerdi Kashani , Mahmoud Aazami , Ahad Khoshzaban , Behnaz Tavakol , Sharmin Kharazi, Seyed Mahdi Rezayat Sorkhabadi. Journal of Nanoparticle Research. (2012) 14:1265.
• A Porous Hydroxyapatite/Gelatin Nanocomposite Scaffold for Bone Tissue Repair: In Vitro and In Vivo Evaluation. Mahmoud Azami, **Shima Tavakol**, Ali Samadikuchaksaraei, Mehran Solati Hashjin, Nafiseh Baheiraei, Mehdi Kamali, Mohammad Reza Nourani. Journal of Biomaterial Science Polymer Edition. (2012) 23: 2353.

PROCEEDING PAPERS

• Termogel nanofiber induces Human Endometrial- Derived Stromal Cells to Neural Differentiation and Improves Motor Dysfunction Following Spinal Cord Injury. 3rd International Road Safety Congress. February 18-19 (2014), Tehran, Iran.
• Investigation on the Biocompatibility of Self-Assembling Peptide Nanofibers. International NanoSafety Congress, February 19-20 (2014), Tehran, Iran.
• Investigating the Effects of Particle Size and Chemical Structure on the Cytotoxicity and Bacteriostatic Potential of Nano Hydroxyapatite/Chitosan and Nano Hydroxyapatite; as a Substitute Bone Biomaterial. International NanoSafety Congress, February 19-20 (2014), Tehran, Iran.
• Investigation on neural differentiation potential of human Endometrial-Derived Stromal Cells via Matrigel nanofiber: in vitro and in vivo studies in rat. Basic and Clinical Neuroscience Congress, December 18-20 (2013), Tehran, Iran. (Oral presentation)
• Derivation of neural-like cells from human Endometrial-Derived Stromal Cells via Noggin supplementation in termogel nanofiber based cell culture system. Basic and Clinical Neuroscience Congress, December 18-20 (2013), Tehran, Iran.
• Bone regeneration Based on nano hydroxyapatite and Hydroxyapatite/Chitosan nanocomposite, International Congress on Nanoscience & Nanotechnology (ICNN2012) 8 - 10 September 2012, Kashan, Iran. (Oral presentation)
• Comparative study of bone regeneration potential between nano hydroxyl apatite- gelatin scaffold and demineralized bone matrix (DBM) in Rat. Nanotechnology students' conference, Gilan, 2011. (Oral presentation)
• Introduction of nanotechnology, the first seminar on nanotechnology and its medical application by AMST, AMST association, Tehran University of Medical Sciences. 2010.(Oral presentation)
• Application of nano in control of air pollution, the 4th nanotech-

nology students' conference, Tehran. 2007.(Oral presentation)
• Application of nano in purification of sewage, abstract book of the 4th nanotechnology student's conference. 2007.
• Embryology summery (Persian) 2014 Publisher; Taaliye Andishe, Tehran, Iran.

PATENT

• Hydrogel based peptide nanofiber containing long motif of laminin for application in medical studies; International category A61, Patent no 82433.
• Biodegradable and biocompatible nanocomposite of hydroxyapatite-PLGA T- plate implant, stem cell compatible for bone fracture, 91.130.2130 (Under filling, US patent).

BOOK (COMPILATION)

• Nanomedicine. 2 chapters,Jahad Daneshgahi, Tehran, Iran.
• Introduction of Physiology (Persian) 2014 Publisher; Taaliye Andishe, Tehran, Iran.

Donald A. Tomalia

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

Dr. Tomalia is the CEO/Founder of NanoSynthons and National Dendrimer & Nanotechnology Center, Distinguished Visiting Professor (Chemistry Department) Columbia University, NY; Adjunct Professor (Department of Chemistry) University of Pennsylvania, PA and Affiliate Professor (Department of Physics) Virginia Commonwealth University, VA. He received his B.A. in Chemistry from the University of Michigan and Ph.D. in Physical–Organic Chemistry from Michigan State University while working at The Dow Chemical Company (1962-1990). He has founded three dendrimer-based nanotechnology companies; namely: NanoSynthons LLC (2011), Dendritic Nanotechnologies, Inc. (2001) and Dendritech, Inc. (1992). Other positions currently held by Tomalia include: Advisory Board CLINAM, European Foundation for Clinical Nanomedicine; Sr. Scientific Advisor to the European Union CosmoPHOS Nano Project (2012-present). Dr. Tomalia also serves as Faculty Member, Faculty 1000 Biology; Associate Editor, Journal of Nanoparticle Research (Springer); Editorial Board, Nanomedicine (Elsevier); Ed. Board of Bioconjugate Chemistry and is a founding member of the Ed. Board for NanoLetters (2000-4).
He is the pioneering scientist/inventor associated with the discovery of poly(oxazolines) (Industrial Research-100 Awards in 1978 & 1986) and dendrimers. His 1979 discovery of dendrimers (dendritic polymer architecture) led to a third R&D-100 Award in 1991 and the Leonardo da Vinci Award (Paris, France) in 1996. He received the International Award of The Society of Polymer Science Japan (SPSJ) (2003) which recognized his discovery of the fourth major macromolecular architectural class; namely, dendritic polymers. He was the invited "Linus Pauling Memorial Lecturer" (2010) Portland, OR and recipient of the Wallace H. Carothers Award (American Chemical Society) (2012).
He has authored/co-authored over 265 peer-reviewed publications with more than >20,300 citations and granted >128 U.S. patents. Over 170 papers are focused in the dendrimer/dendritic polymer field including two monographs entitled: Dendrimers and Other Dendritic Polymers (J. Wiley) co-edited with J.M.J. Fréchet (2001) and more recently Dendrons, Dendrimers, Dendritic Polymers (Cambridge University Press (2012)). His review article entitled: "Starburst Dendrimers: Molecular Level Control of Size, Shape, Surface Chemistry, Topology and Flexibility from Atoms to Macroscopic Matter," D.A. Tomalia, A.M. Naylor W.A. Goddard III, Angew. Chem. Int. Ed. Engl., 29(2), 138 (1990) has > 2,680 citations. Tomalia was inducted into the Thomas Reuters Hall of Citation Laureates in Chemistry (2011) (i.e., top 40 most highly cited scientists in the field of chemistry).
Tomalia is recognized as a pioneer in dendritic polymers and international focal point for activities related to dendrimer-based nanotechnology and nanomedicine. His extensive studies on dendrimers provided a conceptual window to his recent development of a systematic framework for defining and unifying nanoscience. This concept is now accepted by both chemists and physicists as cited in "Developing Superatom Science" (Chemical & Eng. News (USA), April 15, 2013) and "In Quest of a Systematic Framework for Unifying and Defining Nanoscience" (Modern Physics Letters B, 28, (3), 1430002 (2014)). This paradigm proposes the application of traditional first principles to discrete nano-building blocks (i.e., nanoelement categories) which are found to behave much like picoscale atoms by exhibiting stoichiometries, heuristic surface chemistries and nano-periodic property patterns/relationships normally associated with traditional atoms. Tomalia is now applying many of this nano-periodic paradigm and these principles to nanomedicine (J. Internal Medicine, in press (2014)).

Panagiotis N. Trohopoulos

Dr med Panagiotis (Panos) N. Trohopoulos is a Distinction of Excellence Greek (Ellin) Medical Doctor, his Specialty is Cardiologist, and he is based in Thessaloniki, Greece (Ellas). He is also the Founder / Owner / Managing Director of the CosmoPHOS Ltd which is an Innovative Translational Nanomedicine SME (small-medium enterprise) established in Thessaloniki, Greece (Ellas) since 2012. CosmoPHOS Ltd is focused on the Translational Research & Development of Novel Nanomedicine Products for the Early Diagnosis, Targeted Therapy, and Therapy Monitoring of Cardiovascular Diseases, and especially of Atherosclerotic Heart Disease which causes the myocardial infarctions (heart attacks), and of Atherosclerosis in general. Additionally, Dr med Trohopoulos is the Founder and the Scientific / Exploitation / Strategic Coordinator of the CosmoPHOS-nano Project which is a Large-scale EU FP7 NMP Funded Translational Nanomedicine R&D Project in Cardiovascular Diseases, and more specifically in Atherosclerotic Heart Disease. The Project co-funded by the European Union under the FP7 Programme / NMP Theme (Nanosciences, Nanotechnologies, Materials and New Production Technologies) with 8,5 Million Euros, and additionally co-funded by All Project Participants with 4,5 Million Euros, having a total project budget of 13 Million Euros. The CosmoPHOS-nano is a Five-year R&D Project started on March 1, 2013 and will be concluded on February 28, 2018, and it is a Multidisciplinary R&D Project consisting of 19 World-Class Participants, including 13 Universities and Research Foundations and 6 Companies, from 11 European Countries, Japan, and USA, with a wide variety of complementary and cutting-edge scientific, technological and manufacturing expertise and know-how. The CosmoPHOS-nano Project is the World's Largest R&D Project of Nanomedicine in Cardiology aiming to develop a Radical Innovative Theranostic (Diagnostic and Therapeutic) "Smart" Nanomedicine Product, the CosmoPHOS System, to enable: a) Molecular Imaging by using Near-Infrared Fluorescence (NIRF), b) Targeted Therapy by using Photodynamic Therapy (PDT), and c) Real-time and Follow-up Therapy Monitoring of Atherosclerotic Coronary Artery Disease (CAD) of the Heart, which is the number one cause of human death and morbidity in Europe and worldwide. The CosmoPHOS System is anticipated to significantly reduce the number of deaths and the morbidity caused by CAD. This is forecast to result in a significant decrease of the European and global healthcare costs caused by CAD, increase the income of the European healthcare industry from CAD market which is the global largest, and alleviate the European and global society. The CosmoPHOS-nano Project is the First EU FP7 NMP Funded Large-scale R&D Project planning to apply Nanomedicine for Cardiac Patients. It foresees conducting during the final Project-year, a First-in-man Phase-I Clinical Trial in CAD Patients, to evaluate the safety and feasibility of the novel CosmoPHOS System for human use. Dr med Panagiotis (Panos) N. Trohopoulos is also an Executive Board Member and Vice-chair of the Working Group Business of ETPN

(European Technology Platform Nanomedicine), which is an Initiative led by Industry and set up together with the European Commission, addressing the application of nanotechnology to achieve breakthroughs in healthcare. Additionally, Dr med Panagiotis (Panos) N. Trohopoulos is and Advisory Board Member of CLINAM (European Foundation for Clinical Nanomedicine) which is a non-profit institution aiming at advancing medicine to the benefit of individuals and society through the application of nanoscience. Finally, Dr med Panagiotis (Panos) N. Trohopoulos is Founding Member and Steering Board Member of the International Society for Nanomedicine, Member of the European Society for Nanomedicine, Member of the Hellenic Cardiological Society, and Member of the European Society of Cardiology.
(E-mail: panagiotis.trohopoulos@cosmophos.com)

Hans van der Voorn

Hans van der Voorn is the Executive Chairman and CEO for Izon Science Ltd, based in New Zealand. He originally trained as an engineer and previously founded and developed an energy company. Hans was one of the founders of Izon in 2005 and became its fulltime CEO in 2007. He has been the inventor on several Izon patents and has a particular interest in developing high quality and reliable nano-measurement capabilities for biomedical use.
Izon Science has a nanopore based measurement and analysis platform it calls TRPS for Tunable Resistive Pulse Sensing. Its qNano instruments are of particular interest to nanomedicine researchers and developers. Izon's aim is for TRPS to become the world's leading measurement platform for nanomedicines needing to migrate from research laboratories to clinical use. The core capabilities of izon's TRPS system include particle number, real number based size distribution, and more recently, particle by particle charge distribution. Hans' van der Voorn's view is that transparency and objective repeatability of the measurements is necessary for nanomedicines needing approval, and that this is now very achievable.

Peter van Hoogevest

Lipoid GmbH
Frigenstrasse 4, D-67065 Ludwigshafen
Telephone +49 621 53819-63
E-mail: van.hoogevest@lipoid.com

Phospholipid Research Center Heidelberg
Im Neuenheimer Feld 582, D-69120 Heidelberg
Telephone +49 621 53819-63
E-mail: pvanhoogevest@phospholipid-institute.com

PROFESSIONAL EDUCATION

University of Basel (Switzerland)

• 1994 Degree as "Privat Dozent" (Adjunct Professor) in Pharmaceutical Sciences. Title of "Habilitationsschrift": "Recent Advances in the Industrial Research and Development of Liposomal Drugs".

State University of Utrecht (The Netherlands)

• 1984 Ph.D. degree. Faculty of Mathematics and Natural Sciences
Title of Ph.D. Thesis: "Permeability properties of Band 3- and Glycophorin-containing Model membranes", Promoters: Prof. Dr. B. de Kruijff and Prof. Dr. J. de Gier, Department of Biochemistry.
• 1979 Pharmacist certificate.
• 1978 Masters degree. Faculty of Mathematics and Natural Sciences
Main subject: Pharmaceutical Sciences
Ancillary subject: Biochemistry.
• 1975 Certificate B.S. Faculty of Mathematics and Natural Sciences
Main subject: Pharmaceutical Sciences.

WORK EXPERIENCE

• Lipoid GmbH, Ludwigshafen, Germany
January 2012-now: Head Scientific Department
• Phospholipid Research Center Heidelberg, Germany
January 2012-now: Managing Director
• Phares Drug Delivery AG, Muttenz, Switzerland
December 2000- November 2011: Managing Director, Chief Operations Officer (COO), Member of the Board of Directors.
• ADD Advanced Drug Delivery Technologies AG, Muttenz, Switzerland
1998-2000: CEO, Co-founder, Delegate and Member of the Board of Directors.
• Novartis Ltd., Basel, Switzerland: 1996-1998: Laboratory Head, Sterile Dosage Forms.
• Ciba Ltd., Basel, Switzerland:
1994-1996: Section Head Early Development Area/Sterile and Semi-Solid Dosage Forms.
• Chiron Corporation, USA:
1993-1994: Visiting scientist at the Vaccine Research and Development Department at The Biocine Company a Joint Venture between Ciba-Geigy and Chiron (California, USA).
• Ciba-Geigy Ltd., Basel, Switzerland:
1989-1993: Section Head Biotech Products of Sterile and Semi-Solid Dosage Forms Area, Pharmaceutical Development, Pharmaceuticals Division.
1985-1989: Section Head Liposomal Dosage Forms of Novel Dosage Forms Area, Pharmaceutical Development, Pharmaceuticals Division.
1984-1985: Section Head Protein Formulation, Biovet Group, Animal Health, Agrochemical Division.

Rakesh N. Veedu

MSc, PhD

I completed my PhD from The University of Queensland, Australia in May 2006 in the area of Synthetic Organic Chemistry after completing my Masters from Griffith University. In 2006, I commenced my postdoctoral training in my current area of nucleic acid chemical biology at the Nucleic Acid Center, University of Southern Denmark under the guidance of Professor Jesper Wengel, co-inventor of locked nucleic acid (LNA) technologies. Later in 2010, I returned to the University of Queensland to commence my independent research at the School of Chemistry and Molecular Bioscience with a University of Queensland Research Fellowship. I established the first nucleic acid chemical biology laboratory at The University of Queensland where my research focussed on developing novel nucleic acid-based therapeutic candidates for tackling solid cancers and Alzheimer's disease. In February 2014, I again moved to the Nucleic Acid Center, University of Southern Denmark to further strengthen my research activities. Currently, I focus on developing biostable nucleic acid aptamers that can selectively target cancer cells and also developing aptamer-targeted cancer imaging technologies. In 2013, I together with my collaborator developed an aptamer targeted 19F-MRI detection system against B16 melanoma cells.

Viola Vogel

Viola Vogel is a Professor in the Department Health Sciences and Technology (D-HEST), heading the Laboratory of Applied Mechanobiology at the ETH Zurich. After completing her graduate research at the Max-Planck Institute for Biophysical Chemistry, she received her PhD in Physics at the Johann-Wolfgang Goethe University in Frankfurt/Main, followed by two years as a postdoctoral fellow at the University of California Berkeley, Department of Physics where she applied nonlinear optical techniques to analyze fluid interfaces. She became an Assistant Professor in Bioengineering at the University of Washington/ Seattle in 1991, with an Adjunct appointment in Physics. She launched a new program in Molecular Bioengineer-

ing, and was later promoted to Associate (1997) and Full Professor (2002). She was the Founding Director of the Center for Nanotechnology at the University of Washington (1997-2003), and moved to the ETH in 2004.
Her work has been internationally recognized by multiple awards (including Otto-Hahn Medal; NIH FIRST Award; Philip Morris Foundation Research Award; Julius Springer Prize 2006 for Applied Physics; ERC Advanced Grant (2008), major lectureships (including the Lacey Lectureship at CalTech (2007), the Timoshenko Lectures at Stanford University (2011), the International Solvay Chair in Chemistry Brussels (2012), an Honorary Doctorate from the University of Tampere Finland (2012), and served as Panel Member Representing the European Research Council at the World Economic Forum in Davos 2013. She served International Organizations as US Representative on the Council of Scientists of the Human Frontier Science Program (2003-4), as well as by Jury duties for the European Research Council, the British Marshall Fund, the Humboldt Foundation, the National Research Council (USA); NASA, NIH, NSF, DOE and for the German Government (BMBF) as well as for the Max-Planck Society (Rapporteur, Physical-Chemical Technical Division). She was also a member of the Gordon Research Conferences Selection and Scheduling Committee and of the PCAST subpanel that finalized the National Nanotech Initiative (White House). She currently serves on several scientific advisory boards, including the Wyss Institute at Harvard (Boston), the Institute of Bioengineering and Nanotechnology (A-Star, Singapore), the Singapore- MIT Alliance for Research and Technology (SMART), the Max-Planck Institute for Colloids and Interfaces (Golm), the Nano-Initiative-Munich (NIM, DFG Excellence Cluster), CeNIDE Duisburg-Essen, and is a Member of the Hochschulrat (Board of Regents) of the Ludwig-Maximilians-Universität, München. Most recently, she was elected to serve on the Jury to select the Queen Elizsabeth Prize for Engineering awardee 2015.

Lea von Moos

• 2016 ETH Zürich, Switzerland Doctor of Sciences, Dr. sc. ETH Zürich Safety of iron phosphate nanoparticles for food applications
• 2010 ETH Zurich, Switzerland Food Science MSc, Specialization in Nutrition and Health
• 2008 ETH Zurich, Switzerland Agricultural Science BSc
• 2004 Language Links, Perth, Australia Cambridge Certificate in Advanced English (CAE)
• 2003 Kantonsschule Alpenquai Luzern, Switzerland Matura (UK A levels)

PUBLICATIONS

• Glube, N., von Moos L., Duchateau G. (2013). Capsule shell material impacts the in vitro disintegration and dissolution behaviour of a green tea extract. Results in Pharma Sciences 3: 1-6.

PRESENTATIONS

• **von Moos, L.**, Schneider, M., Trantakis, I., Hilty, F., Pratsinis, S., Zimmermann, M., Sturla, S. (2014). Gastrointestinal exposure to nanoscale iron compounds in foods: absorptive pathways and potential toxicity. NRP 64 Progress Report Meeting, ZŸrich, Switzerland. March 2014 (Talk and Poster)
• **von Moos**, L. Safety of Iron Phosphate Nanoparticles for Food Applications. Annual Meeting of the Scientific Advisory Board of the World Food Systems Center, ETH Zürich, Switzerland. January 2014. (Talk)
• **von Moos**, L., Trantakis, I., Rast, P., Hilty-Vancura, F., Zimmermann, M., Pratsinis, S., Sturla, S. (2013). In vitro exposure of human intestinal cells to iron phosphate nanoparticles indicate no direct cytotoxicity. Congress of the European Society of Toxicology (Eurotox), Interlaken, Switzerland, September 2013. (Poster)
• Foltz, M., Glube, N., **von Moos**, L., Knol D., Duchateau, G. (2012). Understanding gastrointestinal behavior of dietary bioactive compounds and potential interactions with product matrices. 1st International Conference on Food Digestion, Cesena, Italy, March 2012

Andreas Wagner

Dr Andreas Wagner is currently the Head of Liposome Technology at Polymun Scientific GmbH. He has significant expertise in the field of liposomal formulation and process development. He studied Biotechnology in Vienna, Austria and earned his Master and Ph.D. degrees in the field of biotechnology and biopharmaceutical technology/liposomology at the Institute of Applied Microbiology supervised by Prof. Hermann Katinger and Prof Karola Vorauer-Uhl. Dr Andreas Wagner is listed as inventor on several patents, like the liposome technology and some product patents of liposomal formulations. Furthermore, he has published several peer reviewed articles dealing with liposomes, the technology, products thereof and their application in preclinical and clinical studies. Since 2001, he built up the liposome technology unit at Polymun Scientific GmbH. Polymun Scientific GmbH is a private Austrian company offering contract development and manufacturing of biopharmaceuticals as well as development and production of liposomal as well as lipid nanoparticle formulations. Its patented liposome technology allows efficient manufacturing of constantly high quality in small and large scale. Polymun is an EMEA-certified manufacturer conducting several own R&D projects. For more information, please visit www.polymun.com

Tony Watts

Tony Watts read a B.Sc. then a Ph.D. in biophysics in the Astbury Department of Biophysics, Leeds University, UK from 1968 - 1975, followed by post doctoral research for 5-years at the Max Planck Institut für Biophysicalische Chemie, Göttingen, Germany. In 1980 he took up a position in the Biochemistry Department, Oxford University, UK where he is now a Professor and the CW Maplethorpe Fellow in Biological Sciences, St Hugh's College. Starting with his PhD, he has had an interest in both model and biological membranes, using a wide range of biophysical methods to characterize vesicular, model, reconstituted and natural membranes, with a recent interest in the application for cellular and clinical delivery of active compounds using novel, polymer-based Lipodisq™ particles.

www.bioch.ox.ac.uk/~awatts

Frank F. Weichold

M.D., Ph.D.

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

Dr. Weichold's experience includes execution of strategic and operational initiatives across the sciences' value chain. Dr. Weichold has led the development of international collaborations and public private partnerships for discovery and early development, implemented global operating and development models, and executed large scale business model transformations. He has accumulated more than a decade of industrial research and medical product development experience while leading teams in Clinical Pharma-

cology, DMPK, as a Director at MedImmune LLC, Gaithersburg, Maryland. Prior, he directed research and clinical development of vaccines at the Aeras Foundation (founded by The Bill and Melinda Gates Foundation).
As a tenured Professor in the University of Maryland system, he developed and managed independent research programs and trained graduate students. He also held faculty positions at the University of Maryland Biotechnology Institute to study signal transduction pathways that affect immune responses, and at the Humboldt University, Berlin (Germany) to teach and study microbial immune modulation. During the five years of postdoctoral education, Dr. Weichold worked at the National Institutes of Health in Bethesda, Maryland, first at the LTCB (NCI) where he researched immune pathologies in HIV infection, then at the Hematology Branch of the NHLBI where bone marrow pathologies, transplantation immunology and gene therapy were the focus of his studies.

Tanja Weil

Tanja Weil studied chemistry (1993–1998) at the TU Braunschweig (Germany) and at the University of Bordeaux I (France) and completed her PhD at the MPI for Polymer Research under the supervision of K. Müllen. In 2003 she received the Otto Hahn Medal of the Max Planck Society. From 2002 to 2008 she advanced from Section Head of medicinal chemistry to Director of Chemical Research and Development at Merz Pharmaceuticals GmbH (Frankfurt). In 2005 she was also appointed to the MPI for Polymer Research and in 2008 she became an Associate Professor at the National University of Singapore. Since 2010 she has been Director of the Institute of Organic Chemistry III and Macromolecular Chemistry at Ulm University. In 2012, Tanja Weil has received an ERC Synergy Grant of the European Research Council. She is a member of the Board of Directors of the International Graduate School for Molecular Medicine (2011) and co-founder of the Ulm Competence Center for Peptide Pharmaceuticals (UPEP) (2012).
To date, she has published over 100 peer-reviewed journal articles, as well as being listed as an inventor on more than hundred individual patent applications published from 2004 to 2014.

Klaus-Michael Weltring

Dr. Klaus-Michael Weltring is a molecular biologist by training with a PhD and a Habilitation degree from the University of Münster. Since 2001 he is the managing director of bioanalytik-muenster responsible for the development of the Münster region into a leading nanobioanalytic location at the European level. He has set-up a local network of researchers from different disciplines and SMEs and organizes the marketing of the region at international events and fairs. Between 2003 and 2008 he was the deputy-coordinator of the Nano2Life Network of Excellence and leader of the "ELSA" Board in this network. Until recently he co-managed the Nanomedicine Round Table and the EuroNanoBio project (FP7 CSA projects). Since 2009 he is a member of the Executive Board of the ETP Nanomedicine leading the ELSA Advisory Group of this platform. Currently he is the Chief Scientific Officer of the Nano-Bioanalytik-Zentrum and manages a ZIM NEMO Network of 12 local companies, which develop new and certified methods for characterization of Nanomaterials in consumer products and biological systems.

Joy Wolfram

E-mail: jvwolfram@houstonmethodist.org

EDUCATION

• 9/2012-present Doctor of Philosophy student (Nanoscience and Technology), National Center for Nanoscience and Technology, Chinese Academy of Sciences, Beijing, China.
• 8/2007-12/2010 Master of Science (Biology), Department of Biosciences, University of Helsinki, Helsinki, Finland.
• 8/2007-5/2010 Bachelor of Science (Biology), Department of Biosciences, University of Helsinki, Helsinki, Finland.

OCCUPATION

• 11/2011-present Research Fellow, Ferrari Group, Department of Nanomedicine, Houston Methodist Research Institute, Houston, Texas, USA. Design and evaluation of nanotherapeutics for cancer therapy.

PUBLICATIONS

1) Shen J, Kim HC, Su H, Wang F, **Wolfram J**, Kirui D, Mai J, Mu C, Mao ZW, Shen H. Cyclodextrin and polyetlylenimine functionalized mesoporous silica nanoparticles for delivery siRNA cancer therapeutics. Theranostics, 2014, accepted.
2) **Wolfram J**, Suri K, Huang Y, Molinaro R, Borsoi C, Scott B, Boom K, Paolino D, Fresta M, Wang J, Ferrari M, Celia C, Shen H. Evaluation of anticancer activity of celastrol liposomes in prostate cancer cells. Journal of Microencapsulation, 2014, accepted (DOI: 10.3109/02652048.2013.879932).
3) Yang Y, **Wolfram J**, Shen H, Fang X, Ferrari M. Polyarginine induces an antitumor immune response through binding to toll-like receptor 4 (TLR4). Small, 2014. (DOI: 10.1002/smll.201302887).
4) **Wolfram J**, Suri K, Celia C, Yang Y, Shen J, Fresta M, Zhao Y, Shen H, Ferrari M. Shrinkage of pegylated and non-pegylated liposomes in serum. Colloids and Surfaces
B: Biointerfaces, 2014C;114:294-300.
5) Yang Y, **Wolfram J**, Shen J, Fang X, Shen H, Ferrari M. Live-cell single-molecule imaging reveals clathrin and caveolae dependent docking of SMAD4 at the cell membrane. FEBS Letters, 2013;587(114):3912-3920.
6) Molinaro R#, **Wolfram J#**, Federico C, Cilurzo F, Celia C, Fresta M. Polyethylenimine (PEI) and chitosan carriers for the delivery of RNAi effectors. Expert Opinion on Drug Delivery, 2013;10(12):1653-1668. (# equal contribution).
7) Gentile E, Cilurzo F, Di Marzio L, Carafa M, Ventura CA, **Wolfram J**, Paolino D, Celia C. Liposomal chemotherapeutics. Future Oncology, 2013;9:1849-1859.
8) Celia C, Trapasso E, Locatelli M, Navarra M, Ventura CA, **Wolfram J**, Carafa M, Morittu VM, Britti D, Di Marzio L, Paolino D. Anticancer activity of liposomal bergamot essential oil (BEO) on human neuroblastoma cells. Colloids and Surfaces B, Biointerfaces, 2013;112:548-553.
9) Shen J, Xu R, Mai J, Kim H, Guo X, Yang Y, **Wolfram J**, Mu C, Xia X, Gu J,Liu X, Mao Z, Ferrari M, Shen H. High Capacity Nanoporous Silicon Carrier for Systemic Delivery of Gene Silencing Therapeutics. ACS Nano, 2013;7(11):9867-9880.
10) Yang Y, **Wolfram J**, Boom K, Fang X, Shen H, Ferrari M. Hesperetin impairs glucose uptake and inhibits proliferation of breast cancer cells. Cell Biochemistry and Function, 2013;31(5):374-379.
11) Yang Y, Wolfram J, Shen H, Fang X, Ferrari M. Hesperetin: an inhibitor of the transforming growth factor-β (TGF-β) signaling pathway.

RECENT ACADEMIC AWARDS

• 2014 Research Grant, Viktoriastiftelsen, Finland (19200 euro)
• 2013 Travel Award, Amgen Scholars Program, Europe (1000 sterling pounds) 2011 Research Grant, Nylands nation, Finland (18800 euro).

Kenneth Wong

Kenneth Wong, MD, PhD, FRCSEd, is a surgeon scientist at the University of Hong Kong. He received medical degree from University of Edinburgh and his Ph.D degree and post-doc training at Imperial College, University of London. He is currently Clinical Associate Professor in the Department of Surgery.
The major research areas of Dr. Wong include clinical applications of nanomedicine using various animal disease models, genome-wide association of childhood congenital disease, tissue repair and regeneration. Dr. Wong has published extensively in peer-reviewed journals and book chapters and is an Associate Editor of Nanomedicne:NBM.

Tina Wong

Associate Professor Tina Wong is a Senior Consultant in Glaucoma at the Singapore National Eye Centre and the head of the Ocular Therapeutics and Drug Delivery Research Group at the Singapore Eye Research Institute. She holds adjunct faculty appointment at the School of Materials Science and Engineering at Nanyang Technological University, where she is co-Director to the Ocular Therapeutic Engineering Centre. Her research interests include the cellular mechanisms of ocular scarring and inflammation towards drug and nucleic acid therapeutics development, and sustained drug delivery platforms for glaucoma and other ophthalmic conditions requiring chronic or intensive medical therapy.

Richard Wooster

Richard Wooster is President and Chief Scientific Officer at Blend Therapeutics. Previously he was Vice President and Head of the Cancer Metabolism Discovery Performance Unit in Oncology at GlaxoSmithKline. In this role he led the evaluation of the metabolic pathways that are deregulated in cancer, led the PI3K portfolio of inhibitors into the clinic and was responsible for the clinical evaluation of the novel antimitotic kinase inhibitors to PLK and CENPE. Before this he led the translational medicine group in Oncology at GSK and worked on Tykerb, Mekinist and Tafinlar. During his academic career he discovered the breast cancer susceptibility gene BRCA2, was one of the founders of the Cancer Genome Project at the Welcome Trust Sanger Institute where, among many achievements, mutations in BRAF were first discovered and he developed the COSMIC mutation database and web site. He has more than 100 peer-reviewed articles and papers in scientific journals, including Nature, Nature Genetics and Science. Richard has a First Class BSc in Biochemistry and a PhD in drug metabolizing enzymes both from the University of Dundee, Scotland.

Jens Würthner

Dr. Würthner is currently working in the department of Oncology Translational Medicine, Novartis, where he is leading two drug development teams who aim to establish the clinical proof-of-concept for targeted anti-cancer medicines. To this end, he and his teams are conducting first in human studies of novel therapeutics to detect pharmacological target engagement and early signs of clinical efficacy in selected cancer indications. After completing his medical degree at the University of Hamburg in 1995, he worked as a physician in a number of academic institutions, including the Universities of Freiburg, Munich and Duesseldorf, and as a post-doctoral researcher and fellow at the National Cancer Institute in Bethesda, MD, USA. Dr. Würthner started his industry-career in 2006, when he accepted an employment offer from AstraZeneca Oncology, followed by a position at GlaxoSmithKline Biopharm R&D, both in the UK. His clinical and research focus over the last 20 years were at the interface of oncology, immunology and in part infectious diseases, which he combines with his interest in bringing new technologies into clinical practice, as exemplified by identification and effective use of biomarkers in pharmaceutical drug development.
Dr. Würthner has established a number of industry-academia collaborations and was appointed as an honorary lecturer of the University of Manchester in 2008, and of Kings College London in 2012. He also serves as a member of the International Committee of the Faculty of Pharmaceutical Medicine, UK. He is board certified in Pharmaceutical Medicine (UK) and Medical Microbiology, Virology and Infection Epidemiology (Germany).

Huanming Yang

Ph.D.
BGI-China, Shenzhen

Dr. Yang is the co-founder and President of BGI-China, one of the major genomics centers in the world. He and his partners have made a significant contribution to the International HGP, HapMap Project, 1000 Genomes Project, and other human -omics research, as well as sequencing and analyzing genomes of many other animals, plants, and microorganisms, with many publications in Science, Nature, Cell, and other internationally prestigious journals.
Dr. Yang obtained his Ph.D. from University of Copenhagen (Denmark) and postdoctoral trainings in France and USA. He has received many awards and honors, including Research Leader of the Year by Scientific American in 2002 and Award in Biology by the Third World Academy of Sciences (TWAS) in 2006. He was elected as an associate member of European Molecular Biology Organization (EMBO) in 2006, an academician of Chinese Academy of Sciences in 2007, a fellow of TWAS in 2008, a foreign associate of National Academies of India in 2009, of Germany in 2012, and of the USA in 2014.

Stefano Zapperi

Stefano Zapperi received a Ph D. in Physics from Boston University in 1998. He is currently senior researcher at CNR-IENI, Milano and group leader at the ISI Foundation, Torino. In the past few years, he has been invited as a visiting scientist or visiting professor at several universities including Cornell University, Aalto University, University of Barcelona, Boston College, Northeastern University. He is an expert in the statistical mechanics of non-equilibrium complex systems and has worked in fracture, plasticity, friction, magnetism

and quantitative biology. Interdisciplinary research activities relevant for biomedicine involve statistical physics modelling of tumor growth, biomechanics of tissues and cells, protein polymerisation in neurodegerative diseases. He is author of over 150 publications in the most prestigious scientific journals (including 4 in Nature, 3 in Science, 3 in Nature Phys., 1 Nature Mat. , Nature Comm and PNAS, and 30 in Phys. Rev. Lett.) receiving more than 3000 citations (h-index=30). He is member of the editorial board of JSTAT. He organized nine international workshops and symposia on applications of complex systems theory, in particular he co-organized two international workshops on the Physics of Cancer, funded by ESF and Cecam. He was invited to be part of the scientific committee of several international conferences. In 2007, he acted as the secretary of the International Conference on Statistical Physics (STATPHYS 23). In 2004 he received the Marie Curie Excellence award. He coordinated the FP6 NEST project on "Triggering instabilities in materials and geosystems" (TRIGS), running from 2007 to 2009, coordinating the ERA-NET Compexity pilot project on "Localizing signatures in catastrophic failure" running from 2011-2013. In 2011 he received an Advanced Grant from the European Research Council on "Size effects in fracture and plasticity" (SIZEFFECTS).
Web: http://www.smmlab.it

Reinhard Zellner

Reinhard Zellner, born in 1944, is Senior Professor of Physical Chemistry at the University of Duisburg-Essen. He studied chemistry and physics at the University of Göttingen and received his Ph.D. in 1971 with a study on fast atom reactions in the gas phase. In the following years he was a postdoctoral researcher in Cambridge / England and a Visiting Professor at the University of Texas/Austin, In 1980 he received the "venia legendi" for physical chemistry at Göttingen. Since then he held professorships at the universities in Göttingen, Hanover and Duisburg-Essen.
His research activities concentrate on reaction kinetics, dynamics and photo-chemistry of gas and surface processes. More recently, microphysical and chemical processes on aerosols as well as interactions of nanoparticles with biological systems entered the scope of his scientific interests. In 2007 he initiated the Priority Program of Deutsche Forschungsgemeinschaft on "Biological Responses to Nanoscale Particles", of which he also acted as a coordinator. He has published more than 250 scientific papers and supervised more than 60 Ph.D. thesis`.

Yuliang Zhao

Professor, PhD
Deputy Director-General, National Center for Nanoscience and Technology of China.
Director, Chinese Academy of Science Key Lab for Biomedical Effects of Nanomaterials and Nanosafety, Institute of High Energy Physics, Chinese Academy of Science (CAS).

Yuliang Zhao holds a BS degree from Sichuan University in Radiochemistry (1985) and a PhD degree in Radiochemistry from Tokyo Metropolitan University (1999). He was a JSPS Postdoctoral Fellow at TMU and a researcher at RIKEN prior to becoming a Hundred Elite professor at Institute of High Energy Physics, Chinese Academy of Sciences in 2001. Prof. Zhao is the founder and director of CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety. He is also the co-founder and co-Director of Research Center for Cancer Nanotechnology at Tianjin Cancer Hospital in China. He holds adjunct faculty appointments at The Methodist Hospital Research Institute, Houston Medical Center (USA). Peking University (Beijing), the Medical School of Jilin University (Changchun), and Sichuan University (Chengdu).

His research interests span both basic and translational research on novel nanomedicine for cancer therapy: making use of nanotoxicology knowledge towards safer nanomedicine by design, focusing on novel nanomedicines (without delivery systems) for low-toxic cancer chemotherapy, especially, proposed the use of non-killing cell mechanism (prison cell not poison cells by low-toxic nanostructure materials) to tenderly inhibit cancer growth, rather than cell-killing which results in innocently normal cells being slaughtered. Prof. Zhao is also well-known for his studies on toxicological properties of engineered nanomaterials and cancer nanomedicines. He proposed the nanotoxicology research in 2001 in China and worldwide was one of the earliest scientists who have pioneered and initiated the researches with innovative ideas on safety issues of engineered nanomaterials.
He is the author of over 270 publications, 10 books, and 20 book chapters. He has delivered more than 180 invited lectures worldwide. He has been recognized for his accomplishments with about 20 national and international awards, including the Outstanding Youth Scholar by National Natural Science Foundation of China (2005); the Chinese Academy of Sciences-Bayer Award (2006), Beijing Award for Science and Technology (2008), National Award for Natural Sciences (2012), and Scientific Chinese Award (2013), etc.
He now serves as a member of National Steering Council for Nanoscience & Nanotechnology of China (MOST), the Discipline Committee of National Natural Science Foundation of China, and the Expert Committee of MOST 973 Program. He is the founder and president of Chinese Society for Nanotoxicology. He was the chairperson of eight international conferences, and four Fragrant Hill Scientific Conferences (hina`s Gordon Research Conference), he serves as Associate Editor/Advisory Editorial Board member for seven scientific research journals in USA, Germany and UK. He have been invited to serve as the expert of nanosafety committee for OECD (2005-), United Nations Environmental Program (UNEP, 2006), European Commission (2007-), National Research Council of Canada (2007-), etc.

CURRICULA VITAE OF POSTER PRESENTERS

Elena Ambrosetti

Via Sant'Isidoro 9, 34151 Trieste (Italy)
E-mail: elena_ambro@yahoo.it
Nationality: Italian
Date of birth: 24/09/1983

WORK EXPERIENCE

• 05/2013 → present
Researcher at Elettra Sincrotrone Trieste S.C.p.A. – Trieste, Italy (Nanoinnovation laboratory)
Study and characterization of biomolecular interactions with in vitro surface and solution techniques; development of nano-immuno arrays for proteomic analysis with Atomic Force Microscopy technology.

• 10/2011 → 09/2012
Researcher at Nerviano Medical Sciences S.r.l. – Nerviano, Italy (Assay development and biochemical screening group)
Enzyme characterization and assays set-up with luminescent and radiometric techniques for biochemical screening of anticancer inhibitors.

• 03/2011 → 10/2011
Junior Researcher at Istituto Italiano di Tecnologia – Genova, Italy (Drug Discovery and Development department)
Enzyme assays set-up for inhibitors potency evaluation with fluorimetric and radiometric techniques; low-medium throughtput screening of inhibitor compounds.

• 02/2009 → 02/2011
Training project "New specialist of advanced technologies for new anticancer drugs research" at Nerviano Medical Sciences S.r.l. – Nerviano, Italy
- At the Department of Life Sciences, University of Trieste, AREA Science Park and SISSA (Trieste): participation in lectures and seminars in the field of Molecular Biology, Biochemistry and Oncology; experimental activity consisting in Surface Plasmon Resonance studies and enzyme assays performed in the laboratory of Anti-infective peptides of the Department of Life Sciences.
- At Nerviano Medical Sciences (Nerviano, MI): participation in lectures in the field of cancer research and anticancer drugs development; experimental activity consisting in Surface Plasmon Resonance studies and enzyme assays performed in the laboratory of Assay development of the Department of Biotechnology.

EDUCATION

• 2008: Master Degree in Industrial Biotechnology and Biocatalysis (110/110 cum laude)
• 2005: Bachelor in Biotechnology – Industrial specialization (110/110)
• 2002: Scientific lyceum diploma (100/100)

PERSONAL SKILLS

• Mother tongue: Italian
• Other languages: English – French (scholastic)

JOB-RELATED SKILLS

• Enzyme characterization and inhibitors potency evaluation with different techniques: molecular binding assays (Surface Plasmon Resonance, fluorescence polarization, thermal stability and ITC); enzyme activity assays (radiometric and luminescence assays).
• Atomic Force Microscopy measurements for the characterization of biomolecular interactions.
• Recombinant proteins purification techniques. Preparation of biological samples for microscopy and histochemical assays.

Patrizia Andreozzi

E-mail: patrizia.andreozzi@gmail.com
patrizia.andreozzi@ifom.eu

EDUCATION / REASEARCH

• August 2011 - Present: Carlo Besta Neurological Insitute Fundation, IRCCS. Laboratory at IFOM-IEO Campus. PostDoc (with F. Stellacci and S. Krol). Experimental work on the development of an in-vitro model for Blood Brain Barrier. Synthesis and surface functionalization using layer by layer techniques on gold nanoparticles for a nanodrug delivery system to the Blood Brain Barrier. Development of new methods to study the cellular uptake of nanoparticles. Physical-chemical characterization of nanoparticles and virus interaction.

• January 2010 - July 2011: National Nanotechnology Laboratory (NNL) - Nanochemistry Division Istituto di Nanoscienze del CNR, in Lecce. Post Doc (with Dr. A. Athanassiou, and Dr. D. Cozzoli). Experimental work on the design, implementation and study of multifunctional nanocomposites-based epoxy resins loaded with colloidal inorganic nanocrystals to improve mechanical, thermal and electromagnetic performance of the materials currently used for aerospace application. I was responsible for the synthesis of inorganic nanocrystals and for the preparation and characterization of polymeric nanocomposites reinforced with nanoparticles.

• March 2009 - December 2009: Department of Chemical Sciences and Technologies of University of Rome Tor Vergata. Post Doc (with Prof. G. Paradossi and Prof. A. Palleschi). Experimental work on development synthesis and physical-chemical characterization of biocompatible polymer functionalized with enzyme for effective removal of proteinaceouscarbohydrate-lipid deposits from contact lens.

• January 2009: Chemistry Department of Chemistry at the Sapienza University of Rome. Post Doc (with Prof. C. La Mesa). Experimental work on preparation and physical-chemical characterization of cat-anionic vesicles.

• November 2005 - December 2008: The Sapienza University of Rome. PhD, Physical Chemistry of Colloid Systems (with Prof. C. La Mesa). Title of the dissertation: "Synthesis and Physical-Chemical Characterization of Functionalized Spherical Nanoparticles".
Experimental work on the physical-chemical properties of colloidal systems: soft and hard nanoparticles, vesicles, micelles stabilized by electrostatic and steric interaction.

• October 1996 - February 2005: The Sapienza University of Rome. Matser of Science. Main field: Physical Chemistry of Colloid Systems. Relators: Prof. Camillo La Mesa and Dr. Roberto Di Leonardo. M.Sc. Thesis: "Colloid Crystals and Colloid Glasses Based on Stabilized PMMA Nanoparticles". (score 110/110). Experimental work on the study of the phase diagram of hard spheres.

Chantal Appeldoorn

PhD

Chantal studied Organic Chemistry at Leiden University. In 1998 she started her PhD at the Department of Biopharmaceutics within the Leiden/ Amsterdam Center of Drug Research. Within this UNYPHAR project (a collaboration between Yamanouchi Pharmaceutical (currently Astellas) and the Universities of Groningen, Leiden and Utrecht) she worked on her thesis entitled "Generation of P-selectin inhibitors for anti-atherothrombotic therapy". After a short post-doc period at the same department focusing on the preparation of new P-selectin ligands for the prevention and visualization of atherosclerosis, she moved to Utrecht University in 2002. At the Department of Medicinal Chemistry she worked within the EU financed POLYCARB pro-

ject synthesizing various monomeric, dendrimeric and polymeric carbohydrates as anti-adhesion compounds against several bacterial infections.
As of 2005 she has been working at to-BBB technologies, currently as a senior scientist. to-BBB is a clinical stage biotechnology company located in Leiden focusing on enhanced drug delivery across the blood-brain barrier. The company is developing novel treatments for devastating brain disorders, such as brain cancer, neurodegenerative diseases and lysosomal storage diseases, by combining existing drugs with the G-Technology®, to-BBB's proprietary brain delivery platform. to-BBB currently has two products in clinical trials: its lead product 2B3-101 is in a Phase IIa trial in Europe and the US for the treatment of primary brain tumors as well as brain metastases; the second product 2B3-201 for MS relapses and other neuroinflammatory diseases is completing a Phase I clinical study.

Leila Arabi

Pharm.D.,Ph.D. candidate
Current Address: School of pharmacy, Mashhad University of Medical Sciences, University Campus- Mashhad-Iran, +98(0)5118823255(office)
E-mail: leilaa.arabi@gmail.com, Arabil881@mums.ac.ir,
Mobile No: +98(0)9153030251

RESEARCH INTERESTS

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

EDUCATION

• August 2012-August 2013
1-year internship at the Molecular Pathology department of the Institute for Pathology (University Hospital Basel). involved in the projects such as the "Molecular regulation of cancer stem cells in gastrointestinal neoplasia", "Anticancer-treatment effects in vitro using real-time impedance measurements on patient-derived samples", "Prognostic and therapeutic implications of the oncogenic miR-17-92 cluster in Osteosarcoma", " Evaluation of putative amplification target genes (Vinculin and KCNMA1) in prostate and breast cancer"
• since 2009: Ph.D. candidate of Pharmaceutical Nanotechnology: School of Pharmacy, Mashhad University of Medical Sciences (MUMS), Mashhad, Iran. Ranks first in Ph.D. based on overall scores (18.90 out of 20) Ph.D. Theses Title: "in vitro and in vivo targeted drug delivery to cancer stem cells isolated from murine colon carcinoma by doxorubicin nanoliposomes conjugated with CD44, CD133, EpCAM monoclonal antibodies".under the supervision of Dr Mahmoud Reza Jaafari.

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

PUBLICATIONS AND PRESENTATIONS

• **L.Arabi**, J.Gsponer, J.Smida, M.Nathrath, G.Jundt, C.Ruiz, L.Quagliata and D.Baumhoer,"Upregulation of the miR-17-92 cluster and its two paraloga in osteosarcoma – reasons and consequences", Accepted in GENES&CANCER, The original submission date remains March 11, 2014
• L.Quagliata, M.Matter, S.Piscuoglio,**L.Arabi**, C.Ruiz, A.Procino, M.Kovac, F.Moretti, Z.Makowska, L.Tornillo, S.Diederich, M.H. Heim, C.Cillo and L.Terracciano,"lncRNA HOTTIP/HOXA13 expression is associated with disease progression and predicts clinical outcome in HCC patients", HEPATOLOGY, Vol. 59, No. 3,MARCH 2014.
• **L.Arabi**, A.Badiee,MR.Jafari. "Isolation and Identification of Putative Cancer Stem Cells in C26 Murine Colon Carcinoma Cell Line", The 1st Middle East Controlled Release Conference (MECRC 2014) and The 6th Iranian Controlled Release Society Conference (ICRC 2014), 25-27th February 2014, Tehran University of Medical Sciences, Iran.
• **L.Arabi**., B.Malaekeh Nikuei, R.Kazemi Oskuee, A.Dehshahri,M. Ramezani, "Polycationic Nanoliposomes as DNA Nanocarriers with Low Toxicity: A Promising Gene Transfer System for Further in Vivo Study", The European Summit for Clinical Nanomedicine (6th CLINAM 2013) , June 23-26,2013Basel- Switzerland
• **L.Arabi**, B.Baschiera, S.Wyttenbach, S.Kustermann, C.Ruiz, A. Zippelius, A.Roth, L.Bubendorf. "The use of patient derived malignant effusions as specific models for in-vitro assays", 5th Freiburg Symposium on Anticancer Drug Discovery, April 24 – 27, 2013, Freiburg, Germany
• **L.Arabi**, L.Quagliata, A.Rufle, L.Terracciano, C. Ruiz and D.Baumhoer. "Prognostic and therapeutic implications of the oncogenic miR-17-92 cluster in osteosarcoma",Non-coding RNA, epigenetics and transgenerational inheritance April 11-12, 2013,Cambridge,UK
• L. Quagliata, M. Matter, **L.Arabi**, S. Piscuglio, Z. Makowska, M.Kovac, L. Tornillo1, M.H. Heim, C.Cillo, L. Terracciano"HOTTIP Expression Levels Predict Patients' Survival and Metastasis Formation In Hepatocellular Carcinoma" Charles Rodolphe Brupbacher Symposium, January 30 - February 1, 2013, University Hospital Zurich, Switzerland
• **L.Arabi**., B.Malaekeh Nikuei, R.Kazemi Oskuee, A.Dehshahri,M. Ramezani "Preparation, characterization and transfection efficiency of polycationic nanoliposomes as DNA nanocarriers with low toxicity"Biovalley Life Science Week- 25th September-2012- Basel-Switzerland

HONORS AND AWARDS

• Award for poster presentation in Biovalley Life Science Week-25th September- 2012- Basel- Switzerland
• Ranks first among candidates, Iranian National "pharmaceutical Nanotechnology" Ph.D. comprehensive Exam based on examination and interview 2011 • • Ranks first among candidates, Iranian National "pharmaceutical Nanotechnology" Ph.D. Entrance Exam based on entrance examination and interview 2008 • Ranks first in Ph.D. based on overall scores (18.90 out of 20) Ranks second in Pharm.D. based on overall scores (18.05 out of 20) 5% upper first in national comprehensive exam of basic sciences.

REFERENCE

Prof. Luigi Terracciano, Molecular Pathology Division, Institute of Pathology, University Hospital Basel, Switzerland, lterracciano@uhbs.ch

Konstantinos Avgoustakis

Associate Professor, Department of Pharmacy, University of Patras, 26500, Greece
Tel.: ++2610-962317, Fax: 2610-997607
Email: avgoust@upatras.gr
http://www.pharmacy.upatras.gr/index.php/en/personell/faculty-members/associate-professors/25-avgoustakis-konstantinos

Konstantinos Avgoustakis obtained his Diploma in Pharmacy from the Aristotle University of Thessaloniki, Greece in 1985 and his Ph.D. in Pharmaceutics/Quality Control of Medicines, from the same University in 1991. He received a scholarship for Ph.D. studies abroad (1989-1992) from Greek State Scholarships Foundation and he obtained in 1992 a Ph.D. degree in Pharmaceutical Technology from King's College, University of London, UK. In 1993-1994 he served as research assistant in the Institute of Radioisotopes in

National Centre for Scientific Research "Demokritos". Since 1994 he has joined the Department of Pharmacy in University of Patras (Greece), where he teaches subjects related to Pharmaceutics and Drug Delivery. His research interests lie on the controlled, targeted drug delivery using engineered nanoparticles based on biodegradable polymers and copolymers and magnetic hybrid inorganic/organic nanocarriers, on the development of novel prophylactic or therapeutic vaccines based on biodegradable and biocompatible, polymeric nano- and micro-particles and on the development of formulations for the efficient delivery of drugs with limited aqueous solubility. He is the author/co-author of 50 articles in peer-reviewed journals and 1 article in biomaterials encyclopedia. He has been Guest Editor for the special issue of "European Journal of Pharmaceutics and Biopharmaceutics" on "engineered polymers and polymeric systems in controlled drug delivery and targeting" (2009). He is also the author (inventor) of 1 European patent. His published research has received over 1000 citations (h index 17). He has participated in 9 research programs in collaboration with academic and industrial organizations (in 7 as coordinator). He serves as reviewer for major pharmaceutical and nanoscience/nanotechnology journals and he is Assistant Editor of "Current Nanoscience" and member of the Editorial Board for the "Open Drug Delivery Journal" and for the "Journal of Excipients and Food Chemicals".

May Elizabeth Azzawwi

Senior Lecturer in Human Physiology
School of Healthcare Science, Manchester Metropolitan University
Manchester M1 5GD; Tel. 0161- 247 3332
Email: m.azzawi@mmu.ac.uk

EDUCATION

• 1991: PhD, Faculty of Medicine, National Heart & Lung Institute, University of London, UK. Thesis entitled "Immunopathogenesis of Bronchial Asthma".
• 1986: MSc, Applied Immunology, Brunel University, London, UK.
• 1985: BSc (Hons) Science 'Physiology& Biochemistry'[2:1]. Univ. E. London, UK.

EMPLOYMENT HISTORY

• 2006- current: Senior Lecturer, School of Healthcare Science, Manchester Metropolitan University, UK
• 2001- 2005: Research Fellow (BHF), part time. Cardiovascular Research Group, Dept. Medicine, University of Manchester, UK.
• 1990- 1997: Postdoctoral Research Associate (MRC, BHF), School of Biological Sciences, University of Manchester, UK.
• 1986- 1990: Research Assistant (MRC), Dept. Allergy & Clinical Immunology, National Heart & Lung Institute, Royal Brompton Hospital, London, UK.

PROFESSIONAL MEMBERSHIPS

Member of the European Forum on Nanomedicine; the British Society for Nanomedicine; the British Society for Cardiovascular Research; the Physiological Society; the British Society for Immunology; the Northern Cardiovascular Research Group; and the British Microcirculation Society. Fellow of the Higher Education Academy.

GRANT FUNDING, Recent

• Erasmus Mundus PhD studentship programme, September 2013 (3 years), €108,000.
"Impaired mobility in aging; the role of antioxidants in improving vascular function".
• Erasmus Mundus PhD studentship programme, September 2013 (3 years), €108,000.
"Effects of home-based training in patients with ischaemic artery disease". Co-Investigator.
• NanoInfoBio, Early adopter fund (EPSRC), November 2009, £10,000
"Nanotoxicological influence of nanoparticles on arterial vessel function".

SCHOLARLY ACTIVITIES, Recent

Invited reviewer for many journals: including the journal of 'Nanomedicine'; Invited reviewer for grant applications: The Wellcome Trust; External and internal PhD and MSc supervisor and examiner. Editorial board member of: Journal of Advances in Biotechnology and Bioengineering.

SELECTED RECENT PUBLICATIONS

• Farooq A, Whitehead D, **Azzawi M**. Attenuation of endothelial dependent vasodilator responses, induced by dye encapsulated silica NPs, in-vitro. Nanomedicine, 2013 Feb 22. [Epub ahead print]
• Farooq A, Al-Jowder R, Narayanaswamy R, **Azzawi M**, Roche PJR Whitehead D. Ultrasensitive gas detection using quenching fluorescence of dye-immobilized silica nanoparticles. Sensors & Actuators: B. Chemica, 2013 [Epub ahead of print].
• Shukur A, Rizvi S, Whitehead D, Seifalian A and **Azzawi M**. Altered sensitivity to nitric oxide donors, induced by intravascular infusion of quantum dots, in murine mesenteric arteries. Nanomedicine-NBM 2013; 9(4): 532-539.
• **Azzawi M**. 'The nanotoxicological influence of nanoparticles; with special reference to the vasculature'. In:Current advances in the medical application of nanotechnology. Bentham Publications, 2012.
• Akbar N, Mohamed T, Whitehead D, **Azzawi M**. Biocompatibility of amorphous silica nanoparticles: Size and charge effect on vascular function, in vitro. Biotechnol Appl Biochem. 2011; 58(5):353-62.

Moria Barlev-Gross

Date of birth: 27/05/1988
Adress: Kibutz Hafetz-Hayim 76817
E-mail: moriagross@gmail.com
Phone number: 054-5971445

EDUCATION

• 2003-2006: Arad High-school, Biology and Biotechnology
• 2009-2012: B.Sc. Medical lab science, Hadassah College Jerusalem
• 2014: Paramedics at Magen-David-Adom

WORK EXPERIENCE

• 2008-2010: senior tour guide at "Society for the Protection of Nature in Israel"
• 2012: Intern at Prof. Chuck Greenblatt's lab. Research of Ancient DNA and Microbiology, Hebrew University
• 2012-today: Lab of Membrane and Liposome Research, Prof. Barenholtz lab, Hebrew University

NATIONAL SERVICE

• 2007-2008: Tour guide at "Society for the Protection of Nature in Israel", Golan Heights

Woźniak Bartosz

Kopernika 22/24, 19-100 Mońki
Mobile: 783116826; bartek_u1@interia.pl
bwozniak@unipress.waw.pl

EDUCATION

Engineer Degree in Management and Production Engineering, Technical University in Białystok, 2014

EXPERIENCE

Laboratory experience in several techniques, including:
• Mean particle size and size distribution by means of DLS;
• Zeta potential; • Specific Surface Area (BET); • Nanoparticles Density

I am responsible for sonochemical synthesis of nanopowders and optimization sonochemical coating process of various surfaces with nanoparticles.

Objectives: To obtain necessary experience allowing the use of my technical and analytical skills.

Yaelle Bavli (Felsen)

EDUCATION

Mar 2012-: Ph.D.: Toxicology of nanoliposomal drugs – the Membrane and Liposome Research now Lab (Prof. Yechezkel Barenholz), The Hebrew University of Jerusalem (HUJI), Israel

• 2005-2007: M.Sc. in Pharmacology (cum laude) – University Louis Pasteur (ULP), Strasbourg, France

• 2002-2005: B.Sc. in Biotechnology and Bio-industry (magna cum laude) – ULP, Strasbourg, France

LABORATORY TRAININGS

• 2007 (7 months): University of Melbourne, Department of Medicine, Lab of Prof. Joe Proietto (under the supervision of Drs Sofianos Andrikopoulos and Barbara Fam), Melbourne, Australia. Assessment of the specificity and inducibility of a muscle specific MerCreMer recombinase in transgenic mice and determination of the physiological impact of the KO.

• 2006 (3 months): University of Strasbourg, INSERM (Medical Research Unit), Strasbourg, France. Optimization of antibody detection to highlight the modifications of neuromuscular junctions' morphology in amyotrophic lateral sclerosis (ALS).

• 2005 (5 months): University of Strasbourg, INSERM, Strasbourg, France, Lab of Dr. Frederique Rene. Expression and colocalization of several proteins after injury in a model of peripheral neuropathy in mice (PCR, surgery on mice, immunohistochemistry and immunofluorescence).

PUBLICATIONS

• Oren Regev, Yechezkel Barenholz, Sivan Peretz, Daniel Zucker, and **Yaelle Bavli-Felsen**. Can carbon nanotube–liposome conjugates address the issues associated with carbon nanotubes in drug delivery? Future Medicinal Chemistry, April 2013, Vol. 5, No. 5, Pages 503-505

• Keren Turjeman, **Yaelle Bavli**, Alex Sigal, Pablo Kizelsztein, Yaelle Schilt, Hiba Kanaan, Reuma Ronen, Nahum Allon, Tamar Blumenfeld-Katzir, Efrat Sasson, Uri Raviv, Haim Ovadia and Yechezkel Barenholz. Sterically stabilized nano-liposomes for the treatment of neurodegenerative diseases that involve an inflammatory component. Submitted

• Hadas Perlstein, **Yaelle Bavli**, Simcha Even-Chen, Tanya Turovsky, Abraham Rubinstein, Dganit Danino, David Stepensky and Yechezkel Barenholz. Improved intestinal absorption of celecoxib by beta-casein nanocarriers.

PRESENTATIONS IN CONFERENCES

POSTERS

• The European Summit for Clinical Nanomedicine 2012 (5th CLINAM 2012)

• The 8th Annual Meeting of the Israeli Chapter of the Controlled Release Society (ICRS) 2012

• NanoIsrael Conference 2012

• NanoIsrael Conference 2014

ORAL PRESENTATIONS

• The 8th Annual Meeting of the ICRS 2012 (An improved Pegylated Liposomal Doxorubicin with Significantly Lower PPE than Doxil®)

Kálmán Benedek

PhD

EDUCATION

• 1982 Ph.D., in IMMUNOLOGY, L. Eötvös University, Budapest, Hungary. Thesis topic: Histamine Receptor Bearing Lymphocytes and Their Functions in the Human Body.

• 1976 M.S., in POLYMER CHEMISTRY and BIOCHEMISTRY, L. Eötvös University, Budapest, Hungary, Thesis topic: Structure of Proteins in the I-Filament of Muscles.

EMPLOYMENT

• 2010- Nano Analytical Laboratory, Institute for Nanomedicine, Semmelweis University, Budapest, Hungary

• 1997- iGORi, Thousand Oaks, CA, Director of Analytical and Assay Development

• 1991-95 Amgen, QA/QC Department, Thousand Oaks, CA, Senior Scientist

• 1990-91 Terrapin Technologies, Inc., San Francisco, CA, Senior Scientist, Project Manager

• 1988-90 Millipore Corporation, Specialty Chemistries, Bedford, MA, Consulting Scientist

• 1985-88 Smith Kline & French Laboratories, Pharmaceutics Dept, Philadelphia, Associate Senior Investigator

PUBLICATIONS

1. **BENEDEK, K**., and Swadesh, J.K., "HPLC of Proteins and Peptides in the Pharmaceutical Industry", in HPLC in the Pharmaceutical Industry , Godwin W. Fong and Stanley K. Lam, editors, Marcell Dekker, New York.(1991)

5. **BENEDEK, K**., "The Application of HPLC for Proteins", in Liquid Chromatography Methods in Biotechnology, Elena Katz, editor, John Wiley & Sons, Ltd (1994)

6. **BENEDEK, K** and Guttman, A., "High Performance Capillary Electrophoresis, an Overview", in HPLC, Practical and Industrial Applications, Swadesh, J.K., editor, CRC Press, Boca Raton (1996)

7. **BENEDEK, K**., "Analytical High Performance Liquid Chromatography" in Methods in Molecular Biology, M. Aguilar editor, Humana Press, submitted (2002)

8. **BENEDEK, K**., "High Performance Hydrophobic Interaction Chromatography" in Methods in Molecular Biology, M. Aguilar editor, Humana Press, submitted (2002)

9. Narhi LO, Jiang Y, Cao S, **BENEDEK, K**, Shnek D. "A critical review of analytical methods for subvisible and visible particles. Curr Pharm Biotechnol. 2009 Jun;10(4):373-81.

Fodor Bertalan

H-3515 University of Miskolc, University Village, Building B3/B4, 2nd floor
Tel.: (+36-46) 565-135
E-mail: fodor@uni-miskolc.hu

Bertalan Fodor MSc, PhD, Dr. habil., EurClinChem, College Professor and Head of Department, at the University of Miskolc, Faculty of Health Care, Department of Nanobiotechnology and Regenerative Medicine. He graduated as microbiologist at the Eötvös Lorand University in Budapest, Hungary in 1993. He took his PhD degree in clinical immunology at the Medical School of Debrecen, University of Debrecen in 2003. At present his group focuses to the liposome research, siRNA containing liposome development and nano toxicological characterization of different types nanomaterials.

André-René Blaudszun

André-René Blaudszun studied biochemistry at the Ruhr-University Bochum (RUB, Germany) where his diploma thesis included studying tumor vaccines in mice. In 2007 he graduated and joined the Korea Institute of Science and Technology (KIST) Europe Forschungsgesellschaft mbH (Saarbruecken, Germany) as a research assistant. From 2008 until 2009 he was also employee without allowance in the division of Translational Immunology of the German Cancer Research Center (DKFZ, Heidelberg) under the supervision of Prof. Dr. Philipp Beckhove, testing a T cell-based drug delivery approach in a human tumor xenograft mice model. Since 2010 he is enrolled as a Ph.D. student at the Saarland University (UDS, Saarbruecken, Germany) under the supervision of Prof. Dr. Claus-Michael Lehr. As a member of the Environment and Bio Group at KIST

Europe and in cooperation with the Department of Biopharmaceutics and Pharmaceutical Technology at the Saarland University his PhD work focuses on T lymphocyte-mediated drug delivery.

PUBLICATIONS

• T. Stöhr, **A.-R. Blaudszun**, U. Steinfeld and G. Wenz: Synthesis of glycosylated peptides by NCA polymerization for recognition of human T cells, Polym. Chem., 2011, 2, 2239-2248

• A. Philippi and **A.-R. Blaudszun**: Living immune cells as drug carriers in cancer therapy, Eur. J. Nanomed. 2014; 6(1): 9-10

Ines Block

Ines Block studied Biochemistry at the Ernst-Moritz-Arndt-University of Greifswald in Germany. During her studies she expanded her knowledge in the development of electrochemical biosensors by performing a research internship at the Mexico State University in Las Cruces (New Mexico, USA). After finishing her Diploma in Biochemistry (equivalent to Msc) she started to work as a PhD student in a project aiming at the generation and application of highly complex peptide arrays in the department of Chip-Based Peptide Libraries at German Cancer Research Center (DKFZ) in Heidelberg (Germany) and the Kirchhoff-Institute for Physics at the University of Heidelberg (Germany). In 2009 she graduated (Dr. rer. nat.) in natural sciences at the Ruprecht-Karls-University of Heidelberg in Germany.

Since March 2009 Ines Block is postdoctoral fellow in the Molecular Oncolgy Group of Prof. Dr. Jan Mollenhauer at the Institute of Molecular Medicine of the University of Southern Denmark in Odense, Denmark. Here she is working on the systematic identification of breast cancer genes and drug targets through targeted functional genomics. Furthermore she is the head of an innovative screening project within the Lundbeckfonden Center of Excellence NanoCAN aiming at the development of a biochip-format for the molecular profiling of cancer stem cells.

Gary Braun

Ph.D

Gary Braun received his B.S. and Ph.D. in Chemistry at University of California Santa Barbara, studying surface enhanced Raman spectroscopy in nanoparticle systems. He then joined the Sanford Burnham Medical Research Institute as a postdoctoral associate in Erkki Ruoslahti's laboratory. Gary is currently studying the use of nanoparticles for various biological applications, including cancer detection, cell transport pathways, and drug screening, and using a multidisciplinary approach between inorganic chemistry, biochemistry, and biology.

Elena-Diana Burghelea

Route du Rawyl 47, Sion 1950, Switzerland
Telephone: +41 27 606 86 39
E-mail: elena-diana.burghelea@epfl.ch

EDUCATION

• Nov 2013:Started the Doctoral School in Chemistry and Chemical Engineering EPFL, Research Project: Intelligent Integrated Systems for Personalized Medicine (ISyPeM II)

• April 2013: Master's degree in Advanced Chemistry Methods, University of Turin (Thesis: 'The use of apoferritin as carrier of GdHPD03A and curcumin for the treatment of hepatic pathologies guided of MRI')

• June 2010: Bachelor's degree in Chemistry at University of Turin, Italy

• 2004-2008: Bachelor's degree in Physical-Chemistry at Faculty of Chemistry, Al.Ioan Cuza University, IASI, Romania

• April 2008: '7th School of Physics and Chemistry of the Actinide', Wroclaw, Poland.

• June 2003: High School Diploma in Pedagogical Sciences, in Birlad, Romania.

EXPERIENCE

• Nov 2013- Present: PhD student at Institute of Life technologies HES-SO Valais, Wallis in the Frame of Doctoral School in Chemistry and Chemical Engineering, EPFL. Theme: Designing assays for Point-Of-Care Therapeutic Drug Monitoring

• October 2011- April 2013: Researcher at Molecular Biochemistry Centre (University of Turin, Italy), on Theranostic agents (diagnosis and therapy) delivery guided of Magnetic Resonance Imaging

PUBLICATIONS

Simonetta Geninatti Crich, **Burghelea Diana**, Juan Carlos Cutrin: Curcumin/Gd loaded Apoferritin: a novel "theranostic" agent to prevent hepatocellular damage in toxic induced acute hepatitis, Molecular pharmaceutics, april, 2013

Francesco Cellesi

Group Leader CEN - European Centre for Nanomedicine; Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Via Pace 9, I-20122 Milan, Italy; Dipartimento di Chimica, Materiali ed Ingegneria Chimica "G. Natta", Politecnico di Milano, Via Mancinelli 7, 20131 Milan, Italy
Email: francesco.cellesi@polimi.it

EDUCATION/TRAINING AND PROFESSIONAL EXPERIENCE

• 01/03/20013 Group leader in the CEN Foundation project "Start-up Packages and PhD program", research group "Podocyte-targeted Therapeutic Nanodelivery to Treat Proteinuric Glomerular Diseases", hosted and cofinanced by Fondazione IRCCS Ca' Granda – Ospedale Maggiore Policlinico (MI), in collaboration with Politecnico di Milano, Dipt. di Chimica, Materiali ed Ingegneria Chimica "G. Natta" and Adjunct Professor at Politecnico di Milano.

• 01/09/2006 Lecturer, School of Pharmacy, University of Manchester, UK

• 30/04/2006 Research Fellow, School of Pharmacy, University of Manchester, UK

• 30/04/2005 PostDoctoral Research associate, School of Pharmacy, University of Manchester, UK

• 20/09/2003 Honorary research associate, School of Pharmacy, University of Manchester, UK

• 30/10/2000 PhD, Doctoral studies Institute of Biomedical Engineering, ETHZ (CH)

• 30/03/2000 Postgraduate fellowship, Institute of Biomedical Engineering, ETHZ (CH)

• 22/10/1999 Master (Laurea) in Chemical Engineering Università di Pisa, Italy

EXPERTISE

Francesco Cellesi is Group Leader at CEN - European Centre for Nanomedicine in Milan, and leads a research group focused on Podocyte-targeted Therapeutic Nanodelivery to Treat Proteinuric Glomerular Diseases. His main research interests focus on the design and processing of nano biomaterials for novel drug delivery systems and diagnostics, as well as cell microencapsulation and tissue engineering. His areas of expertise span from applied physical chemistry, polymer chemistry, colloidal chemistry, to materials science and process engineering. As part of his academic duties, Francesco holds the position of adjunct professor at Politecnico di Milano.

Mayank Chaturvedi

I am final year PhD student at the Nencki Institute of Polish Academy of Sciences in Warsaw Poland, under supervision of Prof. Leszek Kaczmarek in International PhD Program in Neurobiology, in collaboration with Indian Institute of Chemical Technology, India and Vect-Horus, a Marseille based French Biotechnology Company.

My PhD project is at intersection of nanotechnology and molecular neuroscience. During my PhD studies I have developed protein loaded nanoparticles (TIMP-1) for brain delivery and have shown their neuroprotective effects after neuronal toxicity.

PUBLICATIONS (In last three years)

1. **Chaturvedi, M.***, Molino, Y., Bojja, S., Khrestchatisky, M., Kaczmarek, L.; "TIMP-1 loaded PLGA nanoparticles across blood brain barrier". Int J Nanomedicine. 2014; 9 (1):575-588. (5 years Impact: 4.0)
2. **Chaturvedi, M.***, Kaczmarek, L.; "MMP-9 inhibition: A Therapeutic strategy in Ischemic Stroke". Mol Neurobiol. In Press PMID: 24026771. (5 years Impact: 5.5)
3. **Chaturvedi, M.**, Figiel I., Sreedhar B., Kaczmarek L.; "Neuroprotection from Tissue Inhibitor of Metalloproteinase-1 and its nanoparticles." Neurochem Int. 2012 Dec; 61(7):1065-71. (5 years Impact: 2.9)
4. Knapska, E., Balcerzyk, M., Lioudyno, V., Kiryk, A., Górkiewicz, T., Mikosz, M., Michaluk, P., Gawlak, M., **Chaturvedi, M.**, Mochol, G., Wojcik, D., Wilczyński, G., Kaczmarek, L.; "Matrix metalloproteinase-9 is required for appetitively but not aversively motivated operant learning and plastic changes in the central amygdala". J Neurosci. 2013; 33(36):14591-600. (5 years Impact: 7.8)

* Co-Corresponding Author

EXPERIENCE

• Teaching Experience: Three years from 2006-2008 worked as a full time lecturer in Swami Vivekanand College of Pharmacy, Indore India

EDUCATION

• M. Pharm.: 2006 - Specialization in pharmaceutical biotechnology from Department of Pharmaceutical Sciences Dr. H.S. Gour University Sagar (MP) India.
• B. Pharm.: 2003 - From IPS Academy College of Pharmacy, Indore, India

GRANTS / TRAVEL AWARDS / SHORT TERM STAY GRANTS / SCHOLARSHIPS

• January 2014: Preludium grant from National Science Center of Poland for young researchers.
• 2010-2014: Prestigious PhD studentship from Foundation of Polish Science under International PhD program in Neurobiology.
• November 2013: BIOIMAGINE Travel Grant to attend SFN 2013 San Diego USA.
• September 2013: Travel Grant from COST for attending Third Annual Conference of COST Action ECMNET.
• April 2013: Travel Award from ISN to attend ISN-ASN Meeting in Cancun, Mexico.
• August 2012: COST Short term stay grant for working in NICN, CNRS Marseille France.
• July 2012: Travel Grant from COST for attending Second Annual Conference of COST Action ECMNET "Brain Extracellular Matrix in Health and Disease" Barcelona, Spain.

SELECTED TALKS (In last three years)

• 2014, Krakow, Poland: Neuronus -2014 Conference, from 25-27 April 2014.
• 2013, Warsaw, Poland: 1st Nencki PhD Conference, from 16-18 October 2013.
• 2012, Barcelona, Spain: Second Annual Conference of COST Action ECMNET "Brain Extracellular Matrix in Health and Disease" from 12 July- 13th July.

SELECTED POSTER PRESENTATIONS (In last three years)

• 2013, San Diego, USA: SFN Meeting 2013, from 9-13 November.
• 2013, Poznan, Poland: Polish Neuroscience Society Meeting 15-17 September.
• 2013, Cancun Mexico: ISN-ASN 24th Meeting, from 20-24 April.
• 2013, Playa del Carmen, Mexico: Synapse Meeting from 25-28 April.
• 2012, Barcelona, Spain: 8th FENS Forum of Neuroscience from 14 July- 18th July.
• 2011, Warsaw Poland: 8th Parnas Conference from 27-31 August.
• 2011, Florence Italy: 8th IBRO World Congress of Neuroscience from 14-18 July.

Bing-Mae Chen

Institute of Biomedical Sciences, Academia Sinica, Taipei 11529, Taiwan
Tel: 886-2-2789-9152
E-Mail: bmchen@ibms.sinica.edu.tw

EDUCATION

• 1987: B.S. (Food Sciences), National Taiwan Ocean University, Keelung, Taiwan
• 1995: M.S. (Microbiology and Immunology), Soo-Chow University, Taipei, Taiwan

EXPERIENCE

• 1987-1994: Research assistant, Institute of Biomedical Sciences, Academia Sinica, Taipei
• 1995-present: Senior research technician and lab manager, Institute of Biomedical Sciences, Academia Sinica, Taipei

RESEARCH

32 publications in SCI journals

Marlus Chorilli

Pharmacist, Methodist University of Piracicaba (2002), Master (2004) and PhD (2007) in Pharmaceutical Sciences from the Universidade Estadual Paulista Júlio de Mesquita Filho. He is currently Assistant Professor at the Faculty of Pharmaceutical Sciences of Araraquara (FCFar) - Universidade Estadual Paulista Júlio de Mesquita Filho, teaching the disciplines of Pharmacotechniques and Pharmaceutical Technology. Programs accredited by the Faculty of Graduate Studies in Pharmaceutical Sciences and Biotechnology and Biosciences Applied to Pharmacy and leader of the research group of CNPq - Research and Development of Drug Release Systems Based on Nanotechnology. Scholarship Productivity in Innovative Technological Development and Extension CNPq, level 2, since 2012. Chartered journals in the field of Pharmaceutical Sciences and scientific adviser to Brazilian funding agencies (CNPq, FAPESP and FAPITEC/SE) and foreign (FONDECYT-Chile). He has experience in Pharmacy with emphasis in Pharmaceutical Technology, especially with technological development and biological evaluation of nanostructured systems for administration of biopharmaceuticals acting.

List up to more relevant search results and may be scientific articles, book chapters, patents (granted or requested), registered software or other types of publications that consider.

• Santos FK, Oyafuso MH, Kiill CP, Gremião MPD, **Chorilli M**. Nanotechnology-based drug delivery systems for treatment of hyperproliferative skin diseases - a review. Current Nanoscience 2013 9(1) (aceito).
• Carvalho FC, Calixto GMF, Luz GM, Katakeyama I, Gremião MPD, **Chorilli M**. Rheological, mechanical and bioadhesive behavior of

hydrogels to optmize skin delivery systems. Drug Development and Industrial Pharmacy 2012 (aceito).
• **Chorilli M**, Calixto GMF, Rimério TC, Scarpa MV. Caffeine encapsulated in small unilamellar liposomes: characterization and in vitro release profile. Journal of Dispersion Science and Technology 2012 (aceito).
• **Chorilli M**, Salgado HRN, Santos FS, Silva LM. Validation of a HPLC method for determination of glutamine in food additives using post-column derivatization. American Journal of Analytical Chemistry 2012 03: 113-117.
• Souza ALR, Kiill CP, Kolenyak F, Luz GM, Silva HR, **Chorilli M**, Gremião MPD. Nanotechnology-based drug delivery systems for dermatomycosis treatment. Current Nanoscience 2012 8:512-19.
• **Chorilli M** Leonardi GR, Scarpa MV, Salgado HRN. Evaluation of preservative effectiveness of liquid crystalline systems with retynil palmitate by challenge test and D-value. J. AOAC International 2011 94(1): 118-127.
• **Chorilli M**, Prestes PS, Rigon RB, Leonardi GR, Chiavacci LA, Sarmento VHV, Oliveira AG, Scarpa MV. Structural characterization and in vivo evaluation of retinyl palmitate in non ionic lamellar liquid crystalline system. Colloids and Surfaces B: Biointerfaces 2011 85: 182-188.
• Fiorentino FAM, **Chorilli M** Salgado HRN. The use of the challenge test to analyse preservative efficiency in non-sterile cosmetic and health products: applications and critical points. Analytical Methods 2011 3(4): 790-798.
• **Chorilli M** Bonfilio R, Chicarelli RS, Salgado HRN. Development and validation of an analytical method by RP-HPLC for quantification of sibutramine hydrochloride in pharmaceutical capsules. Analytical Methods 2011 3(4): 985-990.
• Guimarães GN, **Chorilli M**, Prestes PS, Leonardi GR, Pires-de-Campos MSM, Polacow MLO. Effects of formulations containing dimethylaminoethanol (DMAE) acetoamidobenzoate and pidolate on the skin. Latin American Journal of Pharmacy 2011 30(4): 641-646.
• **Chorilli M**, Bonfilio R, Louvadini CR, Gonçalves F, Salgado HRN. Development and validation of an LC-MS/MS method for quantitative analysis of mirtazapine in human plasma. American Journal of Analytical Chemistry (AJAC) 2011 2: 650-657.
• Prestes PS, **Chorilli M**, Chiavacci LA, Scarpa MV, Leonardi GR. Physicochemical characterization and rheological behavior evaluation of the liquid crystalline mesophases developed with different silicones. Journal of Dispersion Science and Technology 2010 31(1): 117-123.
• Zague V, **Chorilli M**, Polacow MLO, Pires-de-Campos MSM, Leonardi GR. In vitro ultrasound influence on cutaneous permeation of hyaluronidase. Journal of Dispersion Science and Technology 2010 31(6): 756-759.
• **Chorilli M**, Prestes PS, Rigon RB, Leonardi GR, Chiavacci LA, Scarpa MV. Desenvolvimento de sistemas líquido-cristalinos empregando silicone fluído de co-polímero glicol e poliéter funcional siloxano. Química Nova 2009 4: 1036-1040.
• **Chorilli M**, Udo MS, Rodrigues LAP, Cavallini ME, Leonardi GR. Avaliação sensorial de formulações fotoprotetoras contendo filtro solar de amplo espectro. Latin American Journal of Pharmacy 2009 28(3): 383-392.
• **Chorilli M** Campos GR, Bolfarini PML.. Desenvolvimento e estudo da estabilidade físico-química de emulsões múltiplas A/O/A e O/A/O acrescidas de filtros químicos e manteiga de karité. Latin American Journal of Pharmacy 2009 28(6): 936-940.
• Pires-de-Campos MSM, Leonardi GR, **Chorilli M**, Spadari-Bratfisch RC, Grassi-Kassisse DM. The effect of topical caffeine on the morphology of swine hypodermis as measured by ultrasound. Journal of Cosmetic Dermatology 2008 7: 232-237.
• **Chorilli M**, Rimério TC, Oliveira AG, Scarpa MV. Obtenção e caracterização de lipossomas unilamelares pequenos contendo cafeína. Latin American Journal of Pharmacy 2007 26: 715-722.

Noemi Stefania Csaba

Noemi Stefania Csaba is Assistant Professor at the University of Santiago de Compostela, Center for Research in Molecular Medicine and Chronic Diseases. She is author of 27 scientific articles published in internationally recognized journals, 6 book chapters, 4 patents, over 30 presentations at international symposia. She is editor of the book "Nanostructured biomaterials for overcoming biological barriers" (RSC Publishers) and of the special journal issue "Nanocarriers&drug delivery: rational design and applications" in Current Topics in Medicinal Chemistry. Noemi accumulates over 10 years of expertise in the field of nanomedicine & nanotechnology and has been involved in several national and international collaborative projects (EU-FP7, Euronanomed, Bill&Melinda Gates Foundation etc). She has been principal investigator in two national/ regional projects on nanomedicine and nanovaccine design. She is currently involved in the large scale FP7 EU Project "Trans-Int: Transporting therapeutic macromolecules across the intestinal barrier" as the co-leader of the work package dedicated to nanocarrier design and characterization. She is member of the Supervisory Board of the "Nanofar" Erasmus Mundus Joint Doctorate Programme on Nanomedicine and she is national delegate at the COST TD1004 Action on Theranostics and imaging.

Roberta Dal Magro

Actual position (January 2013 – present): PhD student (PhD Program Nanostructure and Nanotechnology, curriculum Nanobiotechnology and Nanomedicine), University of Milano-Bicocca.

EDUCATION AND TRAINING

• November 2010 – October 2012: Master Degree in Medical Biotechnology (110/110 cum laude), University of Milano-Bicocca. Experimental thesis: Study of the interactions of brain targeted engineered nanoparticles with an in vitro model of brain-blood barrier
• October 2007 – November 2010: Bachelor Degree in Biotechnology, University of Milano-Bicocca.

Key areas of research interest: characterization of nanoparticles as drug delivery systems; study of nanoparticles interaction with in vitro models of cerebral microvascular endothelial cells; identification of the molecular mechanisms involved in the cellular uptake of nanoparticles; in vivo biodistribution studies; evaluation of in vivo inflammatory reactions triggered by nanoparticles.

• 2014: Member of Neuroscience Centre of the University of Milano-Bicocca
• 2014: Member of Italian Society of Nanotoxicology

CONGRESS PRESENTATIONS

• "Solid Lipid Nanoparticles: a strategy to overcome the blood-brain barrier"
R. Dal Magro, F. Ornaghi, I. Cambianica, F. Re, F. Barbero, C. Musicanti, A. Brambilla, E. Salvati, A. Cagnotto, M. Masserini, P.Gasco, G. Sancini. NPMED Meeting, Nanoparticles and Nanotechnologies in Medicine 2013, Bresso (Mi), 19-21st June 2013.
• "Solid Lipid Nanoparticles: a strategy to overcome the blood-brain barrier"
R. Dal Magro, F. Ornaghi, I. Cambianica, F. Re, F. Barbero, C. Musicanti, A. Brambilla, E. Salvati, A. Cagnotto, M. Masserini, P. Gasco, G. Sancini. SIF Meeeting, Porto Novo (AN) 18-21st September 2013.
• "Enhanced brain targeting of engineered solid lipid nanoparticles"
R. Dal Magro1, F. Ornaghi1, I. Cambianica1, S. Beretta1, F. Re1, A. Brambilla1, F. Barbero2, C. Musicanti2, A. Cagnotto3, E. Donzelli4, A. Canta4, M. Masserini1, G. Cavaletti4, P. Gasco2, G. Sancini1. IT-

CRS Meeting, Pavia (PV), 21-23rd November 2013.
• Workshop Tecnobionet, The future of research and clinical applications in neuroscience, Genova (GE), 3-4th April 2014.

László Dézsi

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

László Dézsi PhD, DrHabil., works as Senior Research Associate in the in vivo laboratory of Nanomedicine Research and Education Center of the Semmelweis University since 2012 in Budapest, Hungary.
He obtained his MSc degree in biology at Eötvös Loránd University and his PhD in physiology at Semmelweis University Medical School, Budapest, Hungary. He conducted teaching and research activities at Semmelweis University (1981-1999), while he also received fellowships at Albert Ludwigs Universität, Freiburg, Germany (Prof. E. Bassenge) working in the field of local regulation of blood flow in skeletal and cardiac muscle; as well as at the University of Pennsylvania, Philadelphia, USA, Cerebrovascular Research Center (Prof. M. Reivich, Dr. J. H. Greenberg) working in the field of cerebral blood flow/metabolism and cerebral ischemia/reperfusion in stroke models in animals. He had been head of laboratory, CRO monitor and research project manager in vascular and safety pharmacology at Gedeon Richter Pharmaceutical Plc. (1999-2012). He was manager of Analgesic Research Laboratory (2006-2012), a joint venture of Gedeon Richter and University of Pécs, Department of Pharmacology (Prof. J. Szolcsányi). He participated in curriculum development and had been Secretary of Biomedical Engineering Course Committee at Technical University, Budapest (1994-2000).
He made his habilitation at Semmelweis University in 2005 and became Adjunct Professor in 2006. He established his own teaching course (2008) "Cardiorespiratoric and neurophysiological measuring techniques" at the Department of Human Physiology (Prof. Z. Benyó). Currently he is working in the field of nanomedicine investigating cardiopulmonary and immunological effects of nanoparticles in in vivo models of complement activation related pseudoallergy (CARPA) under the supervision of Prof. J. Szebeni.

Samantha M. K. Donnellan

22 Dean Path Buildings, Edinburgh, EH43AZ
Phone + 44 7747170492
E-Mails: samanthadonnellan@hotmail.co.uk; smd31@hw.ac.uk
http://www.nano.hw.ac.uk/people/phd-students/samantha-donnellan.html
Date of Birth: 22/03/1987

EDUCATION

Heriot Watt University: PhD; Treatment of TB using Nanomedicines Oct 2012-Present
I am currently working on a PhD project under the supervision of Dr K Stevenson, Moredun Research Institute and Prof V Stone, Heriot Watt University.
• University of Edinburgh: MSc; Forensic Anthropology Sept 2010 – Nov 2011
- August 2011: MSc thesis was 'Comparative Morphology of Sharp Force Trauma to the Cranium using High Resolution Casts': Grade awarded: A.
• University of Glasgow: BSc (Hons); Medical Biochemistry (2.1)
- June 2010: Final Year BSc. Honours Project was 'DNA Profiling in the UK Criminal Justice System': Grade Awarded: A.
- May 2010: Dissertation was cancer research: 'The Role of the HER2 Gene in Breast Cancer Development'.

PUBLICATIONS

• Donnellan SM1, Chatzinikolaou F, Kranioti EF, Morphological Analysis of Sharp Force Trauma Patterns Using High Resolution Casts. Proceedings of the 22rd Congress of the International Academy of Legal Medicine, 2013 (in press).
• Donnellan SM1, Chatzinikolaou F, Kranioti EF Morphological Analysis of Sharp Force Trauma Patterns Using High Resolution Casts Int J Legal Med (2012) 126 (Suppl 1):S175-6

PRESENTATIONS

• Podium Presentation, 'Sharp Force Trauma Analysis' Scottish Student Forensic Research Symposium, University of Strathclyde, March 2014.
• Histology CPD Workshop, 'Negative Casting of Bone Trauma' University of Edinburgh March 2013 and 2014.
• Poster Presentation British Association of Human Identification (BAHID) Manchester, December 2012. AWARDED SECOND PRIZE.
• Poster presentation 'Morphological Analysis of Sharp Force Trauma Patterns Using High Resolution Casts' 22rd Congress of the International Academy of Legal Medicine, Istanbul, Turkey 5-8th July 2012.
• Poster Presentation 'Skeletal Trauma and Pathology British Association for Biological Anthropology and Osteoarchaeology (BABAO) University of Edinburgh, September 2011.

WORKSHOPS COMPLETED

• University of Edinburgh- April 2014 Edinburgh Infectious Diseases "Imaging Infection".
• Heriot-Watt University- Jan 2014 Present. 3 short courses: Learning Enhancement and Development Skills (LEADS).
• University of Edinburgh- May 2013 6 week course. CMVM light microscopy course: Beyond Pretty Pictures.
• University of Edinburgh- Feb 2013 Short Course: Statistical/Experimental Design course.
• University of Manchester- Dec 2012 Forensic Recognition Course.
• University of Edinburgh- Mar 2012 Short Course: AMIRA 3D Imaging: Surface scanning and Hands on Amira training.
• Bournemouth University- July 2011 Short Course: Forensic Stimulations: Mass graves- Temporary Mortuary.
• University of Edinburgh- Mar 2011 Short Course: Cross-sectional and Surface HistologyWorkshop: An Application of Anthropological Methods.

SKILLS

• AMIRA software
• Use of SEM
• Excellent computing /written skills
• Teaching/Lecturing
• Researching/Surveying
• Bone Maceration

OTHER (Memberships etc)

• University of Edinburgh Postgraduate Seminar Committee Member Dec 2013-Present
• International Student Volunteers (ISV) University Leader (Jul-Aug 2013 + 2014): taking groups of 15+ undergraduate students on biological field trips to Costa Rica
• Higher Education Academy Accredited (via the Professional HEA Recognition Scheme (Jan 2014))
• Society for General Microbiology, member since 2012.

EMPLOYMENT AND ACADEMIC WORK EXPERIENCE

• Exam Scribe, Reader and Invigilator- Heriot Watt University, 2013-present.
• Laboratory Demonstrator/teaching aid- Heriot Watt University, 2013- present.
• Research Assistant- University of Edinburgh, Jan '12- Oct 2012.
• Experience using CT scanner/SEM- University of Edinburgh
• Field Researcher in Archaeology, Pathology & Trauma- University of Edinburgh, Ibiza, May '11&'12
• Attended & assisted in animal PMs, Edinburgh University Veterinary Dept, Mar '11-2012
• Attended a Forensic Anthropological

Ramy El-Sayed

Mobile: +46700674380
Email: ramy.el.sayed@ki.se

Through my Education and Trainings, I have developed a wide knowledge in different fields including Nanomedicine, Physiology, toxicology, organic and inorganic Nanoparticle synthesis, Polymeric composites, Chemical engineering and Mechanical engineering. My personal interest and PhD is focusing on nanomedicine.

EDUCATION

• Oct. 2012-present:Karolinska Institutet- KI, Stockholm, Sweden, dsdPhD student, Institute of Environmental Medicine-IMM, dfd-Carbon Nanotubes: A biocompatible & multifunctional carrier
• Feb 2011- Oct 2012: Karolinska Institutet- KI, Stockholm, Sweden, dsdPhD student, Department of Laboratory Medicine, dfdCarbon Nanotubes: A biocompatible & multifunctional carrier
• Nov 2009-March10: Kungliga Tekniska högskolan- KTH, Stockholm, Sweden, Research assistant, Functional nano materials division, Drug delivery vesicles
• Aug 2007-jul 2009: Kungliga Tekniska högskolan- KTH, Stockholm, Sweden, Master degree of chemical science and engineering
• Aug 2000-feb 2006: American University in Cairo-AUC, Egypt, Cairo, Bachelor of Science; Mechanical engineering, Double specialization Material science and Industrial Science

SELECTED PUBLICATIONS

• "Thermostable luciferase from Luciola cruciate for imaging of carbon nanotubes and carbon nanotubes carrying doxorubicin using in vivo imaging system." Nano letters (2013).
• "Mechanisms of carbon nanotube-induced toxicity: Focus on pulmonary inflammation." Advanced Drug delivery (2013).
• "Solid formulation of cell-penetrating peptide nanocomplexes with siRNA and their stability in simulated gastric conditions." Journal of Controlled Release (2012).
• "Optical properties of thin films of zinc oxide quantum dots and polydimethylsiloxane: UV-blocking and the effect of cross-linking." Journal of Colloid Interface (2012).

Giulio Fracasso

Verona University, Dept. of Pathology and Diagnostics, Immunology Section, Verona Italy
E-mail: giulio.fracasso@univr.it
Phone:+39-045-8126457-6449

Birthdate: 30th September 1965
Birthplace Verona, Italy

EDUCATION

• 1979-1984: Baccalaureate, Liceo Scientifico Fracastoro, Verona Italy, Scientific Education
• 1985-1992: Master, Pharmaceutical Chemistry, University of Padua
• 1992-1996: Specialization, Biochemistry and Clinical Chemistry, University of Verona
• 1999-2003: PhD School, Biotechnology Applied to Biomedical Sciences, University of Verona

POSITIONS AND HONORS

• 1993-1995: Lecturer of Pharmacology, Ospedale Civile Maggiore, Borgo Trento (Verona), Regional School for trained nurses, Italy
• 1994-1995: Banca Popolare of Verona Research Fellow, Dept. of Pathology, Univ. of Verona, Italy
• 1995-2000: Lecturer of Immunology, School for biomedical laboratory engineer, Faculty of Medicine,Univ. of Verona, Italy
• 1996-1998: AIRC Research Fellow, Italian Association for Cancer Research (AIRC), Dept. of Pathology, Univ. of Verona, Italy
• 1999-2000: Research contract, One year research contract, Dept. of Pathology, Univ. of Verona, Italy
• 2000-2003: PhD Fellow, Dept. Pathology, University of Verona, Italy
• 2004-present: Head degree Technician/Technologist, Dept. of Pathology, University of Verona, Italy

RESEARCH INTEREST

Cancer Immunotherapy, Immuno-Targeting, Antibody Engineering, Nanomedicine, Prostate Cancer.

PEER-REVIEW

• Journal of Biomedical Nanotechnology
• Nanoscale

PUBLICATIONS (last 3 years)

1. Lütje S et al. Dual-Modality Image-Guided Surgery of Prostate Cancer with a Radiolabeled Fluorescent Anti-PSMA Monoclonal Antibody. J Nucl Med. 2014.PMID: 24700882.
2. Amendola V et al. Magneto-Plasmonic Au-Fe Alloy Nanoparticles Designed for Multimodal SERS-MRI-CT Imaging. Small. 2014 Mar 11. doi: 10.1002/smll.201303372.
3. Schmidt S et al. Discriminatory Role of Detergent-Resistant Membranes in the Dimerization and Endocytosis of Prostate-Specific Membrane Antigen. PLoS One. 2013 Jun19;8(6):e66193.
4. Selvestrel F et al. Targeted delivery of photosensitizers: efficacy and selectivity issues revealed by multifunctional ORMOSIL nano - ovectors in cellular systems. Nanoscale. 2013 Jul 7;5(13):6106-16.
5. Frigerio B et al. Single-chain fragment against prostate specific membrane antigen as a tool to build theranostic reagents for prostate cancer. Eur J Cancer. 2013 Jun;49(9):2223-32.
6. Schmidt S et al. Cloning and characterization of canine prostate-specific membrane antigen. Prostate. 2013 May;73(6):642-50.
7. Meneghetti M et al. Plasmonic nanostructures for SERRS multiplexed identification of tumor-associated antigens. Small. 2012 Dec 21;8(24):3733-8.

Doris Gabriel

Apidel SA
14 Chemindes Aulx, 1228 Plan les Ouates
Phone:+41795997554
e-mail:doris00gabriel@gmail.com

AREAS OF EXPERTISE

Pharmacist with in depth experience in the development of drug delivery systems, controlled / bio-responsive drug release and biomaterials

EDUCATION

• Department of Chemical Engineering, Massachusetts Institute of Technology and Harvard Medical School, Children's Hospital Boston, USA, 2010 - 2012: Post-doctoral researcher in drug delivery, Advisor: Prof. Daniel S. Kohane
• Department of Life Sciences, Federal Institute of Technology Lausanne, Switzerland 2009: Post-doctoral research fellow in Biophotonics, Advisor: Prof. Hubert van den Bergh
• Department of Pharmaceutics and Biopharmaceutics, University of Geneva, Switzerland 2004-2008: PhD studies, Thesis: Protease-sensitive polymeric photosensitizer prodrugs Advisors: Prof. Norbert Lange, Prof. Robert Gurny
• Department of Pharmaceutical Sciences, University of Basel, Switzerland 2002: Master degree in Pharmaceutical Sciences
• Department of Chemistry and Biochemistry, University of Bern, Switzerland 1997-2000: Undergraduate studies in Pharmaceutical Sciences

PROFESSIONAL EXPERIENCE

• Apidel SA, Geneva Switzerland
2012 – now: R&D laboratory head, responsible for the set-up and supervision of Apidel's laboratory facilities, as well as managing key research projects and delivering research results for partners
• Seetal Apotheke Seon, Switzerland

2003: Community pharmacist responsible for drug dispensing, prescription verification, formulation work and team management

PROFESSIONAL AFFILIATIONS

Member of the Swiss Pharmacist Association (Pharma Suisse), Member of the Swiss Industrial Pharmacists association (GSIA), Member of the Controlled release society (CRS)

FELLOWSHIPS AND AWARDS

• 2009: Swiss Pharmaceutical Sciences Society (SPhSS): best poster award, Bern 2009
• 2010: Swiss Pharmaceutical Sciences Society (SPhSS): best publication award, Geneva 2010
• 2010: Swiss National Science Foundation: Two-year post-doctoral fellowship for young researchers

Eduard Gatin

Eduard Gatin, 1980-1985 Physicist Education, research in polymer and materials science, dental materials University of Bucharest, Faculty of Physics.1990 started as Assist Professor at Faculty of Physics, University of Bucharest.1994- 2000 Doctor in Biology & Physiology (PhD degree). Area of interest, polymer membranes for blood filtration. I continued with research in material science - polymers, advanced nano materials, ceramics, metal alloys, corrosion, dental materials and tissue regeneration. I was integrated for post graduated studies regarding this field, University of Bucharest, Faculty of Physics. Beginning with 2008 I am dedicated to material science related to Medical Field (dentistry, as: dental restoration materials, corrosion, dental enamel quality, tissue regeneration – dentine). From 2010 – present, Lecturer – Biophysics Department University of Medicine "Carol Davila", Faculty of Dentistry. Teaching classes:Seminars, classes and Biophysics Laboratories, Dental Materials Lab. Research activity: Materials structure, physical / chemical properties, dental enamel, bacteria activity, polymer resin composites, dental ceramics, metal alloys and corrosion studies. Techniques: RAMAN spectroscopy (improved by SERS), SEM, EDX. Between 2010 – 2013 I was postdoctoral student in EU Program PostDoc (to improve research work, EU finance support) and I succeeded to have my own MiniRaman equipment. In 2013 I succeeded to propose a method for quality dental enamel evaluation. Is pending for patent registration.

Agostina Francesca Grillone

Phone: +39 3889261429
e-mail: a.grillone@sssup.it
Agostina.Grillone@iit.it
Place of birth: Catanzaro
Date of birth: November 23th 1986
Nationality: Italian

EDUCATION

• November the 4th 2013: First year of Doctor of Philosophy (Ph.D.) in BioRobotics, Sant'Anna School of Advanced Studies of Pisa (Italy).
Title of the thesis: "Smart nanomaterials to overcome brain tumor resistance''.
Italian Institute of Technology, Center for Micro-BioRobotics @ SSSA (Viale Rinaldo Piaggio, 34 - 56025 Pontedera (Pisa), Italy).
Advisor: Dr. Gianni Ciofani, Tutor: Prof. Paolo Dario
• May the 3th 2013: Master's Degree in Cellular and Molecular Biology LM-6 (110/110).
Title of the thesis: "Development of a new method to assess the activity of potential inhibitors of the p53- HDM2/4 interaction".
Department of Clinical and Experimental Medicine, Unit of Experimental Biology and Genetics, Pisa, Italy. Supervisor: Dr Leonardo Rossi (leoros@biomed.unipi.it) and Dr Barbara Costa. Level of international classification: ISCED 5
• March the 8th 2010: Bachelor's degree in Biological Science (109/110).
Title of the thesis: "Sequencing of the gene SMC1A in patients with Cornelia de Lange Syndrome". Institute of Biomedical Technologies CNR-ITB, Pisa, Italy. Supervisor: Dr Antonio Musio

PUBLICATIONS

An antibody-free strategy for screening putative HDM2 inhibitors using crude bacterial lysates expressing GST-HDM2 recombinant protein. Costa B, Grillone AF, Salvetti A, Rocchiccioli S, Iacopetti P, Daniele S, Da Pozzo E, Campiglia P, Novellino E, Martini C, Rossi L. Drug Test Anal. 2013 Jun 4. doi: 10.1002/dta.1492.

Ana Filipa Guedes

My name is Ana Filipa Guedes, I was born on 26-12-1986 (27 years old) in Vila Pouca de Aguiar, Portugal. I graduated in Genetics and Biotechnology in 2008, at the University of Trás-os-Montes and Alto Douro (UTAD). My final research project was developed in the Portuguese Institute of Oncology (IPO-Porto), with the title "Study of the IGF1R polymorphism +3174 G> A, the risk for Prostate Cancer", with the supervision of Prof. Rui Medeiros. This work resulted in a poster communication and on the submission of a manuscript [1, 2].
I took my Master Degree in Biotechnology for Health Sciences from the same University, with the final classification of 18 out of 20. During my graduation, I presented one seminar [3]. The research project I developed for my Master thesis, entitled "Diagnostic and prognostic markers in gliomas" was carried out at the Centre for Neuroscience and Cell Biology (CNC), at the University of Coimbra, in collaboration with the Hospitals of the same University (HUC), supervised by Dr. Rosário Almeida. I had the opportunity to present that work as a poster communication [4].
Equally enriching for my scientific formation were some voluntary trainings that I performed in the Department of Cytogenetics, at the Hospital Centre of Trás-os-Montes and Alto Douro (Vila Real) and at the Department of Human Reproduction of the University Hospitals of Coimbra. I have also collaborated in the teaching of practical classes of Molecular Genetics at UTAD. I also worked for 3 months in a spin-off, 2CTech (Aveiro, Portugal), that develops male infertility tests and differential diagnosis of dementias.
In 2012, I moved to Instituto de Medicina Molecular, in Lisbon, working under the supervision of Prof. Nuno C. Santos. In 2013, I started my PhD under his supervision, with the project "Molecular characterization of the fibrinogen-erythrocyte interaction as a cardiovascular risk determinant" (Portuguese National funding agency fellowship SFRH/BD/84414/2012). Until now, I presented our work as poster communications in different National and International meetings [REFS], we submitted a paper [REF] and I will soon present an oral communication in an International workshop [5-10].
During my scientific experience, I had opportunity to do several experimental courses, such as on Atomic Force Microscopy (Oporto, Portugal, March 25-28, 2013), Nanomedicine (Lisbon, Portugal, February 18-20, 2013), AFM in Single Cell Adhesion and Mechanics (Berlin, Germany, January 24-25, 2013), Flow Cytometry (Lisbon, Portugal, December 10-13, 2012) and Laboratory Animals Research (Lisbon, Portugal, March-June, 2012).

PUBLICATIONS

[1] Guedes, A.F. et al., Primeiras Jornadas Nacionais de Genética e Biotecnologia, Vila Real, November 21-23, 2008 (poster presentation).
[2] Ribeiro, R. et al. 2014. manuscript submitted to Clin. Cancer Res.
[3] Guedes, A. F., Vila Real, May 25, 2009 (oral presentation).
[4] Guedes, AF, et al., European Association for Cancer Research, Oporto, October, 2012 (poster presentation).
[5] Carvalho, F. A*, Guedes, A. F* et al. 2014. manuscript submitted to ACS Nano.[6] Carvalho, F.A., et al., 23rd International Fibrinogen Workshop, Marseille, July 9-11, 2014 (accepted for oral presentation).

[7] Carvalho, F.A., et al., XXXV Congresso Português de Cardiologia. Albufeira, Portugal, April 27-29, 2014 (accepted for poster presentation).
[8] Malho, IF, et al., iMed Conference 5.0 – Innovating Medicine, Lisbon, Portugal, October 11-13, 2013.
[9] Guedes, A.F., et al., VII PhD Meeting CAML Lisbon, Portugal, December 5-6, 2013.
[10] Guedes, A.F, et al., EBSA 2013 – 9th European Biophysics Congress, Lisbon, Portugal, July 13-17, 2013 (poster presentation). Abstract published on (2013) Eur. Biophys. J.,42, S111.

Robert Hennig

Dipl.-Pharmacist
Universitaetstrasse 31, 93053 Regensburg, D
E-Mail: Robert.Hennig@ur.de
Telephone: +49(0)941-9431731

Robert was born in Halle/Saale in 1986 and studied Pharmacy at the University of Leipzig, from where he graduated in 2009. After he finished his Diploma on "Maleic anhydride-functionalized copolymers" in 2010, he started his Ph.D. course in 2011 at the Department of Pharmaceutical Technology in Regensburg. Since then he is working on nanoparticles for ocular applications with the focus on finding new therapeutic alternatives for highly prevalent proliferative diseases like age-related macular degeneration and diabetic retinopathy. Furthermore, his work includes the investigation of multivalent nanoparticle-receptor interactions to better understand how nanoparticles recognize their target cells.

PUBLICATIONS

• Pollinger, K.; **Hennig, R.**; Bauer, S.; Breunig, M.; Tessmar, J.; Buschauer, A.; Witzgall, R.; Goepferich, A. Biodistribution of Quantum Dots in the Kidney After Intravenous Injection. J. Nanosci. Nanotech. 2014, 14 (5), 3313–3319.
• Loth, T.; **Hennig, R.**; Kascholke, C.; Hötzel, R.; Hacker, M. C. Reactive and stimuli-responsive maleic anhydride containing macromers – multi-functional cross-linkers and building blocks for hydrogel fabrication. Reactive and Functional Polymers 2013, 73 (11), 1480–1492.
• Pollinger, K.; **Hennig, R.**; Ohlmann, A.; Fuchshofer, R.; Wenzel, R.; Breunig, M.; Tessmar, J.; Tamm, E. R.; Goepferich, A.: Ligand-functionalized nanoparticles target endothelial cells in retinal capillaries after systemic application. In: Proc. Natl. Acad. Sci. U.S.A. 2013, 110 (15), 6115–6120.
• Pollinger, K.; **Hennig, R.**; Breunig, M.; Tessmar, J.; Ohlmann, A.; Tamm, E. R.; Witzgall, R.; Goepferich, A.: Kidney podocytes as specific targets for cyclo(RGDfC)-modified nanoparticles. In: Small 2012, 8 (21), 3368–3375.

Cordula Hirsch

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

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

Cristian Iacovita

Oltului, 36, Cluj-Napoca, Romania
Telephone: 004 0743 421 147
E-mails: cristian.iacovita@umfcluj.ro; crissiac@yahoo.com
Sex: M , Birthday: 15/02/1982
Nationality: Romania

PROFESSIONAL EXPERIENCE

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

CURRENT RESEARCH TOPICS

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

Tore Geir Iversen

Tore Geir Iversen is a senior researcher and Project Leader at the Centre for Cancer Biomedicine, The Norwegian Radium Hospital in Oslo, Norway. He received 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 Kirsten Sandvig in 1997, a group that for many years has contributed significantly to our present knowledge about endocytosis and intracellular transport focusing on different protein toxins as tools. In 2006, Iversen turned his focus into studying how nanoparticles are endocytosed and transported in cells. His group was the first to demonstrate that accumulation of nanoparticles within endosomes could induce changes in the normal intracellular transport of the cell. Research interests also include more applied biological studies about nanoparticles and the criteria required for their clinical use in therapy and imaging. Currently, he is co-ordinator of in vitro studies in a national nanomedicine project (RCN-NANO2021): "Biodegradable nanoparticles in cancer diagnosis and therapy"

SELECTED PUBLICATIONS

• **T Iversen**, T.G., N.Frerker, and K.Sandvig. 2012. Uptake of ricinB-quantum dot nanoparticles by a macropinocytosis-like mechanism. Journal of Nanobiotechnology 10:33
• Tekle, C., B.van Deurs, K.Sandvig, and **T.G.Iversen**. 2008. Cellular

Trafficking of Quantum Dot-Ligand Bioconjugates and Their Induction of Changes in Normal Routing of Unconjugated Ligands. Nano Letters 8:1858-1865.
• **Iversen, T.G**., T.Skotland, and K.Sandvig. 2011. Endocytosis and intracellular transport of nanoparticles: Present knowledge and need for future studies. Nano Today 6:176-185.
• Skotland, T., **T.G.Iversen**, and K.Sandvig. 2010. New metal-based nanoparticles for intravenous use: requirements for clinical success with focus on medical imaging. Nanomedicine 6:730-737.

Magdalena Janczewska

Laboratory of Biomedical Engineering, Warsaw University of Technology, Warsaw, Poland; m.janczewska@ichip.pw.edu.pl

In 2012 graduated with honors from Warsaw University of Technology, where majored in Biotechnology with specialization in Industrial Biotechnology. During studies did volunteer internship in Department of Histology and Embryology on Medical University of Warsaw. In 2013 began doctoral studies in the Laboratory of Biomedical Engineering under the supervision of professor Tomasz Ciach. Currently deals with synthesis and characteristics of biodegradable nanoparticles based on natural polymers dedicated to targeted chemotherapy. In 2013 became a member of spin-off NanoVelos sp. z o. o. where is responsible for chemical synthesis of nanoparticles, drug entrapment and research on nanoparticles internalization and transport mechanisms.
In 2013 attended an annual Summer School of Stem Cells Culture organized by Biology Department of Warsaw University and participated in four conferences, both local and international including International Conference on Nanotheranostics ICoN held in Larnaca, Cyprus. Took part also in 1st Warsaw – Cambridge Young Scientist Meeting, Breaking Boundries in Chemistry in Warsaw and 3rd Summer Symposium on Nanomaterials and their Application to Biology and Medicine held in Medical University in Poznan.

Christina Janko

Dr. rer. nat., Section of Experimental Oncology and Nanomedicine (SEON)
Department of Otorhinolaryngology, Head and Neck Surgery, University Hospital Erlangen, Glückstraße 10a, 91054 Erlangen
Tel: +49 9131/8543944
Fax: +49 9131/8534828
E-Mail: christina.janko@uk-erlangen.de

ACADEMIC BACKGROUND

• Since 01/2013: Postdoctoral Research Fellow at the Nanotoxicology Unit at the Section of Experimental Oncology and Nanomedicine, Department of Otorhinolaryngology, University Hospital Erlangen.
• 03/2012- 12/2012: Postdoctoral Research Fellow in the Department of Internal Medicine 3, Immunology and Rheumatology, University Hospital Erlangen.
• 11/2007-02/2012: PhD thesis in the Department of Internal Medicine 3, Immunology and Rheumatology, University Hospital Erlangen.
Title: "The impact of CRP for the clearance of dying cells"
• 10/2006-10/2007: Diploma thesis in the Department of Internal Medicine 3, Immunology and Rheumatology, University Hospital Erlangen.
Title: "Cell death and Immunogenicity of cells treated with high hydrostatic pressure"
• 03/2006 – 08/2006: Internship at Roche Diagnostics GmbH in the area of Screening/Biochemistry (Penzberg): "Analysis of therapeutical components for oncology"
• 10/2002- 10/2007: Studies of Biology, Friedrich Alexander University of Erlangen-Nuremberg

Selvaraj Kunjiappan

Selvaraj K obtained his Bachelor degree in Pharmacy from Tamilnadu Dr.MGR Medical University, Chennai and Master degree in Industrial Biotechnology from Bharath Engineering College, Chennai, India.
Currently a PhD student at Department of Chemical and Bioprocess Engineering, Jadavpur University, Kolkata, India. His area of research is isolation and characterization of flavonoids from natural sources, includingformulation and fabrication of gold and platinum nanomaterials and their hepatoprotective and antioxidant activities of primary hepatocytes.

Dalit Landesman

Dr. Dalit Landesman Milo is a Research associate in Prof' Dan Peer's Laboratory of Nanomedicine in the department of Cell research and Immunology at Tel-Aviv University. She is a project manager taking part in a national consortium in the field of utilizing and developing robust targeted nano delivery systems of therapeutic siRNA for cancer therapy.
Dalit has an extensive background in the industrial biotech field. She established BioArt Ltd, a private biotechnology company that focused on developing and manufacturing of innovative biotechnology based on therapeutic recombinant proteins for preclinical and clinical trials. Prior to that, she was a senior scientist in the bioefficacy team in Compugen Ltd (CGEN). She holds a Ph.D. degree in human genetics from Sackler Medical School, M.Sc degree in Biochemistry and B.Sc degree from Tel-Aviv University.

Miguel A. Lázaro

Sagetis-Biotech, Via Augusta 394,
08017 Barcelona (Spain).
Tel: +34 933097169
E-mail: malazaro@sagetis-biotech.com

PROFESSIONAL EXPERIENCE

• 2012-present: Sagetis-Biotech (Barcelona-SPAIN). Research Scientist at a biotechnology company. Working on drug delivery.
• 2004-2011: Barcelona Science Park (Barcelona-SPAIN). Research Scientist at Unitat mixta PCB-LILLY. Working on drug discovery for an external collaboration of Eli Lilly and Company.
• 2004: Villapharma Research (Murcia-SPAIN). Team leader. Working on drug discovery for an external collaboration of Janssen.
• 1999-2003: Roviall Química S. L. (Murcia-SPAIN). Laboratory manager. Working on syntheses of libraries for an external collaboration of Janssen.

EDUCATION

• 2008: 17th School on Medicinal Chemistry. University of Leiden (Netherlands).
• 1998: Fachbereich Chemie der Philipps-Universität Marburg (Germany). Postdoc of one year at group of Prof. Dr. R. W. Hoffmann with a fellowship of EU. Synthesis of amino acid components for combinatorial approaches to new molecular catalyst.
• 1996: Institut für Organische Chemie und Biochemie der Universität Bonn (Germany) at group of Prof. Dr. E. Breitmaier with a fellowship. Diasteroselective Ireland-Claisen rearrangement.
• 1993-1997: Ph.D. in Chemistry at University of Valencia (Spain) with a fellowship. Synthesis of Sexual Pheromone of Chilo Suppres-

salis and Pheromone Forerunner for a Controlled Emission.
• 1992: University of Valencia (Spain). After bachelor laboratory work for one year.
• 1986-1991: B.S. in Organic Chemistry at University of Valencia (Spain).

Kumaran Letchamanan

PhD candidate
Date of Birth: 29th May 1986
Nationality: Malaysian
E-Mail: kumaran05@nus.edu.sg
kumaran0529@gmail.com

KEY QUALIFICATIONS

• I am a disciplined and responsible person and always able to deliver the given tasks well
• I am independent, fast learner and able to work with minimum supervision
• I am a good team player with excellent interpersonal and communication skills
• Possessed a positive attitude, professional work ethics, and high-level of integrity
• Confidence, eager to learn new knowledge (personal development) and skills, motivation and ability to adapt to new cultures.

EDUCATION

• National University of Singapore (2011-present) ; PhD Student
University Malaysia Pahang (2006-2010); B.(Hons) Chemical Engineering

PUBLICATIONS AND AWARDS

• 2010: Formulation of New Organic Drag Reduction Agent Using Natural Mucilage Extracted From Aloe Vera
• 2010: Silver Medal, International Invention, Innovation and Technology Exhibition
Title: Formulation of New Drag Reducing Agent Using Natural Mucilage Extracted From Hibiscus Rosa-Sinensis Linn Leaves
• 2009: Gold Medal, Bio Malaysia
Title: Aloe Vera Mucilage as New Flow Improving Agent in Pipelines Carrying Liquids in Turbulent Mode

SYMPOSIUM AND POSTER PRESENTATION

• Inaugural GSK-Singapore Partnership for Green and Sustainable Manufacturing (GSM) Symposium 2011
• Inaugural GSK-Singapore Partnership for Green and Sustainable Manufacturing (GSM) Symposium 2013

Xiaochun Li-Blatter

Xiaochun Li-Blatter has worked as a scientific collaborator at Biozentrum, University of Basel since 1997. Together with Professor Anna Seelig, she has quantitatively evaluated the mechanism of activation and inhibition of ATP binding cassette transports (The breast cancer resistance protein, P-glycoprotein and SAV1866) and contributed to the drug formulation strategies to overcome the multidrug resistance problem. She has published 14 publications. Currently she holds a position at the Biophysics Facility at Biozentrum. She got a diploma of organic chemical engineering at Beijing Institute of Chemical Fibre Technology.

HER WORK MAINLY FOCUSES ON THE FOLLOWING FIELDS:

1. Characterization of biomolecules (e.g. drugs and proteins) and nanoparticles with respect to size, conformation, stability and oligomeric states.
2. Determination of binding constants and the stoichiometry of interactions for nanoparticles such as liposomes, polymers and proteins, with ligands which are generally drugs, substances and small biomolecules. Characterization of the interactions with thermodynamic parameters; understanding the binding process at the molecular level.
3. Developing high throughput screening techniques for inhibitors/substrates of proteins and quantifying their potency.

THE MAIN RESEARCH TOOLS

Analytical ultracentrifugation and complementary use of a fluorescence detection system, zetasizer, static light scattering; isothermal titration calorimetry, differential scanning calorimetry; microscale thermophoresis; surface plasmon resonance; monolayer technique; fluorescence-based thermal shift; fluorescence and circular dichroism spectroscopy.

RECENT PUBLICATIONS

1. The brain entry of HIV-1 protease inhibitors is facilitated when used in combination. Marzolini C, Mueller R, Li-Blatter X, Battegay M, Seelig A. Mol. Pharmaceutics, 2013, 10 (6): 2340–2349
2. P-glycoprotein-ATPase modulation: the molecular mechanisms. Li-Blatter X, Beck A, Seelig A. Biophys J. 2012 Mar 21; 102(6): 1383-93.
3. Exploring the P-glycoprotein binding cavity with polyoxyethylene alkyl ethers. Li-Blatter X, Seelig A. Biophys J. 2010 Dec 1; 99(11): 3589-98.
4. Detergents as intrinsic P-glycoprotein substrates and inhibitors. Li-Blatter X, Nervi P, Seelig A. Biochim Biophys Acta. 2009 Oct; 1788(10): 2335-44.

Philippe Lienard

Pharmaceutical Sciences and Chemical Development
19 rue de la perruche, 78117 Chateaufort, F
Telephone : +33 1 58 93 34 56 (office)
+33 6 74 79 74 77 (cellular)
E-mail: philippe.lienard@sanofi.com
50 years, French nationality, married and three children

JOB DESCRIPTIONS

• Recently: Business Development: Sanofi Research / Vitry, France
Business development of the Sterile Solid Nano-particles for IV (SSNIV), unique manufacturing facility in Europe, for various opportunities with partners external and internal.
• Since May 2012: Early Development: Candidate Selection Manager, Sanofi Research / Vitry, France
Interfacing with research departments, I manage the transition between research and development phases elaborating the CMC strategies needed and promoting the innovation for formulation and drug delivery technologies for the new candidate projects.
• From August 2006 to April 2012: Drug Product Anticipation: Pharmaceutical Science, Sanofi-Aventis Research / Vitry, France.
Within a CMC platform, I was in charge of a unit of several formulators and chemists having for mission to assess the properties of the new drug substances upcoming from research, in constant interactions with other departments such as discovery, analytical and chemical sciences. I put in place innovative modeling approaches to anticipate the physico-chemical behavior and the stability of the active compounds in their formulations.
• From August 2005 to July 2006: Head Project Coordinator: Chemical Development, Sanofi-Aventis Research / Bridgewater (NJ)- USA
I had to coordinate the rapid transition of processes from laboratory to the Pilot Plant ensuring safety and quality reproducibility. I created the necessary documentation and followed up production in a timely manner. I oversaw the overall planning for the local chemical development.
• From November 2003 to July 2005: Director, Chemical Development, Sanofi-Aventis Research / Great Valley (PA) - USA
I was in charge of a unit dedicated to oncology drug substance synthesis development at the Great Valley research center; I focused my group on innovative and a creative chemistry approach to design robust and scaleable processes for drug substance production.

Moreover, I managed the drug substance supply for pre-clinical and clinical trials up to phase III including the validation of the synthesis of drug substance, intended to be filled, taking under account quality and HSE aspects. I handled an important out-contracting budget to supply various DS needs.

• From June 1995 to October 2003: Group Leader,Chemical Development Department, Sanofi~Synthelabo / Porcheville - France
In charge of the pilot plant and the chemical warehouse at the Porcheville site, I managed a team of twenty people composed of engineers, technicians and operators and optimized the team's effectiveness. The pilot plant included different equipment such as reactors, tanks, filters, dryers and clean rooms to perform the synthesis of active drug substances at the stage of clinical research and for production according to standard GMP rules. Additional position occupied as Laboratory Head

• From November 1991 to February 1993: Post Doctoral Research Position: University of Sciences - Geneva - Switzerland - Professor W. Oppolzer's

EDUCATION AND TRAINING

• **Ph D in Organic Chemistry – University Paris XI - Orsay - France**
Laboratory work : Asymmetric synthesis of biologically active compounds related to podophyllotoxin and steganon to discover new potential anticancer .
ICSN/ CNRS - Gif-sur-Yvette (Professor H.-P. Husson) - France

SCIENTIFIC PUBLICATIONS

10 publications available upon reques

Neill James Liptrott

Department of Molecular & Clinical Pharmacology, The University of Liverpool, 70 Pembroke Place, Block H (first floor), Liverpool, L69 3GF Tel: +44 (0) 151 794 5919, e-mail: neill.liptrott@liv.ac.uk
Date of birth: May 30th 1979
Nationality: British

PROFILE

My main interest is in immune cell biology and my current research interests are aimed toward understanding the impact of immune signalling factors, e.g. cytokines, on cell physiology. In turn this research extends to how this affects the metabolism of drugs and toxins in a variety of metabolically active cells. As part of this focus I am investigating the interaction of novel nanomedicines with the cellular physiology of immune cells in order to investigate potential impact on cellular function.
I am also exploring immunogenicity issues relating to renal cells developed from stem cells as part of ongoing research in the department of Cellular and Molecular Physiology (University of Liverpool, UK) in collaboration with researchers there who are developing renal stem cell lines to be utilised in drug safety studies and also to clearly define regulatory mechanisms within said cells. This also incorporates novel nanoparticle incorporation to track these cells in vivo, which requires assessment of nanoparticle immunogenicity.
In addition to this work my current research programme encompasses drug induced kidney injury specifically the mechanisms involved in its development and how the immune system may be involved in physiological differences in renal metabolism.

RESEARCH

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

• 2011-2012: Postdoctoral Research Associate - Department of Molecular and Clinical Pharmacology, University of Liverpool.
Determining the interaction between nanoformulated drug delivery systems and the human immune system.

• 2009-2011: Postdoctoral Research Associate - National Biomedical Research Centre for Microbial Disease, Royal Liverpool and Broadgreen University Hospital Trust, Liverpool. Investigating the mechanisms governing the intracellular pharmacology of HIV antiretrovirals in primary immune cells.

• 2007-2009: Research Associate - National Biomedical Research Centre for Microbial Disease, Royal Liverpool and Broadgreen University Hospital Trust, Liverpool, UK.

EDUCATION

• 2004-2007: Ph.D. - Department of Molecular and Clinical Pharmacology, University of Liverpool. Pharmacological and Immunological Factors that Influence Antiretroviral Drug Therapy.

• 2003-2004: M.Sc. Human Immunity, University of Liverpool, UK.

• 1999-2003: B.Sc. (Hons) Molecular Biology, University of Liverpool, UK.

Iraida Loinaz

Head of Biomaterials Unit of CIDETEC. She obtained her MChem degree in the University of the Basque Country in 1998 and she completed her education with two placements in the University of Bergen (Norway) and in the Regional Technical College Galway (Ireland), where she was a research assistant with Dr. Myles Keogh. She got her PhD in Organic Chemistry in the University of the Basque Country in 2004. Since then she is the Head of the Biomaterials Unit in CIDETEC. She focuses her research in the synthesis of biomaterials, especially polymer nanoparticles, nanogels and hydrogels. She has been part of many private and public funded research projects. She has more than 20 publications in a high impact papers and has been inventor in three patents.

Jasna Lojk

I'm a junior researcher in the fields of nanotechnology and nanomedicine.
I graduated in biology at the Biotechnical faculty (University of Ljubljana, Slovenia) in 2011. During my undergraduate studies, I worked on a research project in arthropod histology at the Department of Biology. I did my graduation thesis in the field of innate immunity at the Department of Infectious Diseases and Immunology at the Faculty of Veterinary Medicine (Utrecht University, The Netherlands). Since 2011, I'm working as a PhD student in the Group for nano- and biotechnological applications at the Faculty of Electrical Engineering (University of Ljubljana, Slovenia). My main research topics are the mechanisms of nanoparticle internalization and cytotoxicity in relation to different nanotechnological application, with some results already published in International Journal of Nanomedicine. We also focus on the applications of electrotransfection, mainly on analysis of electroporation mechanisms and method optimization.

Stefan Lyer

STEFAN LYER studied Biology at the Friedrich-Alexander University Erlangen/ Nürnberg. After finishing his PhD thesis at the German Cancer Research Center (DKFZ)/Ruprecht-Karls-University Heidelberg he stayed as a post doc at the Department of Genome Analysis at the DKFZ for another year. In 2008 he moved back to Erlangen starting a post doc position at the group of Prof. Christoph Alexiou at the ENT-Department of the University Hospital Erlangen, which was renamed Section for Experimental Oncology and Nanomedicine (SEON) in 2009. Here, he focussed on the application of nanoparticles in cancer therapy. Since 2011 he has been assistant group leader of SEON.

Alexander Lyskin

Chemist, M.Sc.
Tel. +972-2-642-38-45
+972-54-592-38-45
e-mail : alexlyskin@gmail.com
Birth date: 1982
Place of birth: USSR

EDUCATION

• 2007-2010: M.Sc. in Chemistry, Faculty of Mathematics & Sciences, The Hebrew University of Jerusalem, Israel
• 2004-2007: B.Sc. in Chemistry, Faculty of Mathematics & Sciences, The Hebrew University of Jerusalem, Israel

PROFESSIONAL EXPERIENCE

• 2011-Present: Lipocure Ltd Pharmaceutical Company, Hadassah Ein Kerem Campus, Jerusalem, Israel
• 2010-2011: Institute of Control and Standards of medicinal products, Ministry of Health, Israel;
Department of Analytical and Inorganic Chemistry, The Hebrew University of Jerusalem, Israel

PROFESSIONAL SKILLS

• Specialty of Analytic and Inorganic Chemistry
• Knowledge of HPLC Methodology
• Computer languages JAVA, C++

SPOKEN LANGUAGES

English, Hebrew, Russian

PRESENTATIONS AT PROFESSIONAL MEETINGS

Alexander Lyskin and Eli Grushka, "Characterization of the Cosmosil Cholester column: Lipophilicity measurement and LSER" , The 13th Annual Meeting of the Israel Analytical Chemistry Society, Tel-Aviv, Israel, January, 2010

Tamás Mészáros

Date of birth: 20.01.1982

EDUCATION

• 2011- : Semmelweis University – Ph.d. School, Theme: Liposome, Immunology
• 2002 – 2008: Eötvös Lóránd University, Faculty of Science, Biologist, Immunologist
• 2000 – 2002: Eötvös Lóránd University, Faculty of Primary and Pre-School Education, Biology and Chemistry

WORK EXPERIENCE

• 2009 - : Nanomedicine Research and Education Center; János Szebeni, György Báthori and Benedek Kálmán
• 2007 - 09: Semmelweis University, 3rd Department of Medicine (Research Laboratory); Lilian Varga – György Füst
• 2006 - 07: National Center for Epidemiology (Microbiological Research Group); Ágnes Gyuris – János Minárovits
• 2005 – 06: Eötvös Lóránd University – Faculty of Science (Department of Immunology); József Prechl – Anna Erdei

PUBLICATION

• 2010 April: „C1-inhibitor autoantibodies in SLE" (**Mészáros T**, Füst G, Farkas H, Jakab L, Temesszentandrási G, Nagy G, Kiss E, Gergely P, Zeher M, Griger Z, Czirják L, Hóbor R, Haris A, Polner K, Varga L.; Lupus. 2010 Apr;19(5):634-8. Epub 2010 Jan 13 -2009 Impact Factor: 2.586)
• 2011 January: „High levels of acute phase proteins and soluble 70 kDa heat shock proteins are independent and additive risk factors for mortality in colorectal cancer." (Judit Kocsis, **Tamás Mészáros**, Balázs Madaras, Éva Katalin Tóth, Peter Gál, Lilian Varga, Zoltán Prohászka, George Füst; Cell Stress Chaperones. 2011 Jan;16(1):49-55. Epub 2010 Aug 22. -2009 Impact Factor: 2.167)
• 2012 June: „Hemocompatibility of liposomes loaded with lipophilic prodrugs of methotrexate and melphalan in the lipid bilayer." (Kuznetsova NR, Sevrin C, Lespineux D, Bovin NV, Vodovozova EL, **Mészáros T**, Szebeni J, Grandfils C. ; J Control Release. 2012 Jun 10;160(2):394-400.)

Magdalena Michalak

ul. Tarnogórska 237, 44-105 Gliwice
Phone: (48) 516 716 396
E-mail: magdalenamichalak@onet.pl
Date of birth: 19 September 1989

EDUCATION

• 2012-04.2014(expected): MSc – Biotechnology (biotechnology of pharmaceutical drugs), Warsaw University of Technology, Department of Chemistry
• 2008-2012: MEng Biotehchnology (specialization: bioinformatics), Silesian University of Technology, Department of Automatic Control, Electronics and Computer Science

WORK EXPERIENCE

• 07.2013-01.2014: NanoVelos Sp. z o.o. Warsaw, Poland, Member of scientific group, responsible for cell culture, testing cytotoxicity of dextran nanoparticles and anticancer drugs.
• 08.2012-01.2014: Warsaw University of Technology, Taking part of the research project looking into cytotoxicity of drug carriers..
• 09-10.2011: Silesian Centre of Heart Diseases, Microbiology Lab., Internship. Learning lab diagnostics skills.
• 08-09.2011: Maria Skłodowska-Curie Oncological Institue, Internship. Working with cell culture (glioma steam cells).
• 03-07.2011: Maria Skłodowska-Curie Oncological Institue, Voluntary work. Working in the cell culture lab, performing tests experiments on the tumor cells.

ACHIEVEMENTS

• 12.14.2013: Award on Conference - Influence of Young Scientists For Achievement of Polish Science Against cancer - NANOTECHNOLOGY IN MODERN DRUG ADMINISTRATION SYSTEMS
• 19.04.2013: Second Prize at the European Young Engineers Conference 2013 "Targeting tumor: Delivery of doxorubicin encapsulated in dextran nanoparticles."

PUBLICATIONS

19.04.2013: "Targeting tumor: Delivery of doxorubicin encapsulated in dextran nanoparticles. "Conference materials - European Young Engineers Conference 2013, Authors: Magdalena Michalak, Iga Wasiak

Gergely Milosevits

Dr. med.
166., 2310 Szigetszentmiklos (Hungary)
Mobile: +36308425722
E-mail: ikkuma@gmail.com
Date of birth: 01/01/1988

WORK

• 2012 → : Resident clinical doctor, II. Department of Pediatrics (Semmelweis University, Budapest), Tűzoltó u. 7-9., 1094 Budapest (Hungary)
• 2012 → : Research fellow, Nanomedicine Research and Education Center, Budapest; Institute of Pathophysiology (Semmelweis University, Budapest), 26 Ullői ut, 1085 Budapest (Hungary)
• 2012: Diploma thesis ("Flow cytometric analysis of the physicochemical characteristics and stability of nanopharmaceutical carriers and agents"), Nanomedicine Research and Education Center, Budapest; Institute of Pathophysiology (Semmelweis University, Budapest), 26 Ullői ut, 1085 Budapest (Hungary)

• 2011 Dec 20.: Article published in Chemisty and physics of lipids. "Flow cytometric analysis of supravesicular structures in doxorubicin-containing pegylated liposomes." Milosevits G, Rozsnyay Z, Kozma GT, Milosevits J, Tömöry G, Robotka H, Rosivall L, Szebeni J. Nanomedicine Research and Education Center, Semmelweis University and Bay Zoltán Ltd., Budapest, Hungary.
• 10/2009 → 2012: Student researcher, FACS laboratory, Nanomedicine Research and Education Center, Budapest; Institute of Pathophysiology (Semmelweis University, Budapest)
26 Ullői ut, 1085 Budapest (Hungary)

EDUCATION AND TRAINING

2006 - 2012: Semmelweis University (medical university), Budapest (Hungary)

CERTIFICATIONS

• Semmelweis University Diploma (medical doctor)
• ECDL (European Computer Driving Licence)
• State Accredited Language Examination Certificate in german language (intermediate level)
• State Accredited Language Examination Certificate in english language (advanced level)
• President's Award For Educational Excellence (2002, USA)

Teba Mohamed

School of healthcare Science, Manchester Metropolitan University, Oxford Road, Manchester M1 5GD
07928004019; teyba007@hotmail.co.uk

PERSONAL PROFILE

I am currently a PhD student at Manchester Metropolitan University; I am an individual who is capable of achieving personal objectives as demonstrated by successful completion of my MSc degree. I possess excellent communication skills and an ability to relate to all levels of social contacts. I have numerous transferrable skills which I have gained throughout my academic education. I am extremely ambitious, goal oriented with a clear and concise vision for future objectives. Being a PhD student has taught me to remain calm, efficient and enabled me to find solutions to problems. Communication of my research has allowed me to be part of a successful group, and to participate in developing and introducing new ideas and understandings to the scientific community.

EDUCATION

• 2009-Current: PhD student in Health Science, Manchester Metropolitan University, Title: An investigation into the uptake of gold nanoparticles by isolated cells and whole vessels and their influence on function.
• 2007-2009: MSc in Biomedical Science, Manchester Metropolitan University, Project title: Effect of elevated pressure on function of Small resistance arteries
• 2004-2007: BSc (Hons) in Physiology with studies in Pharmacology, Manchester Metropolitan University (Obtained 2.2).

KEY SKILLS

• Excellent communication skills, both written and verbal.
• Able to collect, analyze and interpret scientific data.
• Able to critically review the literature.
• Present scientific findings through written reports, publications and poster presentations.
• Operate procedures for laboratory machinery and equipment, including scanning electron microscope, UV-Vis spectroscopy, fluorescence spectrophotometer, thermo gravimetric analysis, dynamic light scattering, zeta potential measurements; organ-bath system, Vortex and centrifuge.
• Synthesis various materialised nanoparticles (including silica and gold).
• Train undergraduates and postgraduates on uses of specialised equipment including the organ-bath system and the Malzern Zeta sizer (dynamic light scattering).

CONFERENCES

• **Mohamed T**, Whitehead D and Azzawi M 'The influence of PVP and mPEG- Coated Gold Nanoparticles on vascular function', International Cell Tracking Symposium on the use of nanoparticles as imaging agents for cell tracking, Liverpool University, 2013.
• **Mohamed T**, Whitehead D and Azzawi M 'The Effect of gold nanoparticle stabilisers on aortic vessel function', Alternative Muscle Club 'AMC', Oxford University, 2011.
• **Mohamed T**, Azzawi M, Whitehead D, Jones C, Akbar N,Azhar M 'Attenuation of vasodilator responses, induced by nanoparticle uptake, in rat aortic rings', The physiological society, University of Manchester, 2010.
• Whitehead D, Azzawi M, Farooq A and **Mohamed T**. 'Novel nanoparticle systems for use in bioapplications tested using novel physiological systems', microfluidics and nanotech in Drug Discovery conference, Astrazeneca, 2010.

PUBLICATION

Akbar, N., **Mohamed T**., Whitehead D, Azzawi, M. 'Biocompatibility of amorphous silica nanoparticles: size and charge effect on vascular function, in vitro', Biotechnology and Applied Biochemistry, 2011, 58(5), 353–362.

Kristin Mohr

Dr. Kristin Mohr studied biomedical chemistry at the University of Mainz. In the course of her studies, she traveled abroad to the Institute for Materials Science and Technology of Polymers (MTP) at the University of Twente (Netherlands) where she studied enzymatic reactions confined in polymeric vesicles under Prof. G.J. Vancso. Having completed her Diplom in 2010, Kristin Mohr continued her academic studies at the Institute of Physical Chemistry at the University of Mainz where she pursued her PhD in chemistry under the advisory of Prof. M. Schmidt. She completed her thesis in 2013 which focused on the aggregation and interaction behavior of nanoparticles in blood serum. As a PhD student, Kristin was trained in various physical and biophysical characterization techniques with a special focus on dynamic and static light scattering. During her thesis, Kristin received a scholarship from the academy of literature and science Mainz to work on general protein fractionation and characterization at the Alpert Medical School of Brown University (RI, USA). Since completing her PhD she has spent time as a visiting scientist at the University of Massachusetts, Amherst USA under Prof. M. Muthukumar. As of July 2013, Dr. Mohr became the head of the polymer analytics laboratory under Prof. K. Landfester at the Max Planck Institute for Polymer Research in Mainz. In addition to the advancement of general polymer characterization, her current research focuses on the defined analysis of protein-polymer interactions.

Doris Night Ngongo

Doris Night is currently a PhD registered student of International Business at Jomo Kenyatta University of Agriculture & Technology (JKUAT), currently working at the Kenya Medical Research Institute (KEMRI) in the Marketing Department.

Doris was the winner of the 2007/2008 Bashorun Abiola Prize in Marketing, a prize founded in 1987 by Chief Bashorun Abiola awarded to the student from Africa with the best overall academic performance on the MSc in International Marketing at the University of Strathclyde - Glasgow. Doris was awarded the Top Student Gold Trophy Award in Business Category in 2006 from Edith Cowan University-Australia during the 2006 Graduation Ceremony. Doris was also awarded the prestigious President's Award by H.E. the President of the Republic of Kenya for participating in the PA 2003 Expedition Challenge.

Doris holds Degrees and a Diploma in the following areas; MSc in International Marketing (Customer Management) from University of Strathclyde, Glasgow, United Kingdom, a Bachelor of Business with double Majors in Marketing & Management from Edith Cowan University, Perth, Australia and a Diploma in Marketing & Public Relations from THAMES International (Informatics Group).
Her key competencies are in Marketing, International Marketing, Customer Management, Public Relations and Communications, Strategic Partnerships, Collaborations & Networking, Strategic Planning & Management, Strategic Leadership & Management, Fundraising, Conferencing & Events Management, ISO 9001-2008 (QMS), ISO 13485 (Medical Devices), ISO 17043 (Proficiency Testing) Auditing & Assessment and Web Analysis. Her interests include International Business, Diplomacy & International Relations and Forensics.
She has served in KEMRI since 1994 both in Human Resource Department and currently in the Marketing Department. She has been instrumental on brand positioning of all KEMRI Products/ Services to its target markets through local, regional and international conference participation. She was involved in the drafting of the First KEMRI Strategic Master Plan (2005-2015; revised in 2009), the KEMRI Service Charter and development of the 1st draft of the KEMRI HIV/Aids Policy, 1st draft of Revenue Generation Policy and the 1st draft of the Gender Mainstreaming Policy. Doris sat in the ICT/ERP Committee that saw KEMRI launch its ERP system. She coordinated the KEMRI Workplace Wellness Programme that revolutionized how staff are treated at work places. She was part of the team that planned for the successful take-off of the 1st & 2nd KEMRI Annual Scientific & Health (KASH) Conference. She was instrumental in planning of Ministry Of Health exhibitions at the Kenya at 50 commemorations at Kenyatta International Conference Centre (KICC), Nairobi in 2013.

PUBLICATIONS

1. **Ngongo DN**, Kimotho JH, Ochwoto M, Basiye FL, Wandera CM, Kaiguri PM.; Quantification Of Demand Of Bacteriological Culture Media In Kenya- Journal of Pharmaceutical Society of Kenya Vol.2 No. 17; March 2014.
2. **Ngongo DN**.Banking Relationships: a female perspective of customer experiences (An analysis of the role of social networking sites in enhancing the marketing relationships of Standard Chartered Bank of Kenya with their female customers)-Academic Dessertation: University of Strathclyde, 2007/2008.
3. **Ngongo DN**., C. Kimani C. Documentation and Repackaging of HIV/AIDS Information in Kenya: a Case Study of KEMRI's Aids Review Newsletter-AHILA AIBSA Journal, 2006.

EDITOR AND DISTRIBUTOR OF THE FOLLOWING PUBLICATIONS

KEMRI HIV/AIDS Update, KEMRI AIDS Review, Banking on Life Magazine, UNILEVER Vitality, Training Manual, African Health Sciences Journal & Africa Confidential Newsletter.
Abstract reviewer:
KEMRI Annual Scientific and Health Conference (KASH)-2012.

Adkhamjon Paiziev

Institute of Electronics Uzbek Academy of Science, Durmon Yuli str. 33 Academgorodok, Tashkent 100125, Uzbekistan
Phone:/Fax:(+998-71) 262-7940
(+998-71) 262-8767
E-mail: adkhampaiziev@gmail.com

EDUCATION

- 08/07/1994: PhD, Physical Electronics, Institute of Electronics, Uzbekistan
- 15/06/1972: MSc, Theoretical physics, Tashkent State University, Uzbekistan

WORK EXPERIENCE

- 2011-present: Head of Biophotonics Lab.
- 27/03/1978-2011 Senior Scientist, Applied Physics , Institute of Electronics Tashkent, Uzbekistan

25/12/1976-27/03/1978: PhD student, Physical Electronics, Institute of Electronics Tashkent, Uzbekistan

- 02/01/1974-25/12/1976: Research worker, Theoretical Physics, Leningrad, Former USSR
- 30/08/1972-02/01/1974: Engineer, Physics, Institute of Electronics Tashkent, Uzbekistan

FELLOWSHIPS

- 02/03/2009-26/05/2009: Plant genetics and physiology, Hebrew University Jerusalem (Izrael)
- 09/11/2005-10/05/2006: Plant cell biology, Wageningen University (Wageningen, The Netherlands)
- 17/01/2005-28/02/2005: Bioluminescence of plant cells, Catania University (Catania, Italy)
- 02/01/1974-25/12/1976: Theory Auger process in the atomic collisions, Ioffe Physical-Technical Institute (Leningrad, Former USSR)

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

AWARD

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

FIELD OF INTEREST

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

LIST OF PUBLICATIONS

More 70 papers in scientific journals and books.

Irene Pereira de Sousa

Irene Pereira de Sousa was born in Pordenone (Italy) in 1987. She studied Pharmaceutical Chemistry and Technology at the University of Padova. During her studies she spent six months at the University Miguel Hernandez of Elche (Spain) as Erasmus student. She graduate in March 2012 with a thesis entitles: "Pullulan as a new/ old macromolecular drug carrier: cell interactions and pharmacokinetic profiles". The project, supervised by Prof. Paolo Caliceti, dealing with the formulation and evaluation of Pullulan derivatives as tumor drug delivery systems, was further carried out by Irene during a five months postgraduate scholarship at the University of Padova. In September 2013 Irene started her PhD in Pharmaceutical Science at the University of Innsbruck under the supervision of Univ.-Prof. Mag. pharm. Dr. rer. nat. Andreas Bernkop-Schnürch. She is working for the EU-Project Alexander (FP7) aimed to develop mucus permeating nanoparticulate drug delivery systems. Up to now, she had developed a virus-like nanoparticulate system, she is investigating the benefit of nanoparticles PEGylation and she is evaluating the efficacy of mucolytic-nanoparticles for overcoming the mucus barrier.

Sivan Peretz Damari

Bialik 11, Rama-Gan, Israel
Email : siv6786@gmail.com
Cell-phone : +972546763655
Birth Date : 6.7.1986

EDUCATION HISTORY

• M.Sc., Chemical engineering, 2012-present, Ben-Gurion University.
• B.Sc., Chemical engineering, Advanced Materials, 2008-2012, Ben-Gurion University.

RESEARCH AND PROFESSIONAL EXPERIENCE

Ben-Gurion University, Beer-Sheva, Israel
• Master Research - Department of Chemical Engineering, 2012-present
• B.Sc. Research student - Department of Chemical Engineering, 2010-2012
- Research in the area of drug delivery under supervision of Prof. Oren Regev.
- Experienced with laboratory practices, equipment, and advanced analysis techniques (independent operation of TEM, Cryo-TEM and UV-VIS).
Rotem Amfert Negev LTD, Israel
• Lab technician - R&D Department, 2009 - 2010, Experienced with common laboratory practices, equipment, and analysis techniques.

RESEARCH INTERESTS

• Drug delivery-Carbon nanotubes and liposome conjugation.
• Matlab based image analysis of TEM micrographs.

PUBLICATIONS

• **Peretz S**, Regev O. Carbon nanotubes as nanocarriers in medicine. Current Opinion in Colloid & Interface Science 2012; 17:360-368.
• Regev O, Barenholz Y, **Peretz S**, .et al., Can carbon nanotube-liposome conjugates address the issues associated with carbon nanotubes in drug delivery? Future Medicinal Chemistry, 2013. 5(5): p. 503-505.

PRESENTATIONS AT CONFERENCES

• Carbon nanotubes liposomes conjugate for advanced drug delivery system, Innovation and Entrepreneurship in Chemical Engineering, May 2013, Tel Aviv, Israel.
• Liposome Size Distribution and Morphology Analysis by Cryogenic Transmission Electron Microscopy, Russell Berrie Nanotechnology Institute (RBNI) conference, February 2014, Hagoshrimt, Israel.

SCHOLARSHIPS, AWARDS AND STIPENDS

• Student Research award by the Israel Institute of Chemical Engineers, 2013.

Elizabeth Pietrzykowska

Elizabeth is a materials science engineer. She graduated from the Faculty of Materials Science and Engineering at the Warsaw University of Technology in Poland. Now she is studying for a PhD at the Warsaw University of Technology and working at the Laboratory of Nanostructures for Photonic and Nanomedicine at the Institute of High Pressure Physics of the Polish Academy of Sciences. Her work is about composite materials for regenerative implants.
The focus is on forming, under high pressure, nanoceramic powders and the characterisation of the obtained materials (structure, morphology and mechanical properties). The major subject of the Laboratory group is nanopowders' synthesis and their practical applications in medicine.

PUBLICATION

Patent application P-399701, Lojkowski et al, The method of bone implants fabrication and the bone implant.

Wojciech Konstanty Podleski

International, Independent Clinical Investigator, Consultant, with leading expertise in Immunology, Food Allergy, Clinical Immunopharmacology, Hypersensitivity Diseases and Bronchial Asthma.
TEMPORARY CONTACT ADDRESS, where all correspondences and query should be directed:

Route de Fribourg 15 CH-1723 Marly, SWITZERLAND
E-mail: prof.dr.med.w.k.podleski@gmail.com
Phone/FAX : + 41 (0) 26 535 54 39 Pesonal Cell Phone: + 41 (0) 79 583 79 17
Permanent (24/24, 7) secretarial assistance : + 41 (0) 79 869 40 50
Permanent residence address : ul.Kopernika 14/m.11 PL-40-064 Katowice, POLAND
Languages : English, French, Polish, Russian, Mandarin (some)
Hobbies: Opera & Classical Music, Hiking, Skiing, Snorekling

EDUCATION AND TRAINING

• 1965: M.D., Medical School, Wroclaw, Poland
• 1972: Ph.D. in Medicine, University of Lausanne, Switzerland, Medical Faculty, Diploma No 2092
• 1971: Visiting Fellow, Summer School of Immunology, British Society for Immunology, Edinburg, England
• 1972: International Seminar in Allergy and Clinical Immunology, Swiss Society for Allergy and Clinical Immunology, Geneva, Switzerland
• 1974 – 1976: Fellowship, University of Colorado, Denver, Colorado,U.S.A., Medical School and Affiliated Hospitals, clinical training in Allergy, Bronchial Asthma and Clinical Immunology (Head : Prof.Dr.med. Richard Farr)

RESEARCH & CLINICAL SERVICES EXPERIENCES

• 1967-1970: Director, Polish Academy of Sciences, Reference Laboratory for Thyroid Diseases, Wroclaw, Poland
• 1970-1972: Research Fellow, World Health Organization, International Reference Center for Immunoglobulins Lausanne, Switzerland (Head: Prof.Dr. David Rowe -discovered IgD)
• 1978-1992: Director, International Institute for Clinical Immunopharmacology, Allergy and Bronchial Asthma, Denver, Colorado U.S.A.

ACADEMIC APPOINTEMENTS

• 1967-1970: Full Staff Member, Attending Physician (Assistant), The Second Internal Medicine Clinic, Medical School, Wroclaw Poland
• 1973-1974: Assistant Professor of Immunology, State University of New York at Buffalo, Center for Immunology, Buffalo, U.S.A.
• 1976-1978: Assistant Professor of Medicine, Department of Medicine, Medical School, University of Colorado, Denver, U.S.A.

MEDICAL LICENSES TO PRACTICE MEDICINE

• 1967: Lowersilesian District, Wroclaw, Poland , No 1735
• 1974: Educational Council for Foreign Medical Graduates (ECFMG), U.S.A., Certificate No 202 507 0
• 1976: Federal License Examination U.S.A, The Colorado State Board of Medical Examiners, No 20361
• 1976: The Colorado State Board of Examiners in Basic Sciences, Certificate No A 1067
• 1977: US Drug Enforcement Agency (DEA), Permission for Narcotics Prescriprions, DEA No AP 727 9967.

CLINICAL EXPERIENCE

• 1964-1965: Educational Stage, Pulmonology Clinic, Hospital of Saint Anthony, University of Paris, Sorbonne, Paris, France (Head ;Prof.Dr.med. Raoul Kourilsky)
• 1965-1967: Residency Training in : Internal Medicine (Prof. Dr.med.Antoni Falkiewicz), Pediatrics (Prof.Dr.med.Wanda Klinowska), Surgery (Prof.Dr.med.Wiktor Bross), Obstetrics & Gynecology (Prof.Dr.med. Kazimierz Nowosad), Medical School, Wroclaw, Poland
• 1967-1970: Full Staff Member, Attending Physician (Assistant), The Second Internal Medicine Clinic (Head, Prof.Dr.med.Antoni Falkiewicz), also being incorporated to educational duties as volontariate services since 1962, under personal supervision of Prof.

Dr.med. Antoni Falkiewicz, Medical School, Wroclaw, Poland
• 1970-1972: Clinical – Research Assistant, Medical Clinic Hospital NestlŽ, Medical School, University of Lausanne, Switzerland (Head: Prof.Dr.med. Alfredo Vannotti)
• 1973-1974: Courtesy Staff, Chest Clinic, E.Meyer Memorial Hospital, Buffalo, New York, U.S.A.
• 1978-1992: Private Specializing Practice in Allergy, Bronchial Asthma and Clinical Immunology, Full Staff, Attending Physician in Lutheran Medical Center Hospital,
Saint Anthony Hospital, Sweedish Medical Center Hospital, Presbyterian Hospital, Denver, Colorado, U.S.A.
• 1979-2005: United States Federal Government Service, Department of Transportation, Federal Aviation Administration (FAA), Designated Aviation Medical Examiner, exclusive medical examination for licensing of American Civil Airlines Pilots, Certificate No 14804 – 1
• 1970: Member, Immunology Club, World Health Organization, Geneva, Switzerland
• 1974: Fellow , The American Association for Clinical Immunology and Allergy
• 1979: Fellow , The American Academy of Allergy and Immunology
• 1980: Affiliate Member, The American Society of Ophthalmologic and Otorhinolaryngologic Allergy
• 1982: Fellow , The American College of Allergy and Immunology
• 1984: Member , Buffalo Collegium of Immunology, Buffalo N.Y., U.S.A.

CONSULTANT AND PRIVATE INVESTIGATOR IN IMMUNOPHARMACOLOGY FOR INTERNATIONAL PHARMACEUTICAL COMPANIES IN THE ATTEMPTS TO ESTASBLISH NEW MEDICAL COMPOUNDS

• 1968-1970: CIBA, Basel, Switzerland. Creating and confirming the concept of beta adrenergic blocking agents. Proved by introduction to the World Pharmaceutical Market the first beta blocking adrenergic agent, TRASICOR
• 1970-1995: OM Pharma, Geneva-Meyrin, Switzerland. Formulating and successfuly constructing immunological protective responses profile to prevent respiratory infection by oral immunobiotherapeutic („ vaccine „), BRONCHO-VAXOM
• 1969-1970: PFIZER, New York, U.S.A. Clinical assessment of respiratory aerosol for antibiotic inhalation therapy, TERRAMYCIN
• 1982-1992: SANDOZ, Basel, Switzerland. Clinical and experimental efficacy and safety studyin bronchial asthma and hypersensitivity diseases of ZADITEN (KETOTIFEN)
• 1988-1992: MERREL DOW, Cincinati, U.S.A. Clinical trials of the first non-sedating anti-histamine agent, (does not cross brain barrier), TERFENADINE (SELDANE)

PARTICIPATION AND CONSULTATION ON INTERNATIONAL ADVISORY PANELS

• 1992 -now: Consultant,World Health Organization, Geneva, Switzerland
• 2006 – now: Medical Advisory Panel to the United Nation Offices, Geneva, Switzerland
• 2007-now: Medical and Scientific Advisory Board, SANKOM, Swiss International Nutritional Company, DelŽmont, Switzerland, Hong Kong, China

HONORS AND AWARDS

• 1986: Election to the INTERNATIONAL „ WHO's WHO OF INTELLECTUALS „ International Biographical Center, Cambridge, United Kingdom
• 1989: Honorary Medal of Lomonosow, Russian equivalent of Nobel Prize, Russian Academy of Sciences, Moscow, Russia
• 2004: Nominated as GREAT MIND OF 21st CENTURY, American Biographical Institute, Raleigh, North Carolina, U.S.A.
• 2007: Nominated as 2007 MAN OF SCIENCES, American Biographical Institute, Raleigh, North Carolina, U.S.A.

SELECTED , THE MOST IMPORTANT PUBLICATIONS

More than 80 papers in the specialty area of hypersensitivity diseases (allergy) , bronchial asthma , clinical immunopharmacology and clinical immunology are listed as primary reference source articles in the Library of World Health Organization Headquarter, Geneva , Switzerland.

Eugenio Redolfi Riva

98 Ippolito Rosellini rd, Pisa 56124, Italy
Date of Birth: 16/04/1985, Piombino (Li)
Nationality: Italian; Phone: +39 3391089876
e-mail: e.redolfiriva@sssup.it
eugenio.redolfi@iit.it

WORK EXPERIENCE

• November 2011, onwards: PhD Student in "Innovative Technologies of Information and Communication Engineering and Robotics" of Scuola Superiore Sant'Anna, Pisa, Title of PhD Thesis: "Study and development of innovative nanomaterials for targeted diagnostic and therapy"
Publication: Redolfi Riva, E., et al. (2013). PMMA/polysaccharides Nanofilm loaded with adenosine deaminase inhibitor for targeted anti-inflammatory drug delivery. Langmuir, 29(43), 13190-13197.
Name and address of employer: Polo Sant'Anna Valdera, 34 Rinaldo Piaggio st, 56025 Pontedera, (Pi), Italy Advisor: Dott. Virgilio Mattoli (virgilio.mattoli@iit.it) Tutor: Prof.ssa Arianna Menciassi (arianna@sssup.it)

EDUCATION

• Date: 27/09/2011: Master Degree in Biomedical Engineering (110/110) Title of Master Thesis: "Study and development of a Drug Delivery System based on nanofilm and nanoparticles" Name of Organization: Università di Pisa Level of international classification: ISCED 5
• Date: 22/07/2008: Bachelor Degree in Biomedical Engineering (103/110), Title of Bachelor Thesis: " DNA microarray for characterization of mutations occurred in p53 protein and their implication in neoplastic diseases" Name of Organization: Università di Pisa, Level of international classification: ISCED 5

Antonella Rocca

Address via Volturno, 40 56126 Pisa (Italy)
Email antonella.rocca@iit.it
Date of birth 20/03/1987
Nationality Italian

EDUCATION AND TRAINING

• 2015, 04/11 (planned): Ph.D. in Biorobotics, Sant'Anna School of Advanced Studies of Pisa (Italy), Thesis: Smart nanomaterials for the stimulation and the differentiation of mesenchymal stem cells
• 2012, 29/11: Professional habilitation (Biologist)
• 2012, 04/05: Master's Degree in Molecular and cellular biology - Università di Pisa (Pisa, Italy), Thesis: Development of tissue substitutes for the middle ear by in vitro culture of mesenchymal stem cells on biocompatible polymer matrices
• 2009, 14/12: Bachelor's Degree in Molecular biological science - Università di Pisa (Pisa, Italy)
Thesis: Genetic susceptibility in papillary thyroid carcinoma: analysis of the polymorphism rs3764340 in the WWOX gene
• 2006, 21/7: Classic High School Diploma - Liceo Classico F. Fiorentino of Lamezia Terme (Italy)

ISI PUBLICATIONS

• G. Ciofani, S. Del Turco, A. Rocca, G. de Vito, V. Cappello, M. Yamaguchi, X. Li., B. Mazzolai, G. Basta, M. Gemmi, V. Piazza, D. Golberg, V. Mattoli. Cytocompatibility evaluation of gum Arabic-coated ultra-pure boron nitride nanotubes on human cells. Nanomedicine UK, on line, doi 10.2217/nnm.14.25
• Rocca, V. Mattoli, B. Mazzolai, G. Ciofani. Cerium oxide nanoparticles inhibit adipogenesis in rat mesenchymal stem cells: potential therapeutic implications. Pharmaceutical Research (revision required)

CONFERENCE ABSTRACT

• Rocca, G. Ciofani, V. Mattoli, B. Mazzolai. Evaluation of the effects of Barium titanate nanoparticles (BTNPs) on human osteoblast-like cell line SaOS-2 proliferation and differentiation. 3rd Nano Today Conference – Biopolis, Singapore, December 8-11, 2013

• Rocca, G.i Ciofani, V. Mattoli, B. Mazzolai. Assessment of the effects of Cerium oxide nanoparticles on rat mesenchymal stem cells (MSCs) stimulation and differentiation. The 2013 Stem cell event trilogy – London, 4-6 June, 2013

• Rocca, D. D'Alessandro, F. Chiellini, D. Dinucci, D. Puppi, L. Trombi, S. Berrettini, A. Dolfi, S. Moscato. In vitro study on the generation of tympanic membrane substitutes via tissue engineering. 66° Congresso Nazionale S.I.A.I. Società Italiana di Anatomia e Istologia - Pistoia, dal 20 al 23 settembre 2012

• S. Danti, A. Rocca, S. Moscato, S. Barachini, M. Petrini, G.i Ciofani. Piezoelectic nanoparticles for mesenchymal stem cell stimulation. 66° Congresso Nazionale S.I.A.I. Società Italiana di Anatomia e Istologia - Pistoia, dal 20 al 23 settembre 2012

• Mota, S. Danti, D. Dinucci, D. D'Alessandro, L. Trombi, A. Rocca, C.A. van Blitterswijk, F. Chiellini, S. Berrettini, L. Moroni. Development of tissue engineered 3D fiber-deposited scaffolds for ossicular chain repair. 3rd TERMIS World Congress "Tissue Engineering and Regenerative Medicine" – Vienna, September 5-8, 2012

AWARDS

• Winner of Spin Your Thesis! Campaign (2013), promoted by ESA (European Space Agency) presenting the experiment Combination of hypergravity and nanotechnology for the improvement of the differentiation of mesenchymal stem cells into osteoblasts (A. Rocca, A. Marino, V. Rocca, G. Ciofani)

• Winner of Spin Your Thesis! Campaign (2014), promoted by ESA (European Space Agency) presenting the experiment Implementation of hypergravity in mammalian cell transfection procedures (G. Genchi, A. Rocca, A. Grillone, A. Marino, S. Gualtieri, G. Ciofani)

Franziska Rönicke

Franziska Rönicke (born 1986, Freiburg) studied Biology at the Technical University Darmstadt, where she finished her diploma 2011 at the Department for Cell Biology of the (Epi) genome. During this time she focused on the purification and characterization of constitutive heterochromatin in its native 3D structure. She then started to work as a research assistant at the Ruprecht-Karls-Universität Heidelberg, Medical Faculty Mannheim. Within this project, she spent six month at the Technion, Israel, working at the Institute of Biomedical Engineering on an interdisciplinary project that aimed at killing selectively cancer cells by ultrasound. In 2013 she started her PhD at the Karlsruhe Institute of Technology in the laboratory of PD Dr. Ute Schepers in the field of Chemical Biology. Her thesis is focusing on the identification of brain specific transporter molecules via a high throughput screening in adult zebrafish. Previous publications are: A. Hörner, D. Volz, T. Hagendorn, D. Fürniss, L. Greb, F. Rönicke, M. Nieger, U. Schepers, S. Bräse (2014) Switchable Fluorescence by Click Reaction of Azidocarbazole Dye , RSC Advances and Dominik K. Kölmel, Anna Hörner, Franziska Rönicke, Martin Nieger, Ute Schepers, Stefan Bräse (2014) Cell-penetrating peptoids: Introduction of novel cationic side chains, Eur J Med Chem.

Matthias Roesslein

Matthias W. Rösslein (born 30. April 1962) has studied chemistry at the University of Basel from 1981 to 1985. Here, at the Institute of Physical Chemistry, he also got his PhD degree with "Summa cum laude" in 1989. Afterward he spent 22 months as a Postdoc at the University of Chicago. From 1991 to 1995 he was then an assistant professor at the Physical-Chemical Institute of the University Zürich. Since 1996 Matthias Rösslein has a permanent position at Empa, Swiss Laboratories for Materials Science and Technology. In 2006 he was appointed a position as 'Senior Scientist'. Thenceforward he specialized as one of the experts in 'evaluation of measurement uncertainty and metrology'. First the main focus of his work was on applying these principles in analytical chemistry. Also in 2006 he joined the Empa laboratory for "Materials-Biology Interaction" focusing on two major subjects: the standardization of in vitro assays to elucidate the effect of nanoparticles on different cell types as well as next generation sequencing in a very close collaboration with Marc Salit and his group at NIST. Since 2008 he has the status as a foreign guest researcher at NIST with regular visits and participations in a number of joined projects. The most recent projects focus on the standardization of basic measurements techniques used for in vitro assays.

Julia Rogowska-Tylman

Laboratory of Nanostructures for Photonics and Nanomedicine INFORMATION,
Institute of High Pressure Physics,
Polish Academy of Sciences
Sokołowska 29/37, 01-142 Warsaw
phone +48 22 876 04 29
e-mail: rogowskatylmanjulia@gmail.com

EDUCATION/ EXPERIANCE

• 2011-2013: MSc Eng. in Material Science and Engeneering, Warsaw University of Technology, specialization: Biomaterials

• 2008-2011: BSc degree in dental technology, Medical University of Warsaw, First Faculty of Medicine / Dentistry Division

• 2012: Internship student, National Insitute for Materials Science, International Center for Materials Nanoarchitechtonics (MANA), Biomaterials Unit, Biometals Group, Tsukuba, JAPAN

FIELD OF INTEREST

• biomaterials surface modification

• bone substitute materials

• nanoparticles sythesis and characterization (Zeta potential, DLS, BET, cytotoxicity tests)

Agata Patrycja Roguska

Ph.D., Mazovia Center for Surface Analysis, Institute of Physical Chemistry, Polish Academy of Sciences

DEGREES

• 2012 - Ph.D. Faculty of Materials Science and Engineering, Warsaw University of Technology

• 2006 - M.Sc. Faculty of Materials Science and Engineering, Warsaw University of Technology

POSITIONS HELD

• 2012 – since: chief specialist on laboratory equipment, Institute of Physical Chemistry, Polish Academy of Sciences, operator of Microlab 350 instrument (AES and XPS techniques), operator of Nova NanoSEM 450 (SEM, EDX, WDX)

• 2011 – 2012: technical specialist

• 2006 – 2011: chemical technician

RESEARCH INTERNSHIPS, TRAININGS AND FELLOWSHIPS

• 2008 (6 months):National Institute for Materials Science, NIMS Junior Researcher Fellowship under the "WUT-NIMS Joint Graduate School Program", Tsukuba, Japan

• 2008 (1 week): Institute for Surface Science and Corrosion (LKO), Department of Materials Science, Friedrich-Alexander University, Erlangen, Germany, research stay

• 2007 (6 months): National Institute for Materials Science, NIMS

Junior Researcher Fellowship under the "WUT-NIMS Joint Graduate School Program", Tsukuba, Japan
• 2013: The Foundation for Polish Science Fellowship (START Program)

RESEARCH GRANTS

• Research project under the ERA-NET EuroNanoMed initiative co-financed by The National Centre for Research and Development, Faculty of Materials Science and Engineering, WUT, investigator
• Research project DEC-2011/03/N/ST5/04388, National Science Centre, Faculty of Materials Science and Engineering, WUT, head of the project
• Research project DEC-2011/01/B/ST5/06257, National Science Centre, Faculty of Materials Science and Engineering, WUT, investigator
• Research project IP2010 0350 70, Ministry of Science and Higher Education, Faculty of Materials Science and Engineering, WUT, head of the project
• Research project N N507 491 138, Ministry of Science and Higher Education, Institute of Physical Chemistry, Polish Academy of Sciences, investigator
• Research project N N507 355035, Ministry of Science and Higher Education, Faculty of Materials Science and Engineering, WUT, principal investigator

PUBLICATIONS

Co-author of 27 scientific publications, including 18 in the journals of the so-called Philadelphia list, for which the Hirsch index is 8 and the total number of citations is 137 (according to the Web of Science database)

RESEARCH AREAS

surface analysis (AES, XPS, SEM, EDX, WDX), surface modification of titanium for biomedical purpose metal oxide nanotubes characterization and functionalization for SERS applications

Ricardas Rotomskis

Ricardas Rotomskis is a professor at the Vilnius University. MS Vilnius University (1976) Diploma in Physics (Solid state physics), obtained his PhD in Biophysics (time resolved laser spectroscopy of biological objects) at Moscow M.V.Lomonosov State University (1982-1985; Moscow, Russia). 1987-1988 postdoctoral work in the Photosynthesis Department of Huigens Laboratory of Leiden University, Leiden (the Netherlands). He joined Department of Quantum Electronics of Vilnius University, Vilnius Lithuania in 1985, associate professor in 1993, professor in 2000, head of the laboratory of Biomedical Physics at Vilnius University Institute of Oncology from 2004.
He has co-authored over 300 papers and reports dealing with spectroscopy of primary processes in organic semiconductors, photosynthesis, dye molecules, aggregates and biologically active molecules, primary photophysical and photochemical processes and photostability of sensitizers, quantum dots, Au-clusters, photochemotherapy, optical biopsy of cancer in vivo and in vitro, atomic force microscopy of nanostructures. 2 monographs, 8 textbooks were published with co-authors.
Main scientific interests: biophysics, biomedical physics, nanophotonics, biophotonics, nanomedicine, lasers application in life sciences and spectroscopy, photophysics and photochemistry of biologically active molecules in model and biological environment, lasers application in life sciences particularly those used in photosensitized tumour therapy; fluorescence methods in biomedical diagnostics and visualization; biomedical applications of nanotechnologies etc..
Member of European Society for Photobiology (ESP), member of Vilnius University Studies Committees for the education in Biophysics and Medical physics, head of Biophysics studies committee at Physics Faculty, a member of Lithuanian Doctoral studies committees for Biophysics, member of Mirror Group of the European Tecnhnology platform "NanoMedicine" representing Lithuania, a member of the LALS International Advisory Board, an expert of Lithuanian Science Council and Singapore National Medical Research Council.
Dr.Theodore Maiman Award at SPIE's International Biomedical Optics'93 Symposium in Los Angeles (USA). Scientific activity in the field of photosensitized tumour therapy in 1986-2001 was awarded Lithuanian Science Award in 2002.

Giulio Sancini

EDUCATION/TRAINING

• 2008: Specialist in Applied Pharmacology at University of Pavia. 2003, University Degree in Pharmacy, University of Milano, Italy
• 1992-2003: undergraduate Training Student at the Dept. of Neurophysiology of the National Neurological Institute IRCCS "C. Besta" of Milan.
Research Fields: Neurophysiology and Neuroscience

ACADEMIC, TEACHING AND RESEARCH APPOINTMENTS

• 2013-present: Member of Italian Society of Nanotoxicology
• 2012-present: member of the Editorial Board of "World Journal of Respirology
• 2011-present: member of the Italian Society of Physiology
• 2001-present: member of the American Physiological Society
• 2004-present: Assistant Professor of Human Physiology, Dept. Health Sciences, at School of Medicine, University of Milano-Bicocca
• 2012-present Participating Scientist at the PhD Program in Neuroscience
• 2012: INTERNATIONAL PATENT, Re F.; Masserini M.; Sancini G.; Salmona M.; Forloni G. (Univ. Milano Bicocca) " Liposomes containing acid lipids and functionalized with a peptide eliciting an efficient removal of beta-amyloid peptide burden from the brain of animal models (transgenic mice) of Alzheimer disease. Patent Number 20120001; Key Words: liposomes —beta-amyloid —brain
• 2010-present: Participating Scientist at the PhD Program in Nanostructures and Nanotechnology
• 2004-2009: Participating Scientist at the PhD Program in Translational and Molecular Medicine, University of Milano-Bicocca
• 1993-2004: permanent position - Laboratory Technician at the Dept. of Neurophysiology, National Neurological Institute IRCCS "C. Besta".

FUNDING

Dr. Sancini research work has been funded by European FP7 (NAD Project, nanoparticles for diagnosis and therapy of Alzheimer's disease, winner of The Best Project award in the field of Industrial Technologies) and FP6 (BONSAI project). G. Sancini is head of the physiology unit currently engaged in a scientific research project to investigate metalloprotease ADAM10 as a new potential target of htt in the HD brain founded by Italian MIUR agency (PRIN 2012, prot. 20128XWKTX "A molecular and functional study of ADAM10 at the Huntington's Disease synapse"). GS research activities in the field of nanomedicine and inhalation toxicology have been founded by CARIPLO Foundation (MISPAN , TOSCA, OVERNANOTOX projects)
Awards: NAD Project, nanoparticles for diagnosis and therapy of Alzheimer's disease, winner of The Best Project award in the field of Industrial Technologies

SCIENTIFIC INTERESTS

Dr. Sancini has focused his research activity mainly on neurosciences, nanomedicine, nanotoxicology and respiratory physiology. Sancini's main research activity was fully oriented to the study of the functional alterations of the blood-brain barrier (BBB) related to Alzheimer's disease, of the assessment of pulmonary and systemic response to particulate air pollution (PM) exposure in in vivo models and the study of the transmembrane voltage-gated currents and postsynaptic potentials in cultured neurons and in cerebral slices incubated in vitro.

ONGOING RESEARCH PROJECTS

1)Molecular and functional study at the Huntington's Disease synapse
2)Nanoparticles for therapy and diagnosis of CNS disease
3)Development of a nanoparticles-based drug delivery system for lung disease therapy.
4)Disclose the inner relationship between ischemia/vascular damage and Amyloid Precursor Protein (APP) processing in brain microvascular endothelial cells and neurons
5)Health risk assessment for nanoparticles and airborne pollutants

ii) TOTAL NUMBER OF PUBLICATIONS in peer-reviewed journals: 40.

Carmen Seidl

Carmen Seidl (born 1988 in Schwäbisch Gmünd, Germany) studied Chemical Biology at the Karlsruhe Institute of Technology (KIT), where she finished her Master of Science in 2012 at the Institute of Toxicology and Genetics. Her interest in nanoparticulate drug delivery systems was aroused during a six month internship at Abbvie, Ludwigshafen. During this time, she assisted in a project dealing with the nanosized encapsulation of monoclonal antibodies targeting the brain. In January 2013, she became part of the BioInterfaces International Graduate School (BIF-IGS) and started her PhD in the laboratory of Priv.-Doz. Dr. Ute Schepers in close collaboration with the Institutes of Organic and Inorganic Chemistry. Her interdisciplinary project is focussed on the development of different nanoscalic drug delivery systems for multimodal antitumortherapy. Carmen Seidl's publication list includes original articles in Glycobiology (Langhauser M., 2012) and Chemical Communications (Ungelenk J., 2014). To finance her PhD work, she is supported by scholarships of FAZIT and the Landesgraduiertenförderung of Baden-Württemberg.

Lorena Simón Gracia

EDUCATION

• 2011: PhD with the highest distinction (Suma Cum Laude), Title: Nanoconjugates as anti-cancer drug delivery systems: PEG-based dendrimers and PMPC-PDPA polymersomes. Supervisors: Prof. Fernando Albericio and Dr. Miriam Royo. Institution: Combinatorial chemistry for the discovery of new compounds. University of Barcelona, Institute for Research in Biomedicine (IRB), Barcelona Science Park

PROFESSIONAL EXPERIENCE

• Oct 2012-present: Postdoctoral Researcher. Institution: Laboratory of Cancer Biology. University of Tartu, http://cancerbiology.ee
• 2011-2012: Researcher. Institution: Combinatorial chemistry for the discovery of new compounds. University of Barcelona, IRB, Barcelona Science Park
• 2007-2011: PhD student. Institution: Combinatorial chemistry for the discovery of new compounds. University of Barcelona, IRB, Barcelona Science Park
• 2010: Predoctoral stay abroad. Supervisor: Prof. Giuseppe Battaglia, University of Sheffield (UK), Department of Biomedical Science
• 2007-2008: Teacher assistant in chemistry laboratory practices. Institution: University of Barcelona, Department of Organic Chemistry

GRANTS AND FELLOWSHIPS

• 2007-2011: PhD Grant (FI-DGR 2007-Formació de personal investigador) Institution: Catalan Government (Generalitat de Catalunya)
• 2010: PhD Grant for foreign stays (BE-DGR 2010-Beques per a la recerca a l'estranger) Institution: Catalan Government (Generalitat de Catalunya)
• 2000: University scholarship for honours, Institution: Catalan Government (Generalitat de Catalunya)

SCIENTIFIC PUBLICATIONS

P1. Lorena Simon Gracia, Daniel Pulido, Christian Grandfils, Fernando Albericio, and Miriam Royo. Biocompatible, multifunctional, and well-defined OEG-based dendritic platforms for biomedical applications. Org Biomol Chem. 2013, 11, 4109
P2. Carla Pegoraro, Denis Cecchin, Lorena Simon Gracia, Nicholas Warren, Jeppe Madsen, Steve Arms, Andrew Lewis, Sheila MacNeil, and Giuseppe Battaglia. Enhanced drug delivery to melanoma cells using PMPC-PDPA polymersomes. Nanotherapeutics. 2013, 334, 328
P3. Luis J. Cruz, Felix Rueda, Begoña Cordobilla, Lorena Simón, Leticia Hosta, Fernando Albericio, and Joan Carles Domingo. Targeting nanosystems to human DCs via Fc receptor as an effective strategy to deliver antigen for immunotherapy. Molecular Pharmaceutics, 2011, 8, 104
P4. Luis J. Cruz, Felix Rueda, Lorena Simón, Begoña Cordobilla, Fernando Albericio, and Joan Carles Domingo. Liposomes containing NY-ESO-1/tetanus toxoid and adjuvant peptides targeted to human dendritic cells via the Fc receptor for cancer vaccines. Nanomedicine (Lon), 2013 (Epub ahead of print).

Romina Spera

Dr Romina Spera is aPhD student in Pharmaceutical Science at "Sapienza" University of Rome.
She obtained her master degree cum laude in Pharmacy from "Sapienza" University of Rome (Italy) in 2011.
Her research activity deals with drug controlled release from liposomes in cooperation with Department of Information Engineering, Electronics and Telecommunications, Sapienza University of Rome and UT BIORAD-RAB of ENEA Casaccia Research Center. In particular, the objective of her work is the preparation and characterization of liposomes integrating magnetite nanoparticles in their aqueous core and the study of the release profile upon low intensity alternating magnetic field.

The research group is also involved in the characterization of poorly soluble drugs/cyclodextrins inclusion complexes.

Marie Spitzner

My name is Marie Spitzner. I am German and studied at the University of Rostock "Medical Biotechnology".
In 2010 I finished my Bachelor Thesis on the topic "Studies on the quantitative determination of polystyrene nanoparticles in aqueous media and biological matrices" and in 2012 I graduated the Master of Science with my Thesis performed at the Johns Hopkins University in Baltimor, MD, USA on the topic "Magnetically labeled mesenchymal stem cells as theranostic platform for cancer".
Since October 2012 I work as a PhD student at the Karlsruher Institute for Technology in the Institute of Toxicology and Genetics (ITG) in the lab of Prof. Dr. Maria C. Mione. The title of my project is " The hippo tumor suppressor as a target of oncogenic ras: insights from zebrafish cancer models".
My publication list includes original articles published in BMC Evolutionary Biology (Thalmann, O. 2011), Diabetologia (Zechner, D. 2012), American Journal of Pathology (Bobrowski, A. 2013) and a review published in the Journal of Cell Sciences (Vacaru, A.M. 2014).
To finance my studies at the University and my PhD work I received scholarships from the DAAD, FAZIT and the "Studienstiftung des deutschen Volkes".

Ali-Mohammad Tamaddon

Pharm.D, Ph.D, Associate Professor, Department of Pharmaceutics and Pharmaceutical Nanotechnology, Faculty of Pharmacy and Center for Nanotechnology in Drug Delivery, Shiraz University of Medical Sciences, Shiraz 71345, Iran;
amtamadon@gmail.com;amtamadon@sums.ac.ir;
+98-711-242-4127 (Ext. 257);
http://pharmacy.sums.ac.ir/en/departments/pharmaceutics/ali-mohammad-tamaddon.html

EDUCATIONAL RECORDS

Research fellowship on "Efficiency and specificity of antisense oligodeoxynucleotides and small interfering RNA (SiRNA) delivered by cationic nanovectors in Ewing sarcoma", PROTHETS project, Partner #8, UMR CNRS 8121, Vectorologie et Transfert de Genes, Institut Gustave Roussy, Villejuif under supervision of Prof. Claude Malvy and Prof. Patrick Couvreur; Ph.D thesis on "Effect of bilayer destabilizing agents on the cytoplasmic release of antisense oligonucleotides from PEG-stabilized cationic nanoliposomes and their cytotoxicity on tumor cells", Shaheed-Beheshti University of Medical Sciences, School of Pharmacy, Tehran under supervision Prof. Hamidreza Moghimi and Prof. Farshad Hosseini Shirazi.
Current academic appointments: Academic member, Department of Pharmaceutics, Faculty of Pharmacy, Director of the Center for Nanotechnology in Drug Delivery and Head of Department of Pharmaceutical Nanotechnology, Shiraz University of Medical Sciences.

ACADEMIC MEMBERSHIPS

Editor of nanotechnology section of Trends in Pharmaceutical Sciences, The journal of Faculty of Pharmacy, Shiraz University of Medical Sciences; Council of Advanced Biomedical Sciences and Technologies and Council of Pharmaceutical Technology Incubator, Shiraz University of Medical Sciences; National Association of Board of Pharmaceutical Nanotechnology and Council of Nanotechnology Network, Ministry of Health and Medical Education; Iranian Controlled Release Society, Iranian Association of Pharmaceutical Scientists and European Society for Nanomedicine (ESNAM).

HONORS & AWARDS

Distinguished Faculty Member, Shiraz University of Medical Sciences; National Young Scientist Award, Ministry of Health and Medical Education; Ph.D grant awarded by National Nanotechnology Initiative, Iran; Ranked 1st top in Comprehensive Board Exam, Shaheed-Beheshti Medical University, Tehran; Participation Grant from European Science Foundation (ESF), Nanomedicine Conference, Sant-Feliu-de-Guixols.

PUBLICATIONS

18 original and review articles in academic journals, 14 oral presentations and invited lectures and 59 abstracts in scientific national and international conferences.

Lee Tatham

Department of Molecular and Clinical Pharmacology, University of Liverpool,
70 Pembroke Place, Block H, Liverpool,
L69 3GF; Tel: +44 (0) 151 794 5919
e-mail: l.tatham@liv.ac.uk

PROFILE

My main research interest is in the development and assessment of nanoscale drug delivery systems, particularly associated with anti-infective applications. I have previously investigated the efficacy and suitability of antibacterial and antifungal nanoformulations against pathogenic microorganisms. Subsequently, I investigated the transcriptional response of bacteria following nanoparticle exposure, allowing comparisons with conventional formulations. My current research interests are aimed towards assessing the pharmacological suitability of hyperbranched polydendron materials for oral and targeted drug delivery to particular cell types. I am currently attempting to investigate the mechanisms of cellular uptake associated with different material formulations.

EDUCATION AND RESEARCH

• 2011-present: Postdoctoral Research Associate, Department of Molecular and Clinical Pharmacology, University of Liverpool, UK. Investigating new nanoscale drug delivery systems and their application to HIV/AIDS treatment. Principal Investigator: Prof. Andrew Owen
• 2007-2011: Ph.D. Institute of Integrative Biology, University of Liverpool, UK. Analysis of the inhibitory activity and mode of action of novel antimicrobial organic nanoparticles. Supervisors: Prof. Clive Edwards and Prof. Alan McCarthy
• 2004-2007: B.Sc. (Hons) Microbiology (I), University of Liverpool, UK

Benjamin Theek

Benjamin Theek, works as a Ph.D. candidate at the Department for Experimental Molecular Imaging at the University Hospital RWTH Aachen and the Institute for Biomedical Engineering at RWTH Aachen, since 2012.
He holds a M.Sc. on Biomedical Engineering, B.Sc. on Molecular Biology and B.Sc. on Applied Biology. With his interdisciplinary educational background he works on developing new tools for image analysis as well as novel theranostic applications.
In the "Nanomedicine and Theranostics" working group he is working on Dr. Dr. Twan Lammers European Research Council starting grant "Neoadjuvant nanomedicines for vascular normalization" and is project leader of his own starting grant, a cooperation project on a "3D printed biomimetic tumor angiogenesis model".
Thus, he focuses on the design and evaluation of polymeric and liposomal nanomedicines for vascular normalization, in order to improve the efficacy of combined modality anticancer therapy. In addition, he develops methods for using ultrasound-based perfusion monitoring to reduce the interindividual variability in image-guided drug delivery and tumor targeting studies. Furthermore, he is involved in several different studies focusing on (theranostic) microbubbles and angiogenesis.

Rudolf Urbanics

Mobile +36208259691
E-mail: urbanicsr@gmail.com

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

He was the Deputy R&D Director and Head of CNS Pharmacology Department at Biorex R&D Co.(1997-2003), worked at IVAX/ Drug Research Institute Budapest, as Scientific Adviser, Leading researcher in Safety and CNS Pharmacology and later in IVAX/Drug Research Institute, Subsidiary of TEVA as Head of In Vivo Pharmacology Group (2003-2008).
Currently, he is working with in vivo models of nano drug - nano carrier induced, complement activation related pseudoallergic reactions (CARPA), clarifying their immune-toxicological and safety hazards.

Rajaletchumy Veloo Kutty

PhD candidate
E-Mail: rajaletchumy@nus.edu.sg
v.rajaetchumy@gmail.com
Date of Birth: 28th February 1986
Nationality: Malaysian

KEY QUALIFICATIONS

Through my PhD research work, I have gained valuable knowledge in development of nanomedicine for cancer treatment such as to design, synthesize and characterize nanoparticles from new materials for both in vitro and in vivo applications. My research interest focus on nanomedicines as multifunctional/theranostic platforms for molecular imaging and therapy of cancer, and targeted nanomedicine that tackles problems related to diagnosis/treatment of for triple negative breast cancer.

EDUCATION

- 2011-present: National University of Singapore, PhD Student
- 2006-2010: University Malaysia Pahang, B.(Hons) Chemical Engineering (Biotechnology)

EMPLOYMENT RECORD

- Research and Development (R&D) Engineer
- Teleflex Medical, Malaysia (2010-2011)

RESPONSIBILITIES

- Leading / Assisting in generation of protocol, execution and report writing for validation of process, product and method validation. (Design V&V, OQ, PQ)
- Manage new product / formulation development process via interface with purchasing, manufacturing, logistics,QA & RA functional groups

PUBLICATIONS AND AWARDS

- 2013: Kutty RV, Feng SS. Cetuximab conjugated vitamin E TPGS micelles for targeted delivery of docetaxel for treatment of triple negative breast cancers. Biomaterials. 34(38):10160-71 (2013) (IF:7.60)
- 2010: Best design award (Title: Production of 120000 MT/Annum of Bio-ethanol from rice straw)
- 2010: Industry Award, from BASF Petronas Chemicals Sdn Bhd
- 2010: Silver Medal in International Invention, Innovation and Technology Exhibition, ITEX

Iga Wasiak

MSc Eng
Lives in Warsaw POLAND
Born in 1985 in Leczyca (Poland)
languages: Polish, English, Spanish
Major capabilities: conscientiousness, good o work organization and planning
Major interest: cell culturing and biology

EDUCATION

- 10.2009 till now: Warsaw University of Technology, Faculty of Chemical and Process Engineering PhD study
- 10. 2008 –11. 2011 Higher School of Management, Department of Management and Marketing, Faculty of Production Engineering and Management, Systems Management Specialization quality in manufacturing processes. BSc
- 10. 2004 – 12. 2009: Warsaw University of Technology, Faculty of Chemistry, Biotechnology course with specialization in Industrial Biotechnology MSc

EXPERIENCE

Plants breeding and acclimatization institute o -intern in the Department of Plant Biotechnology and Cytogenetics assistance in carrying out preparations in the field of in vitro somatic embryogenesis, of androgenesis and genetic transformation 6 -9.2007

PUBLICATIONS

- "Evaluation of algiante –chitosan – algiante chondrocytes encapsualtion procedure" I.Wasiak, T. Ciach Engineering of Biomaterials 106 - 108 (14) 2011 s167-171
- "Preparation of polialdehyde – dextarn coated silver nanoparticles" I.Wasiak, T. Ciach Engineering of Biomaterials 106-108 (14) 2011 s172-176
- "Preparation of silver core nanoparticles with polysaccharide shell" I. Wasiak, T. Ciach Challenges of modern technology 2012 3 (2) s15 -17
- „Nanocząstki polisacharydowe, jako nośniki leków przeciwnowotworowych" I. Wasiak, T. Ciach Młodzi naukowcy dla polskiej nauki cześć IV Nauki Inżynieryjne 1 2012 s 95 -103 in polish
- "Encapsulation of chondrocytes in hydrogel systems effect of chitosan viscosity and microcpaule form" I.Wasiak T.Ciach Inżynieria Chemiczna i Procesowa 2012 vo 33 nr 4 s 529 - 538
- "Carboxymethyl Cellulose Oxidation to Form Aldehyde Groups" A. Kulikowska, I.Wasiak, T. Ciach Challenges of modern technology 2013 4 (2) s 11-18
- Dextran/Albumin hydrogel sealant for Dacron® vascular prosthesis" A. Lisman, B. Butruk, I. Wasiak, T. Ciach Journal of Biomaterials Applications Epub ahead of print

PATENT

Co-author of "A method for preparing polysaccharide nanoparticles", I. Wasiak, T. snip, submitted to the UP-RP 14.03. 2012 P, 398450

MONOGRAPH

„Nanocząstki – mechanizmy pobierania przez komórki nowotworowe" I. Wasiak Nowe trendy w naukach inżynieryjnych 2 (2) 2012 s 103-112 in polish

AWARDS

Award of the Polish Society of Theoretical and Applied Mechanics for a paper on "Preparation of silver nanoparticles with core polysaccaride shell"

Yihang Wu

PhD Student, Institute of Toxicology and Genetics, Hermann-von-Helmholtz-Platz 1
76344 Eggenstein-Leopoldshafen, D
+49-72160823295 (office)
yihang.wu@kit.edu

EDUCATION

- Ph.D. candidate, Biology, Karlsruhe institute of technology, Germany, Expected in 2015
- M.S. Biophysics, Southeast University, P.R. China, 2011
- B.E. Bioengineering, Tianjin University of Science and Technology, P.R. China, 2007

RESEARCH EXPERIENCE

- Karlsruhe Institute of Technology, Institute of Toxicology and Genetics, Supervisor: Dr. Gary Davidson, Dr. Pavel Levkin
- Design and synthesis of combinatorial lipidoid libraries
- Developed potent lipid nanoparticles for both pDNA and siRNA delivery

• Design and synthesis of combinatorial polymer libraries
• Developed potent polymer nanoparticles for both pDNA and siRNA delivery
• Southeast University, Department of Biological Science and Medical Engineering, Supervisor: Prof. Yu Zhang
• Design and synthesis of ultra-small particles of iron oxide for medical diagnosis

PATENT

• Yihang Wu, Linxian Li, Gary Davidson, Pavel A. Levkin, Xin Du, Girish Shankara, "Cationic lipids, their synthesis and uses thereof," EU pending patent. 2013. In preparation

PUBLICATIONS

• Yihang Wu, Linxian Li, Pavel A. Levkin, Gary Davidson. Lipidoid cocktail: combining single and double tail lipidoids for synergistic transfection. In preparation
• Linxian Li, Fengjian Wang, Yihang Wu, Gary Davidson, Pavel A. Levkin. (2013) Combinatorial Synthesis and High-Throughput Screening of Alkyl Amines for Nonviral Gene Delivery. Bioconjugate Chemistry, 2013, 24 (9), 1543–1551
• Yihang Wu, Mengjie Song, Zhuang Xin, Xiaoqing Zhang, Yu Zhang, Chunyu Wang, Suyi Li and Ning Gu. (2011) Ultra-small particles of iron oxide as peroxidase for immunohistochemical detection. Nanotechnology, 2011 April 1; 22, 225703
• Ting Qiao, Yihang Wu, Jing Jin, Wei Gao, Qiaozhen Xie, Shuang Wang, Yu Zhang, Huihua Deng (2011) Conjugation of catecholamines on magnetic nanoparticles coated with sulfonated chitosan. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2011 March 5; 380, 169-174
• Ting Yang, Yihang Wu, Xiaoqing Zhang, Yu Zhang, Ning Gu. (2010) Effect of surface modifications on the peroxidase-like activity of iron oxide nanoparticles. Journal of Southeast University (Medical Science Edition), 2010 June; 29(3): 242-247

PRESENTATIONS

• Yihang Wu. Lipidoid cocktail: combination of lipidoids for synergistic gene delivery. Helmholtz Association Biointerfaces International Graduate School Retreat. 2013 (Oral presentation)
• Yihang Wu. Combinatorial synthesis and high throughput screening of lipidoids for pDNA and siRNA delivery. Helmholtz Association Biointerfaces International Graduate School Retreat. 2012 (Poster presentation)

AWARDS

• 2011 State Scholarship Fund supported by Chinese Scholarship Council
• 2008 and 2009 Class III National Scholarship, Southeast University

Yuning Zhang

Ph.D student, IMM Institute of Environmental Medicine, Nanosafety & Nanomedicine Laboratory, Karolinska Institutet, SE-17177, Stockholm, Sweden
Email: yuning.zhang@ki.se

RESEARCH INTERESTS

My research focuses on developing polymer based theranostic (therapy + diagnostic) nanoparticles for cancer treatment. This includes evaluation of different types of nanoparticles (self-assembled polymer, inorganic, and protein-lipid structures) as drug delivery systems. These are tested in different types of cancer models, including breast cancer, glioma, and pancreatic cancer, both in 2D and 3D formats to evaluate nanoparticles efficacy and to investigate the mechanism of cellular uptake and tracking the distribution of nanoparticles intracellularly.

EDUCATION

• 2011: Registered as a PhD student in nanomedicine, conducted at the department of Neuroscience, Karolinska Institutet and transferred to Division of Molecular Toxicology. IMM Institute of Environmental Medicine, Karolinska Institutet in July 2013.
• 2011: Master Degree in Applied biotechnology, Uppsala University, Uppsala, Sweden
• 2008: Bachelor Degree in Biology Engineering, School of Life Science and Technology, Xian Jiaotong University, Xian, China

PUBLICATION LIST

1. Zeng, X. H.; Zhang, Y. N.; Wu, Z. H.; Lundberg, P.; Malkoch, M.; Nyström, A. M., Hyperbranched copolymers micelles as delivery vehicles of doxorubicin in breast cancer cells, J Polym Sci Pol Chem 2012, 50 (2), 280-288.
2. Wu, Z. H.; Zeng, X. H.; Zhang, Y. N.; Feliu, N.; Lundberg, P.; Fadeel, B.; Malkoch, M.; Nystrom, A. M., Linear dendritic polymeric amphiphiles as carriers of doxorubicin – in vitro evaluation of biocompatibility and drug delivery, J Polym Sci Pol Chem 2012, 50 (2), 217-226.
3. Zeng, X. H.; Zhang, Y. N.; Nystrom, A. M., Endocytic uptake and intracellular trafficking of bis-MPA based hyperbranched copolymer micelles in breast cancer cells, Biomacromolecules, 2012, 13 (11), 3814-3822.
4. Lundberg, P.; Lynd N.A.; Zhang, Y. N.; Zeng, X.H.; Krogstad, D.V.; Paffen, T.; Malkoch, M.; Nyström, A.M.; Hawker, C.J., pH-triggered self-assembly of biocompatible histamine-functionalized triblock copolymers, Soft Matter 2013, 9, 82-89
5. Porsch, C.* ; Zhang, Y. N.*; Östlund, Å.; Damberg, P.; Ducani, C.; Malmström, E.; Nystrom, A. M., In vitro Evaluation of Non-protein Adsorbing Breast Cancer Theranostics based on 19F- Polymer containing Nanoparticles, Part. Part. Syst. Charact. 2013, 30, 381-390.
6. Hed, Y*; Zhang, Y. N.*; Andrén O.C.J.; Zeng, X.H.; Nyström A.M.; Malkoch, M., Side-by-Side Comparison of Dendritic-linear Hybrids and their Hyperbranched Analogues as Micellers Carriers of Chemotherapeutics, J Polym Sci Pol Chem 2013, 51(19), 3992–3996.

ABSTRACTS OF THE INTERVENTIONS

NANOMEDICINE: ETHICAL ISSUES AND SOCIETAL EXPECTATIONS

JOHANN S. ACH

In its first part the paper provides a brief overview of more general ethical aspects of nanomedicine (environmental, health and safety risks; ethical, legal and social issues; political and security risks; cultural and anthropological concerns). These ethical and social challenges are quite well known from other areas of modern medicine and not specific to nanomedicine .
The central thesis in the second part of the talk, then, is that we are currently experiencing a cultural shift, in the course of which our understanding of medicine and the idea of the doctor/patient relationship will change fundamentally. Nanomedicine as part of the emerging "converging technologies" in medicine, contributes to this process, which can be summarized under the headings acceleration, automation, individualization, prevention orientation, etc. The ethical and social consequences that arise from this "convergence" of various (medical) technologies must be taken more clearly into view than at present (not only in medical ethics) seems to be the case.

NBTXR3: CLINICAL DEVELOPMENT AND MEDICAL BENEFIT

RAFIK AIT SARKOUH
Clinical Research Associate, Nanobiotix

Nanobiotix is a clinical-stage nanomedicine company pioneering novel approaches for the local treatment of cancer. The Company's first-in-class proprietary technology, NanoXray, is at the forefront of a new era of nanomedicine, where nanoparticles are not just a vehicle for targeted drug delivery, but have become the principal active element. NanoXray products enhances radiotherapy efficacy in the tumor without increasing healthy tissues damages to provide a new, more efficient treatment for cancer patients. NanoXray products are compatible with current radiotherapy treatments and are meant to treat a wide variety of cancers via multiple routes of administration. The NanoXray technology has the potential to treat numerous cancer indications.
Nanobiotix's lead product NBTXR3, based on NanoXray, is currently under clinical development for advanced soft tissue sarcoma (STS) and locally advanced head and neck cancer. The aim of NBTXR3 is to facilitate complete tumor resections and provide a significant clinical benefit to patients.
Nanobiotix will present data from its clinical trial evaluating NBTXR3 in advanced soft tissue sarcoma.

TARGETED DRUG DELIVERY WITH MAGNETIC NANOPARTICLES – THE SEON-CONCEPT

ALEXIOU C, Lyer S, Janko C, Cicha I, Matuszak J, Dürr S, Tietze R, Zaloga J, Unterweger H, Friedrich R
Department of Otorhinolaryngology, Head and Neck Surgery, Section of Experimental Oncology and Nanomedicine (SEON), University Hospital Erlangen, Waldstraße 1, 91054 Erlangen, Germany

Magnetic Nanoparticles are used for a variety of applications in medicine. This ranges from in vitro diagnostic tests, in vivo imaging, targeted drug delivery and tissue regeneration.
To translate basic findings into clinical trials several requirements such as detailed synthesis and characterization of the nanoparticles, nanotoxicological testings, ex vivo models to simulate in vivo conditions for appropriate adjustment of the necessary parameters and pre-clinical animal studies have to be addressed. These results are of pivotal importance to start with respective GMP production and approval, which is essential for translating these products into clinical trials (figure 1).
SEON (Section of Experimental Oncology and Nanomedicine) addresses these issues with a special focus on drug delivery in oncology1 and their promising potential applications in cardiovascular2, regenerative medicine3 and imaging4. The aim is the translation of the preclinical results into clinical trials and the respective steps necessary to gain this ambitious object.

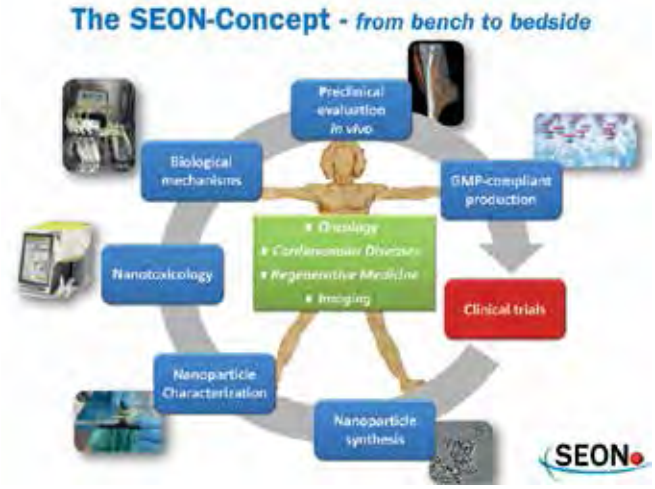


Figure 1

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1. Tietze R, Lyer S, Struffert T, Schwarz M, Engelhorn T, Eckert E, Göen T, Vasylyev S, Peukert W, Wiekhorst F, Trahms L, Dörfler A, Alexiou C: Efficient drug delivery using magnetic nanoparticles – biodistribution and therapeutic effects in tumour bearing rabbits. Nanomedicine (NBM), 9:961-71, 2013
2. Cicha I, Garlichs C, Alexiou C: Cardiovascular therapy through nanotechnology – how far are we still from bedside? European Journal of Nanomedicine, 2014 in press
3. Tripal P, Zaloga J, Friedrich R, Dürr S, Schreiber E, Weigel B, Tietze R, Nowak J, Odenbach S, Lyer S, Alexiou C: Magnetically controlled cell seeding for vascular tissue enineering using endothelial cells loades with iron oxide nanoparticles. Biomaterials, 2014 submitted
4. Lyer S, Tietze R, Dürr S, Struffert T, Engelhorn T, Schwarz M, Dörfler A, Lubos B, Hess A, Schmidt W, Jurgons R, Alexiou C: Diagnostic Imaging in cancer therapy with Magnetic Nanoparticles. Magnetic Particle Imaging (T.M. Buzug and J. Borgert (Eds.) SPPHY 140 pp. 197-201, Springer Verlag Berlin Heidelberg 2012

ACKNOWLEDGEMENT

The authors are grateful to the Else Kröner-Fresenius-Stiftung, Bad Homburg v.d. H., Germany, the German Research Foundation (grant reference AL 552 / 5-1), the German Federal Ministry for Education and Research (grant reference FKZ:01EX1012B), the Bavarian State Ministry of Environment and Consumer Protection (reference number U8793-2012), the Emerging Fields Initiative of the University Erlangen-Nürnberg and the EU (project FP7-NMP-2012-LARGE-6-309820, NanoAthero) for financial support.

OVERCOMING THE TRANSLATION GAP; TAKING NANOMEDICINES FROM PRECLINICAL ANIMAL MODELS TO CLINICAL TRIALS

DR. THERESA MARY ALLEN, FRSC, Professor Emeritus, Pharmacology and Oncology, University of Alberta, Edmonton; Co-founder and Strategic Advisor, Center for Drug Research and Development, Vancouver (CDN)

Applications of nanotechnology have become widespread, and drug delivery systems were some of the first products to come from nanotechnology. Lipidic nanoparticles were among the first nanomedicines to make the transition from concept to clinical application, and they are now an established technology platform with considerable clinical acceptance. Nanomedicines such as Ambisome® and Doxil®/Caelyx® appeared on the market in the 1990s, and are now starting to appear in generic versions such as Lipo-Dox®. Nanomedicines have, in the last couple of decades, been used to for the in vivo delivery of everything from small molecule

therapeutics, to gene medicines such as siRNA. There are over a dozen products in the clinic, and many more in clinical trials. As more products come to market, the principles that guide the development and approval of successful nanomedicine formulations are becoming clearer.

Increasingly, we are seeing nanomedicine formulations becoming more complex, with multi-functional, multi-component nanoparticles being explored in the research laboratory, and entering into the clinical development pathway. Future formulations are contemplated to include, within the same particle, not only a therapeutic, but also combinations of therapeutics, plus one or more molecules for site-specific targeting, biomarker and imaging capabilities, and sensing molecules that can respond to external or internal triggers to control the rate of drug release. The development costs for complex, multi-component nanomedicines will be higher, and often much higher, than those traditionally seen for small molecule therapeutics. Also, as the complexities of the formulations increase, so do the expenses and difficulties associated with their manufacture and quality control. Establishing control over all of the intellectual property associated with complex formulations will also increase costs.

There is starting to be a backlash against the high cost of new molecularly targeted and/or biological products, many of which show only marginal clinical benefits. A new paradigm is emerging where payers are now asking not only 'is this drug safe?' and 'is this drug efficacious?', but also 'is this drug's level of efficacy worth its price?' Hence, right from the beginning of the development of a complex multi-component nanoparticle, there should be a realization that the gains in therapeutic outcomes for such systems must be substantial, and the gains must offset the higher intrinsic costs of development of such systems. Due to the often poor correlation between therapeutic effects seen in animal models, and clinical outcomes, only highly significant increases in efficacy in animal models will likely translate to clinical benefits that justify the development costs and the high cost of treatment. For example, good progress has been made recently in the treatment of haematological malignancies such as human B lymphoma, with conventional combination chemotherapy (e.g., R-CHOP). Even with high cure rates in animal models, it will be hard (i.e., long and expensive) to power a clinical trial that will show significant increases over current combination therapies.

Another point, arising from the previous example, is that cancer chemotherapy almost always employs combinations of drugs or drugs and biologics. Yet, to date, all approved nanomedicines are monotherapies (i.e., have only one therapeutically active ingredient) that have to compete with combination chemotherapy regimens that are widely accepted in clinical practice. For currently approved nanomedicines that show increases in therapeutic indices (efficacy over toxicity), decreases in toxicity appear to contribute more to the increase than increases in efficacy. Yet, regulatory agencies such as the FDA seem to look more favorably on increased therapeutic outcomes than on decreased toxicities.

What are some practical suggestions for dealing with the challenges of maturing nanomedicine formulations in the rapidly changing environment of drug development?

Early on in development look for applications with high unmet medical need and reasonable population sizes.

Look for applications where the properties of the delivery system have significant advantages, or are enabling, compared to the alternatives, e.g., for the delivery of gene medicines that cannot be delivered by conventional means. Working with consultants or experienced nanoparticle manufacturing companies may help to focus and streamline product development.

Understand what kind of testing will be required for the product. For example, most early clinical trials in oncology are done in patients with advanced disease that has spread widely. This may not be the right niche to prove effectiveness for ligand-targeted therapeutics that, in animal models, seem to work better against micrometastatic disease, requiring testing in an adjuvant setting.

Try to take advantage of new regulatory authority programs, e.g., orphan drugs or neo-adjuvant programs, where there may less competition, and financial or regulatory help.

Consider applying principles of combination therapy when developing nanomedicines (e.g., two or more drugs, two or more ligands, two or more targets), but be aware that these will be more costly to develop, so consider the cost/benefit ratio early in development.

Finally, be aware of the rapid emergence of personalized medicine that seeks to tailor therapy to the pharmacogenomic and other 'omics' profiles of individual patients. Understanding where the niche is for drug delivery in this changing environment may provide the key to successful product development.

POLYMER NANOCARRIERS FOR ORAL PEPTIDE DELIVERY: THE TRANS-INT CONCEPT

MARÍA JOSÉ ALONSO
CIMUS Research Center, University of Santiago de Compostela, Spain

Peptide drugs are expected to have a great impact on the treatment of important diseases. Unfortunately, so far these peptides cannot be administered by the oral route, they need to be injected. The availability of an oral form of administration could lead to a great improvement in the treatment of pathologies such as diabetes. Among other groups, we, at the USC, have attempted to deliver peptides such as insulin and salmon calcitonin by the oral route. For this, we have used either nanoparticles made of chitosan or PLGA or nanocapsules consisting of an oily core surrounded by a polymer coating. The results obtained are promising, however, we believe that significant advances need to be made in order to have a clear idea of the potential of nanotechnology for oral peptide delivery. Indeed, in general the design of oral peptide nanomedicines has been based on a "trial and error" approach and there is a weak understanding of the mechanistic behaviour of nanocarriers.

Taking this into account, a few years ago we decided to build the TRANS-INT consortium, which integrates a number of leading experts on nanotechnology, barriers biology, immune-toxicology and preclinical and clinical drug development. According to the view of this consortium, the basis for the definition of oral peptide carriers relies on the in-depth knowledge of their interaction with the biological system in a systematic manner. Thus, the TRANS-INT motto is: "Understand the barrier; understand the carrier".

The concept behind TRANS-INT is the rational design of these oral nanomedicines based on integrative knowledge, networking intelligence and creativity. The design of TRANSINT nanomedicines is being driven by:

- Biopharmaceutical criteria: we want to understand how nanocarriers interact with the intestinal mucosa.
- Safety criteria: we use biomaterials with a good safety profile or are in clinical evaluation
- Bioengineering criteria: we manipulate biomaterials in order to produce nanocarriers with the required functionalities
- Pharmaceutical technology criteria: we make sure that the production technology is scalable that the final nanomedicine has adequate stability

We are expecting a number of out-comes from the TRANSINT consortium, namely to develop analytical tools for the understanding of the interaction of nanomaterials with the GI environment and to understand the potential of nanotech approaches for oral peptide delivery. Beyond these technical outcomes, our aim is to promote the training of talented researchers in a multidisciplinary environment and to contribute to the grown of existing small industries in connection with the big pharmas.

COMBINATORIAL DEVELOPMENT OF SYNTHETIC SIRNA DELIVERY SYSTEMS

DANIEL ANDERSON

High throughput, combinatorial approaches have revolutionized small molecule drug discovery. Here we describe our work on high throughput methods for developing and characterizing siRNA delivery systems. Libraries of degradable polymers and lipid-like materials have been synthesized, formulated and screened for their ability to delivery siRNA, both in vitro and in vivo. A number of siRNA delivery formulations have been developed with in vivo efficacy, and show potential therapeutic application for the treatment of genetic disease, viral infection, and cancer.

NOVEL HEPATITIS B TREATMENT WITH RNAi

DR. CHRISTOPHER R. ANZALONE

Hepatitis B virus (HBV) is the world's most common serious liver infection, with an estimated 350 million patients worldwide that are chronically infected. HBV can lead to cirrhosis of the liver and is responsible for 80% of primary liver cancers globally. Current therapies for chronic HBV include reverse transcriptase inhibitors and interferon. These therapies either require life-long administration or have significant side effects and limited efficacy, and no current therapy is capable of consistently decreasing viral proteins, which is thought to be a necessary step in achieving a functional cure. We have developed an RNAi-based therapeutic against chronic HBV: ARC-520. This approach is unique because it enables knock down of viral RNAs, including the pre-genomic RNA from which the replicative intermediates are derived, thus reducing both the viral load and the viral proteins that result in disease and negatively impact the immune system's ability to eliminate the virus. We completed a Phase 1 clinical study in healthy volunteers, and ARC-520 was shown to be safe and well tolerated at all doses studied. We are currently conducting a Phase 2 study in chronic HBV patients. ARC-520 has induced multi-log repression of viral RNA, proteins and viral DNA with long duration of effect in transient and transgenic mouse models of chronic HBV infection, without toxicity. Similarly, it has been demonstrated to be safe and effective at decreasing circulating viral DNA and proteins in a chimpanzee with chronic HBV.

HOW TO INTRODUCE NANOMEDICINE PRODUCTS AND BRING THEIR IMPACT TO THE MARKET

DR. CHRISTOPHER R. ANZALONE

Nanotechnology is a potentially powerful tool for next generation therapeutics, diagnostics, and devices because matter can react differently at the nanoscale and because precise size and shape may confer unique properties. However, these advantages also pose unique challenges to bringing new products to market. One can think of commercialization as a path through (at least) seven sets of challenges. They are: the basic science that enables a product; manufacturing; regulatory; clinical trial design/patient enrollment; scale-up; sales & marketing; and reimbursement. The pharmaceutical and biotech industries were built around understanding these challenges as they relate to small molecules, but nanotechnology requires new perspectives and skill sets for each of them.

SCIENCE-BASED INNOVATIONS SHOULD RESPECT THE LAWS OF NATURE AND LONG-TERM SUSTAINABILITY

PROF. WERNER ARBER

The use of effective and accurate research methodologies can reveal hitherto unknown laws of nature. Any acquired scientific knowledge represents cultural values. These can be of relevance both for the world-view of the human beings and for potential applications. The latter innovations can contribute to shape the future. Therefore, innovations should generally take note of expectations of the civil society, in particular a long-term sustainability of the development. This goal can best be reached by respecting the laws of nature.

NANOMEDICINES - AN INDUSTRY PERSPECTIVE

MARIANNE ASHFORD
Pharmaceutical Development, AstraZeneca, Silk Road Business Park, Charter way, Macclesfield, Cheshire, SK10 2NA.

Attrition rates within the Pharmaceutical Industry continue to be high with values of ~90 % being quoted from compound nomination to launch. Reducing attrition rate of drug candidates is seen as one of the greatest challenges and opportunities for the Pharmaceutical Industry. ~70% of drug failures have been reported to be due to safety or efficacy issues. By increasing the therapeutic index, the potential to improve the patient experience and establish combinations with improved efficacy is also increased. Nanomedicines or systemically targeted drug delivery technologies have the ability to remain in the vascular compartment for prolonged periods and passively accumulate in "leaky" tissues such as solid tumours via the enhanced permeability and retention (EPR) effect thus improving efficacy and through the changing the drug's bio distribution, improving its safety profile. In addition, instead of relying on medicinal chemistry to optimise drug solubility, ADME properties and toxicity, often major hurdles in a lead optimisation programme, nanotechnology approaches can be used to aid delivery, modify drug pharmacokinetics, and improve efficacy and tolerability. This talk will discuss where nanomedicines can and have been used in the drug discovery-development process to address therapeutic index challenges.

"PUBLISH OR PERISH" AKA. SCIENTIFIC PUBLISHING IN NANOMEDICINE (AN EDITOR'S VIEW)"

LAJOS P. BALOGH
PhD, Editor-in-Chief, Nanomedicine NBM (Elsevier)

Nanomedicine and nanobiotechnology are two rapidly emerging interdisciplinary areas. There are many challenges for these paradigm-changing fields, especially in the area of scientific communication. Ways and means of communication is rapidly changing and publishing is also undergoing dynamic changes. While researchers are under tremendous pressure to prove themselves by publishing "novel, significant, and original" papers in "high impact" journals, the essential question for publishers is how to determine value of research before and/or after making it public, and how to monetize this value. This discussion is now expanding and several articles have recently appeared in well-known journals (Science, Nature, The Economist, etc.) questioning the value and methods of science including translation of publications into products.
Getting published is crucial for academicians and researchers. In this talk the speaker will summarize major changes in business models (traditional, open access, and hybrid), describe the use and abuse of Impact Factor, and introduce the latest scientific methods used for determining the value of journals. Examples will be provided to explain how these tools may be used correctly to evaluate publication activities of individual authors, groups, institutions, and countries. In addition to comparing leading nanomedicine journals, a Q&A opportunity will also be provided.

REPROTOX - EFFECTS OF ALLOY NANOPARTICLES ON REPRODUCTION

STEPHAN BARCIKOWSKI*[1], Christoph Rehbock[1], Jurij Jakobi[1], Lisa Gamrad[1], Selina van der Meer[1], Daniela Tiedemann[2], Ulrike Taylor[2], Wilfried Kues[2], Detlef Rath[2]
[1]Technical Chemistry I, University of Duisburg-Essen and Center for NanoIntegration Duisburg-Essen CENIDE, Universtitaetsstrasse 7, 45141 Essen, Germany
*[1]Technical Chemistry I, University of Duisburg-Essen and Center for NanoIntegration Duisburg-Essen CENIDE, Universtitaetsstrasse 7, 45141 Essen, Germany, E-Mail: stephan.barcikowski@uni-due.de, Tel : +49 (0)201 183 3150, Fax : Tel : +49 (0)201 183 3049
[2]Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut (FLI), Federal Research Institute of Animal Health, Hoeltystrasse 10, 31535 Neustadt, Germany

In order to correctly assess toxicological effects of nanoparticles released from medical implants, testing systems with high purity are required. Unfortunately, nanoparticles obtained from chemical synthesis are frequently contaminated with artificial ligands remaining from synthesis, which may interfere with toxicity assays [1]. Furthermore, chemical reduction methods in aqueous solutions fail to generate alloy nanoparticles with homogeneous ultrastructure [2] [3].

In order to overcome these limitations, ligand-free colloidal nanoparticles were fabricated by pulsed laser ablation in liquid [4]. This method predominantly yields relatively broad size distributions, though precise control of particle size and particle composition are of paramount importance. To overcome this drawback we controlled particle size by addition of low salinity electrolytes during the nanoparticle formation process [5], while the utilization of artifical ligands was completely avoided. Furthermore, pulsed laser ablation in liquid was used to synthesize nanoparticles from binary and ternary alloy targets, while the resulting nanoparticles possess a homogeneous ultrastructure down to a single particle level and their overall composition well represented the implant alloy target. Additionally, the model system AuAg was used to systematically vary the nanoparticle composition and to correlate it to toxicological effects observed in bacterial and mammalian cell cultures.

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THE UNIQUE ROLE OF TUMOR MICROENVIRONMENT IN DRUG RELEASE OF LIPOSOMES

YECHEZKEL BARENHOLZ and Lisa Silverman
Department of Biochemistry, Institute for Medical Research Israel-Canada, Hebrew University-Hadassah Medical School, P.O.B. 12272, Jerusalem 91120, Israel

PURPOSE

To determine the mechanism of action (MoA) by which drug-loaded pegylated nano-liposomes such as Doxil®, which accumulate at the tumor site due to the EPR effect, release drug at a level sufficient for therapeutic efficacy, and to investigate how the tumor microenvironment is involved in, and accelerates, this release process.

MATERIALS AND METHODS

We studied and compared the physicochemical properties of two nano-drugs, both long-circulating pegylated liposomes. The first and more studied formulation is the pegylated liposomal doxorubicin (PLD) Doxil (Caelyx) and its generic version Lipodox, which is not thermo-sensitive. Doxil was remote loaded with doxorubicin by a trans-membrane ammonium sulfate gradient. Doxil demonstrates a very slow drug release in plasma, both in vitro and in vivo. The second type of liposomal formulation are those in which the drive for doxorubicin loading is trans-membrane proton gradient using intraliposome aqueous phase of 300 mM citrate buffer at pH 4.0 including (i) the thermo-sensitive (TS) liposomes (PLDTS), similar to ThermoDox®, which demonstrate a very fast burst, and almost complete, drug release at temperatures above Tm (420C), and a slow (though faster than Doxil) release rate at 370C and (ii) non TS Myocet® like formulation. A third type of liposomal formulation that was studied was large multivesicular liposomes (LMVV) remote loaded with doxorubicin by the same trans-membrane ammonium sulfate gradient as Doxil. The LMVV have a trapped aqueous volume that is at least 15-fold larger than that of Doxil.

RESULTS

Our results show that two low molecular weight species present in the tumor micro-environment play a major role in doxorubicin release. Substantial and similar release rates occur for both nano-liposomes used in this study, irrespective of the type of the trans-membrane ion gradient driving the drug remote loading. However, the LMVV exhibit an almost 3-fold slower release rate, pointing to the unique contribution of the nano-volume of the nano-liposomes to the rate of drug release at the tumor.

CONCLUSIONS

Tumor micro-environment plays a major role in the release of amphiphatic weak bases drugs from liposomes in which these drugs were remote loaded by trans-membrane gradients of either ammonium or hydrogen ions.
Liposomal trapped volume is an important factor in drug release rate. The larger the trapped volume, the lower the drug release.
Use of the principle of MoA of our findings may lead to improving drug release at tumors from liposomes remote loaded by trans-membrane ammonium ions and/or pH gradients.

EMPIRICAL MODEL-BASED IDENTIFICATION OF CRITICAL QUALITY ATTRIBUTES IN THE PRECLINICAL DESIGN OF NANOSTRUCTURED LIPID CARRIERS

T. BASTOGNE[1,2,3], J. Bruniaux[4,5,6,7], F. De Crécy[4], F. Navarro[4]
[1]Centre de Recherche en Automatique de Nancy (CRAN), Université de Lorraine, CNRS~UMR~7039, BP 70239, F-54506 Vandoeuvre-lès-Nancy Cedex, France, thierry.bastogne@univ-lorraine.fr
[2]INRIA BIGS, BP 70239, F-54506 Vandoeuvre-lès-Nancy Cedex, France
[3]CYBERnano, 9 av. de la forêt de Haye, Campus Médecine, BP 184, F-54505 Vandoeuvre-lès-Nancy
[4]CEA, LETI-MINATEC, Département des Technologies pour la Biologie et la Santé, 17 rue des Martyrs, F-38054 Grenoble, France
[5]CEA, DSV iRTSV, Biologie à Grande Echelle, Biomics, 17 rue des Martyrs, F-38054 Grenoble, France 3 INSERM, U1038, F-38054 Grenoble, France
[6]INSERM, U1038, F-38054 Grenoble, France
[7]Université Joseph Fourier-Grenoble I, U1038, F-38041, France

In this study, an empirical modelling-based method is proposed and implemented to identify the critical quality attributes and speed up the formulation optimization of a nanostructured lipid carrier.

DESIGN OF EXPERIMENTS

A mixture design with five factors, each associated with the nanoparticle formulation, was used: cationic lipid concentration (X1), fusogenic lipid concentration (X2), PEG surfactant concentration (X3), lecithin concentration (X4) and the hydrodynamic diameter (X5). The first four factors are dependant of each other and obey to a constraint equation about the nanoparticle composition. The nanoparticle properties considered were polydispersity (Y1), stability (Y2) and transfection efficacies on the Hela (Y3) and PC3 cell lines (Y4).

RATIONAL METHODOLOGY OF NANOPARTICLE DESIGN

The empirical modelling methodology was split up into three consecutive steps. In the first part, we show that only the hydrodynamic diameter of the nanoparticle has a significant influence on the polydispersity response and we deduce its design space. In the second step, an empirical model of the nanoparticle stability is obtained, which allows us to identify two main contributors: the nanoparticle size and the concentration of surfactant PEG. A stability region in the (X3, X5) space is derived from this model. In the final part of this study, two response surface models are computed from the experimental data and are used to determine the optimal values of three formulation factors of the nanostructured lipid carrier.

RESULTS

Two different formulations have been synthetized and their in vitro properties have corroborated the predicted values provided by the previous models. This study confirms that a rational and rigorous engineering of nanoparticles is possible, owing to statistical design of experiments and empirical modelling techniques. Such approaches can drastically reduce the preclinical development duration of nanotechnologies in medical applications.

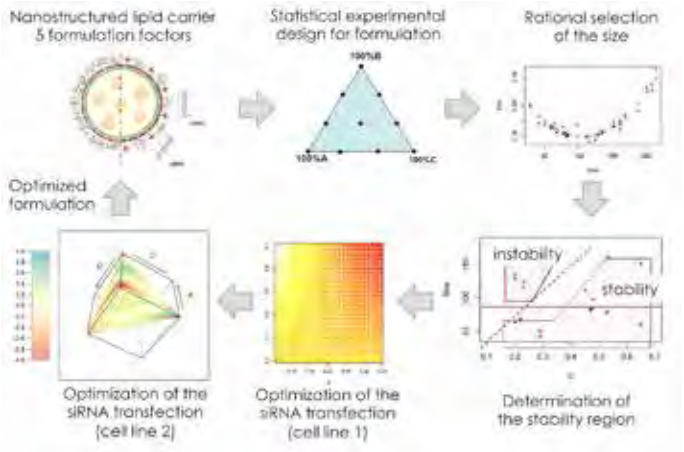

Fig.1 Empirical Model-based methodology to speed up the formulation optimization process of engineered nanoparticles.

POLYMERIC NANOVESICLES GENERATING REACTIVE OXYGEN SPECIES FOR EFFICIENT CANCER THERAPY

P. BAUMANN[1], M. Spulber[1], A. Dinu1, V. Balasubramanian[1], O. Onaca-Fischer[1], A. Sienkiewicz[2], C.G. Palivan1, W. Meier[1]*
[1]Department of Chemistry, University of Basel, CH-4056 Basel, Switzerland, cornelia.palivan@unibas.ch
[2]Institute of Physics of Complex Matter, Faculty of Basic Sciences, École Polytechnique Fédéral de Lausanne, CH-1015 Lausanne, Switzerland

Theranostic is a modern approach in medicine, which profits from its dual-functionality – combining diagnostic with treatment. Polymeric compartments encapsulating active molecules, which serve both to detect and treat a pathologic condition act as efficient multifunctional nanoreactors. The exchange of desired molecules through the compartment membrane, a feature essential for an in situ reaction of nanoreactors, is obtained by a selective permeable membrane. A smart combination of stimulus-responsive polymer compartments, and encapsulated active molecules supports a nanoreactor activity 'on demand', when the stimulus is present in their environment.

We present here stimulus-responsive nanoreactors based on encapsulated photosensitive conjugates in polymer vesicles with sizes in the nanometer domain. Upon irradiation with a specific wavelength, the photosensitive conjugates produce in situ reactive oxygen species (ROS) serving for photodynamic therapy [1]. Encapsulation of rose bengal conjugated to bovine serum albumin inside the cavity of polymer vesicles served to: i. improve the local concentration of photosensitizer, ii. protect the photosensitizer from degradation, iii. decrease its intrinsic toxicity, and iii. support the detection via the fluorescence signal of rose bengal. We selected as polymer compartments polymethyloxazoline-b-polydimethylsiloxane-b-polymethyloxazoline (PDMS-PMOXA) because these polymer vesicles were up-taken by various cell lines without being toxic, and possess a ROS permeable membrane [2]. ROS production was turned on/off by irradiation with a wavelength specific to the photosensitizer. ROS amount in HeLa cells increased significantly due to the activity of the nanoreactor, and induced cell death in the region where the nanoreactors were irradiated. Our nanoreactor represents an efficient candidate for theranosic approaches in photodynamic therapy because of its dual-function: generation of ROS, and easy detection by the fluorescent signal of the photosensitizer.

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NANODRUGS IN THE POST-BLOCKBUSTER WORLD – CRITICAL FDA REGULATORY AND PATENT ISSUES

RAJ BAWA
MS, PhD
President and Patent Agent, Bawa Biotech LLC, Ashburn, Virginia, USA
Adjunct Professor, Rensselaer Polytechnic Institute, Troy, New York, USA
Founding Director, American Society for Nanomedicine, Ashburn, Virginia, USA

Nanomedicine is part of the high-risk, high-payoff global nanotechnology phenomenon. One of the greatest impacts of nanomedicine is taking place in the context of drug delivery where novel nanodrugs and nanocarriers are addressing various fundamental problems of traditional drugs ranging from poor water solubility, toxicity issues, low bioavailability and a lack of target specificity.

In today's global economy, pharmaceutical companies are under enormous pressure to maintain profitability in light of numerous challenges ranging from revenue losses due to patent expirations on blockbusters to enhanced regulatory oversight to an ever-increasing challenge from generic manufacturers. This coupled with the fact that there are numerous market forces and drivers dictating a change in pharma's quest for discovering, developing and delivering novel therapeutics, is altering the pharma landscape. Clearly, new ground rules and competitive business strategies are needed in the post-blockbuster world.

Emerging technologies, like nanomedicine, also bring with them concerns and uncertainties about how they should be regulated. While complex nanodrugs and nanosimilars hold great promise for addressing some of the most challenging issues in nearly every medical specialty, the US Food and Drug Administration (FDA) has yet to formulate "official" regulatory guidelines for these nanoproducts. The safety and efficacy of nanodrugs can be influenced by minor variations in their structure, composition and the bioenvironment of use – areas that are still poorly understood. Therefore, many experts believe appropriate characterization of these medicines may, in some cases, require clinical trials to ensure the safety of patients. It is generally accepted that regulation of nanomedicine must balance innovation and R&D with the principle of ensuring maximum public health protection. The FDA has struggled to handle the issue of nanogovernance. Clearly, guidance is critically needed to provide clarity and legal certainty to manufacturers, policymakers, healthcare providers and the consumer. However, the "baby steps" the FDA has undertaken over the past decade are generally considered inadequate and have contributed to regulatory uncertainty.

As nanomedicine gains a firmer foothold and progress at various levels (technical, legal, societal, ethical) continues, there are certain issues that have come to the forefront. With this backdrop, my presentation will briefly address these current critical issues in reference to the FDA regulation and Patent law:

- Issue 1: Definition of "nano" – Lack of Universal Nomenclature
- Issue 2: Safety and Toxicity
- Issue 3: Lack of Effective Coordination (US Government, EU, Nano-societies, etc.)
- Issue 4: US FDA + Baby Steps = Regulatory Uncertainty on a Bumpy Road
- Issue 5: Are Most Nanoproducts combination products?

Issue 6: New Drug Application (NDA) or Abbreviated New drug Application (ANDA)?

- Issue 7: The US Patent Office and Patent Law issues
- Issue 8: Different "Nano" Terms - Same Materials
- Issue 9: Consumer Confidence, Societal, Ethical Issues, Unintended Consequences, etc.
- Issue 10: Nanosimilars and Generic Nanomedicines – The Future

UNRESOLVED ISSUES IN NEURODEGENERATIVE DISEASE

FRANÇOIS BERGER
Prof. Dr. med., Director of CLINATEC, INSERM, UJF, CEA-LETI,
Grenoble University hospital-France

Neurodegeneration (ND) is the generic term for the progressive loss of structure or function of neurons, including death of neurons. The main ND are Parkinson's and Alzheimer's diseases. Taking into account the "longevity revolution", between the year 2000 and 2030, the number of people over age 65 is expected to double. With 16% of its population being over 65, Europe has the world's highest proportion of older persons in the world and this is going to increase to 25% by 2030.

There is a strong link between age and the neurodegenerative diseases, and thus the number of people suffering from these conditions is constantly on the increase. The existing treatments and early detection modalities for neurodegenerative diseases, on the other hand, are limited and they mostly treat the symptoms rather than the causes. Thus, the importance of the nanomedicine research into this group of diseases is paramount. Detecting neurodegeneration before symptomatic signs, finding the active pathway to implement a precise molecular medicine targeting the neurodegenerative process inside the brain as well as to be able to detect the disease and monitor therapy in the blood circulation are the main unresolved problems in ND.

Alzheimer's disease has two main pathological hallmarks: Excessive amounts of the protein β-amyloid (Aβ or amyloid-beta) clump together to form plaques between cells in the brain; and the protein tau twists to form neurofibrillary tangles within neurons. In fact, diagnosis of definite AD in a demented patient requires detection of abundant plaques and NFTs in the postmortem brain or a brain biopsy. Parkinson's disease is characterized by dopaminergic cells degeneration in the substancia nigra. Nanomedicine tools provide us a unique strategy to access to the ND brain in a non-lesional micro-nano-invasive way to decipher the mechanisms of diseases. Neurodegenerative diseases share many common pathways and mechanisms. Many targeted therapies developed in oncology could be translated in ND, taking into account that many pathways involved in oncogenesis are also involved in brain post-mitotic ND. In situ micro-nano-bio-harvesting is probably mandatory to investigate the individual complexity of the ND brain, as biopsy is mandatory for the targeted therapy of cancer. Many nanotechnology developments provide efficient strategies to explore at the periphery the "nano-poly-omic circulating brain", which is mandatory to follow quantitatively the ND process and therapy. Nanoparticles also provide unique platforms to access to the brain for therapy, after systemic, or local delivery may be in association with physical modalities such as ultrasounds. The perspective of regenerative medicine using the functional interaction of the nanostructure with the extracellular space as well as with the neuronal stem cells is a major perspective for therapy.

The recent failure of Anti-amyloid drugs in phase III trial after inaugural miracle in mice strongly imposes us to re-evaluate our methodology to translate innovation at the bedside. Nanomedicine should integrate this, and may be a pioneering actor to define new « translational technology methodology » accelerating and making it safer.

NANOPARTICLES IN CONTEXT TO THE BBB

FRANÇOIS BERGER
Prof. Dr. med., Director of CLINATEC, INSERM, UJF, CEA-LETI,
Grenoble University hospital-France

Brain accessibility is a major issue from diagnostic to therapy in several pathological entities from cancer to neurodegenerative diseases. Blood Brain Barrier (BBB) is one of the main protecting factors, using a clever and functional association of astrocytes, pericytes and vessels. The treatment of neurodegenerative diseases remains a major challenge due to the limited access of molecules across the blood-brain barrier. As a result, at most, a small percentage of a drug that is administered systemically will reach the central nervous system in its active form. Moreover, in absence of tissue accessibility, these drugs do not have a real rational to target active pathways expressed in the neurodegenerative brain. Contrasting with oncology, fresh tissues obtained during surgery are not available to decipher active pathways that could be targeted with monoclonal antibodies or small molecule inhibitors as well as gene therapy products such as siRNA. Absence of early detection is an other crucial issue: when tremor or memory impairment are detected in Parkinson disease or Alzheimer, neuronal cell death is major and mainly irreversible.

To late and inaccessible: how could nanomedicine impact this devasting panorama providing innovative tools for an efficient theranostic strategy?

Several approaches have been developed using nanoparticles to penetrate the BBB. Intravascular and intranasal deliveries have been developed at the preclinical level. Success was highly efficient in animal models but translation at the bedside very low after decades of research. A major perspective to enhance brain delivery toward the BBB is the use of physical modalities including ultrasound or magnetic stimulation acting in synergy with devoted nanoparticles. Nanoparticles were also designed for direct brain delivery or intra-cerebral fluid delivery. These nanoparticles can be used for drug/gene delivery but also for regenerative actions modulating neuronal stem cells compartment. Strong perspectives are also available to use these nanoparticles for early detection by the highly remote sensitive bio-harvesting of the "circulating nano-polyomic brain".

Cell and animal models relevance is low to predict the preclinical nanomedicine efficacy. We cure the mice but human patients poorly benefited from these innovative therapies. A strong reevaluation of our methodology to develop innovation for human disease is mandatory: big animal models need (Primate, Pig alternative …), need for low dose tissue nanopharmacology, direct translation in human patients of drug validated for safety using cognitive phase 0 nanomedicine trials ? A strong ethical discussion and societal interaction will be indispensible to move toward this new but mandatory translational nanomedicine model.

ANTIBODY DRUG CONJUGATES – ACTIVE TARGETING – STATE OF THE ART

IWAN BERTHOLJOTTI

THE TARGETED CANCER THERAPY – ANTIBODY DRUG CONJUGATES

With the personal behavior we can positively influence the Cancer formation, but realistically we will see a further increase of the number of cancer patients in the world caused by the growth of the world population, the generally improved medical care for other disease and the increase in the average age of the population in developed countries.

There is a high demand for further improvement of existing Oncology Therapies by the development of targeted medicines with high selectivity, improved efficacy and substantially reduced site effects. The increase in selectivity and the increase in potency allow to optimize the therapeutic index, which is the difference between the Maximum and Minim Effective Dose.

The development in the last 20 years of biological active substances as monoclonal antibodies, which are after the humanization well tolerated and safe, provides the necessary basis to think about advanced concepts. One promising "old" idea is to combine targeted biologics with high potent drugs in cancer therapy. This idea gained momentum by the development of new highly potent drugs and stable linkers to chemically bind them with the targeting antibody. The stable linkers allow to form safe and well tolerated Antibody Drug Conjugates. New ADC's successfully showed improved safety and efficacy against standard treatments in the clinical tests. First products on the markets are Kadcyla (Genentech) and Adcetris (Seattle Genetics). These two drugs are the frontrunners of approximately 30 additional new ADC's currently in the clinical phase. In addition there is a high number of ADC's in the pre-clinical phase.

THE ANTIBODY PART OF AN ADC

Cancer Cells often show markers on the cell surface which are overexpressed in comparison with healthy cells. This fact allows to develop well tolerated humanized monoclonal antibodies who can specifically bind to these markers also called antigens. The biotechnology developed manufacturing concepts to produce monoclonal antibodies in a consistent and controlled manner. Monoclonal Antibodies (Mab's) are able to induce cancer cell death, but the efficacy is typically not good enough so that in cancer therapy Mab's are often used in combination with standard chemotherapeutics. These combined treatments are successful and several Mab's reached a blockbuster status.

Standard Mab's can be linked via the Cysteine and Lysine groups to form ADC's. The chemical reaction of Linkers to monoclonal Antibodies needs to be reproducible and consistent. This can be achieved with existing well controlled processes but the resulting ADC's consist of a defined distribution of ADC's with different number of cytotoxic molecules attached to the antibody. To get a homogeneous ADC the industry works to develop new generation antibodies with defined functional groups in the antibody sequence to allow specific binding. This is just one example of different ongoing developments to further improve ADC's.

THE CYTOTOXIC PART OF AN ADC

The cytotoxic drug bio availability to kill a cancer cell is substantially reduced if the cytotoxic molecule is attached to an antibody. The reason for this is that the cytotoxic part will be only efficiently released if the ADC binds to the target cell and will be successfully internalized into the cell. This whole mechanism is selective but reduces the bio availability. To overcome this drug availability issue, drug developers identified cytotoxic drugs which are at least 100-times more potent than standard chemotherapy drugs. The higher potency combined with the necessary solubility to generate a stable ADC allows today to develop selective and effective drugs. The manufacturability of these new extremely high potent cytotoxic and complex molecules generates an additional challenge to manufacture these drugs in a consistent and reliable way. Cytotoxics often used in new developed ADC's are Auristatins, Maytansinoids, Calicheamicins, Duocarmycin, Pyrrolobenzodiazepine (PBD).

THE LINKER TO BUILD THE ADC

In the past hydrazone Linkers were used, which allowed a pH depended drug release. The pH difference between the blood stream (pH 7.4) and in the endosomes and lysosomes of a cell (approximately pH 5) was used to achieve the cytotoxic molecule release. Unfortunately the hydrazone linkers were not stable enough and a time dependent slow release of the drug resulted in a systemic toxicity.

New developed Linkers (e.g. Peptidic, Sulfid-Bond) show a high stability to prevent this systemic toxicity and allow the binding to the cytotoxic molecule and the monoclonal antibody. The Linker molecule is a small molecule which is typically not difficult to manufacture, but which is a key technology part needed to develop a state of the art ADC.

THE COMPLEX ADC SUPPLY CHAIN

The ADC Supply Chain is important since these biological drugs need to be manufactured in a consistent way with the necessary quantity and quality. The Supply chain needs to be established early in the development process and is already relevant if material will be produced in the pre-clinical phase. It is typical that up to 18 months are needed to produce all building blocks, to conjugate the building blocks and to fill the drug into the form used in the treatment. Manufacturer of ADC's have to establish and maintain the necessary infrastructure. Specific investments into dedicated facilities are required especially where cytotoxics are handled. Different technologies are required to develop and manufacture ADC's as for example mammalian cell culture, microbial fermentation, different small molecule chemical technologies, Peptide synthesis, highly potent drug handling, ADC conjugation – handling of cytotoxic drugs in a biopharma environment. The technology know-how and trained personnel have to be in place to minimize the failure rate and to ensure safe handling.

To establish a reliable supply chain on time is a key success factor and is the responsibility of a pharma company if they bring a drug to the market. Delayed authority approval and market stock out can be a result of underestimation of the complexity and the im-

THE COMPLEMENTARY APPROACH TO WATSON – "DEFINIENS ONCOLOGY"

GERD BINNIG

Big Data approaches are established methods for sorting and creating knowledge in an automated fashion. In medicine this potential has by far not been fully leveraged. Research in this field focuses on genomics but now starts to gain momentum also in text (IBM, Watson) and image mining (Definiens). For genomics those approaches are relatively straight forward, whereas images and texts clearly represent less structured data. Data mining methods require structured meaningful data as input and the conversion of images and texts into minable data is by far not trivial. Through advanced image analysis this transformation became feasible for images and a new area of research might arise, which we call -if related to histology- "Tissue Phenomics". This new discipline could develop in a similar way as genomics. It is expected that compared to genomics tissue phenomics might have somewhat less impact on understanding cell biology but will have a bigger impact on patients and their treatments. In particular for the understanding and treatment of cancer genomics is closer to the fundamental mechanisms on the molecular level, whereas tissue phenomics is closer to the health state of the patient.

In the end the different big data approaches, that will develop in the future, will merge into one. Texts and images and all the other types of information will complement each other leading to a much more comprehensive understanding of biology and better treatments of patients.

UNDERSTANDING AND HANDLING COMPLEXITY IN MEDICINE - CAN MACHINE INTELLIGENCE HELP?

GERD BINNIG

Digitizing medical data is a prerequisite for making use of machine intelligence. In radiology the transformation from analog to digital is nearly completed and digital pathology has at least started to emerge. Genetic data are available in digital form and other types of medical information like medical reports or scientific publications will probably soon be completely digitized. Many type of data like most of the texts and all kind of images are unstructured and do not represent minable data. In those cases machine intelligence is already required to convert the data into meaningful structures. Once this conversion is done and data are available for the next level of automated logical considerations and intelligent processing the door is wide open for a new quality of knowledge creation, which differs quite dramatically from the conventional bottom up way of achieving scientific progress. The study of correlations of various types of data with specific ones like clinical outcome data will play here a central role. It is a kind of top down approach more suitable for the discovery of behavior than understanding the behavior. In a next step those discoveries can be implemented e.g. in form of novel diagnostic tests. Additionally bottom up research will be stimulated.

ETPN - ENABLING THE DEPLOYMENT OF NANOMEDICINE IN EUROPE

PATRICK BOISSEAU
CEA-Léti, Chairman of the Board of the ETPN, Grenoble (F), Chair

During Horizon 2020, the research and development in the nanomedicine domain will increasingly progress from the pre-clinical to the early clinical testing stage, consolidating thus the efforts initiated under FP7 and providing European stakeholders with R&I programs, funding schemes and infrastructure for the effective trans-

lation of nanomedicines. The ETPN strives for this shift by further providing strategic input to the community and decision makers and by implementing the concepts elaborated within the Platform and in the framework of the NANOMED2020 Coordination and Support Action.
With a pool of 500+ nanomed SMEs and 1500+ academic teams, Europe represents a huge potential for nanomedicine. However, despite a high scientific quality of academic research, very few products successfully reach the market. After a deep analysis of the value chain in nanomedicine under the Nanomed 2020 FP7 projects, the ETPN and its many collaborators have elaborated a White paper on Nanomedicine under Horizon 2020 that fix guidelines and recommendations for facilitating and enabling the translation of nanomedicine from public to private stakeholders, from labo to the clinic, from clinic to the market, in short from an academic discovery to a marketed product.
The session put together six distinguished international speakers from North America and Europe, all with a recognised expertise in some aspects of nanomedicine and pharmaceutical development. Technical aspects like nanocharacterisation, but also clinical, economical, industrial and ethical aspects will be presented and discussed.

WHERE WORLDS MEET: PARTICLE-IMMUNE SYSTEM INTERACTION

GERRIT BORCHARD
PharmD, PhD
School of Pharmaceutical Sciences Geneva-Lausanne, Geneva, CH
gerrit.borchard@unige.ch

Nanomedicine has the potential to improve therapy by manipulating drug or drug carrier interaction with the target cell or tissue down to a molecular scale. Nano-sized carrier systems such as liposomes, micelles or nanoparticles are used to modify the ADME-T profile of a drug, e.g., by passive targeting of a cytotoxic drug to a solid tumor taking advantage of the ERP effect (enhanced retention and permeation), thus enhancing efficacy and reducing toxic side effects.
The efficacy of such nanomedicines thus depends on both, the pharmacological activity of the drug delivered, as well as the physicochemical properties of the vector ultimately determining the ADME-T profile. Nanomedicines being of particulate nature, they are prone to be recognized by the immune system, including activation of opsonization and capture by the reticuloendothelial system (RES), or activation of the innate immune system through pathogen-pattern recognition receptors (PRR). Distribution and deposition of nanomedicines in the body, unlike drugs of small molecular weight, therefore largely depends on recognition by and interaction with the immune system.
Recognition is largely determined by processes involving the surface of the particulate nanomedicines. By altering the surface properties the attempt could be made to modify the way nanomedicines are interacting with the immune system, e.g., prolong time to recognition (stealth effect) or targeting to specific cell populations. A rational approach would be to identify factors (size, surface charge, hydrophilicity, guided opsonization) that need to be modified to achieve an intended effect. To achieve this goal, a deep understanding of both the physicochemical properties of nanomedicines, as well as mechanisms involved in immune recognition are essential and need to be combined.

HAPTEN-BINDING BISPECIFIC ANTIBODY PLATFORM FOR TARGETED AND PRE-TARGETED DELIVERY OF TOXINS TO TUMOR CELLS

ULRICH BRINKMANN

Bispecific antibodies (bsAb) that bind tumor associated antigens (LeY) as well as the hapten digoxigenin were applied as delivery vehicles for protein toxins. Toxin payloads were variants of Pseudomonas exotoxin A (PE38 without binding domain and PE25 with additional deletions/mutations), recombinantly produced and coupled to digoxigenin. These toxins bind to bsAbs and become as part of the antibody complex delivered to antigen expressing cells. The conceptual difference to established antibody-conjugation or fusion-protein approaches is the non-covalent vehicle-to-payload connection. Cells expressing the cognate antigen are killed upon exposure to LeY-targeting complexes, as demonstrated in vitro and in vivo. One interesting feature of this platform is that it does not require pre-formation of antibody-toxin complexes prior to administration. Pre-dosing of targeting vehicles followed by separate toxin administration causes assembly of targeting complexes with potency in vitro and in vivo.

WHY DO WE DO NANOMEDICINE? – AN ETHICAL CASE

DONALD BRUCE
Edinethics Ltd, Edinburgh, Scotland

Nanomedical researchers and clinicians working in nanomedicine take for granted that there is a strong justification behind what they do. But what is it? This talk will set out an ethical case in support of nanomedical research. At a first level it is the same ethical justification for doing medicine. This is the moral imperative, in so far as we can develop the skills and understanding to do so, to seek to alleviate physical and mental human suffering, to cure and prevent disease, to promote the health and well-being of our fellow humans. To be part of this goal is among the highest human vocations, both for healthcare professionals and support staff, and those involved with research.
But why nano-medicine? Again, it is part of a continuum of scientific knowledge about the human body and its functions and dysfunctions, and of the clinical interventions which them become possible, based on that scientific understanding. But this knowledge is always limited to what can be found out using the available techniques and equipment. Nanotechnologies offer the extension of that knowledge and intervention into a detail and specificity hitherto inaccessible. This is especially important for diseases that have hitherto no remedy or effective treatment, and that are either fatal, or if not fatal, cause long term distress and disability. The EC NanoAthero project provides a good example. Nanomedicine offers tools and ways to enable many long-standing medical goals. Thus, pre-symptomatic diagnosis and targeted drug delivery are not new ideas, but nanotechnologies now offer interventions towards these goals that represent a step change from what has been possible before.
The further we delve into the workings of the body, the more we must handle our interventions with care. The more information people get about their health, the more we also need to consider how to help them handle the knowledge appropriately. So with these exciting possibilities comes a commensurate responsibility on the part of those in nanomedicine. But the responsibility is also to the world's disadvantaged. When nanomedicine was first discussed ethically about ten years ago, concerns were raised about a nano-divide, making high tech interventions that only the rich could afford. While this remains a concern, not least for those who handle healthcare budgets, evidence will be presented of examples where nanotechnologies can make possible to address long standing problems in global medicine for the world's poor.

NEGLECTED DISEASES, CURRENT STATUS AND FUTURE NEEDS

RETO BRUN

Neglected tropical diseases (NTDs) represent a health burden to a significant part of our world's population. Diseases caused by viruses, bacteria, protozoa and helminths kill millions of people each year and are responsible for vast morbidity and disability. The existing diagnostic tools and medications are inadequate for most of these diseases, especially drugs which lack efficacy and safety or require long and complicated application. Product-Development-Partnerships i.e. the Foundation for Innovative New Diagnostics (FIND), the Medicines for Malaria Venture (MMV) or the Drugs for Neglected Diseases initiative (DNDi) closed the R&D gap for NTDs and took over the role the pharmaceutical industry played before.

The main goal is to bring new products that are safe, effective and affordable to patients in resource poor countries.
Several NTDs are ear-marked for world-wide elimination by WHO and the international community. According to the roadmap guinea worm disease, leprosy, lymphatic filariasis, blinding trachoma and African sleeping sickness are targeted for elimination while for schistosomiasis, river blindness, Chagas disease and visceral leishmaniasis control is in the focus for the year 2020. Efforts to eliminate NTDs can greatly benefit from improved PoC diagnostics and new effective and safe oral drugs. In the case of vector borne diseases control strategies for the insect vector or intermediate hosts (e.g. in schistosomiasis) are also crucial elements for elimination.
Nanotechnology is currently not being used much for NTDs but has great potential for rapid diagnostic tests and to target drugs to reach infected cells or organs.

LAB-ON-CHIP DEVICES FOR ANTICANCER DRUG ACTIVITY EVALUATION BASED ON LONG-TERM 3D CELL-CULTURE

BRZOZKA Z., Kwapiszewska K., Jastrzebska E., Chudy M., Dybko A.
Department of Microbioanalytics, Institute of Biotechnology,
Faculty of Chemistry, Warsaw University of Technology, Poland

PRESENTATION SUMMARY

The presentation will concern applications of microfluidic devices for cell culture and testing. Long-term two-dimensional and three-dimensional cell culture on-chip for anticancer drugs activity evaluation and photodynamic therapy testing will be discussed.

ABSTRACT

We designed and fabricated microfluidic cell culture systems which were applied for monolayer cell culture, three-dimensional cell culture, cell based cytotoxicity assays (5-fluorouracil, celecoxib) and photodynamic therapy procedures (5-aminolevulinic acid). Novel method of three-dimensional microfabrication of the microdevices in polydimethylsiloxane (PDMS) and glass was applied. The designed geometry of the microdevices includes cell culture microchambers or microwells and a concentration gradient generator (CGG). The CGG enables to obtain different concentrations of tested drugs in a single step, which is a significant simplification of cytotoxicity assay procedure. In the designed microsystems three various cell lines (normal and carcinoma) were cultured and analyzed. Microsystem for three-dimensional cell culture enabled long-term spheroid cultivation (over 4 weeks) in the in vivo-like microfluidic environment. Application of this type of microfluidic devices is expected to have a significant influence on biological and engineering studies. It can be a user-friendly device applicable in biological laboratory.

SITE-SPECIFIC DELIVERY OF CHEMOKINE RECEPTOR ANTAGONIST ENTRAPPED IN VCAM-1 TARGETED LIPOSOMES ATTENUATE THE INFLAMMATORY PROCESS IN ATHEROSCLEROSIS

MANUELA CALIN, Daniela Stan, Cristina Ana Constantinescu, Viorel Simion, Mariana Deleanu, Emanuel Dragan, Ana Maria Gan, Monica Pirvulescu, Elena Butoi, Ileana Manduteanu, Maya Simionescu
Institute of Cellular Biology and Pathology "Nicolae Simionescu" of Romanian Academy, Bucharest, Romania

INTRODUCTION

Chemokines are critically involved in the development of chronic inflammatory-associated diseases (such as atherosclerosis). Therefore, the development of new and innovative therapeutic approaches to manipulate selectively the chemokine(s) function in a pathophysiologic context may have few or no adverse effects on the immune system. Target-sensitive liposomes (TSL) can be potentially used as carriers for chemokine receptor antagonist to specific pathological sites. The aim of this study was to exploit the overexpression of vascular cell adhesion molecule-1 (VCAM-1) on the surface of activated endothelial cells covering the developing atheromatous plaque and to develop VCAM-1 targeted liposomes able to deliver chemokine receptor antagonist (CCR2 antagonist) at sites of activated endothelium.

MATERIALS AND METHODS

TSL encapsulating a CCR2 antagonist (Teijin compound 1) have been prepared by coupling a peptide with high affinity for VCAM-1 (Vp) to TSL (TSL-Vp), namely to the distal end of PEGylated phospholipid via a thioether bond. The TSL-Vp were characterized for size (by dynamic light scattering), the amount of peptide coupled at the surface of liposomes and CCR2 release (by HPLC). To confirm the targeting potential, the fluorescently labeled liposomes were either incubated with endothelial cells or intravenously administrated to ApoE-deficient mice and investigated by flow cytometry and an IVIS 200 imaging system (Caliper Life Sciences), respectively. To follow the therapeutic effect of Teijin entrapped TSL-Vp on the development of atherosclerotic plaque, seven weeks old ApoE-deficient mice were fed with high-fat diet and intravenously injected three times/week for six weeks with free Teijin or with Teijin encapsulated into VCAM-1 targeted or non-targeted liposomes. PBS-treated animals were used as control. At the end of the experiment, explanted aortas were stained with Oil Red O and atherosclerotic lesions were quantitated using AxioVision 4.8 Software (Carl Zeiss MicroImaging, Inc.).
Results. 1) The hydrodinamic diameter of liposomes was ~150 nm; 2) the coupling efficiency of the peptide to the liposome surface was ~10 µg peptide/µmol liposomes; 3) VCAM-1 targeted liposomes bound specifically to the surface of activated endothelial cells both, in vitro and in vivo; 4) Teijin release from target sensitive liposomes was higher (~ 80 % after 6 hours) for VCAM-1 targeted liposomes as compared with non-targeted liposomes (~ 30% after 6 hours); 5) release of Teijin from target sensitive liposomes decreased monocytes transmigration (~70%) through activated endothelial cells; 6) the atherosclerotic lesions area was reduced (by ~25 %) in ApoE-deficient mice injected with VCAM-1 targeted liposomes entrapping CCR2 antagonist as compared to control groups.

CONCLUSION

VCAM-1 targeted liposomes bind specifically to activated endothelium covering atherosclerotic lesions of ApoE-deficient mice. Upon binding, the specific delivery of the CCR2 antagonist entrapped into target-sensitive liposomes reduces the transmigration of monocytes into the vascular wall and, consequently decreases the atherosclerotic lesions.

ACKNOWLEDGEMENTS

This work was supported by UEFISCDI, contract no. 4_001, NANODIATER project under the frame of EuroNanoMed, PNII-PCCE-ID-2011-2-0028 project and the Romanian Academy.

TRANSATLANTIC COOPERATION ON NANOTECHNOLOGY AND BEYOND

CHRIS CANNIZZARO

The United States and the European Union (EU) have established strong and successful cooperation on nanotechnology regulatory issues and research in support of regulation, implemented in a wide variety of fora, and together play a leading role in international organizations involved with nanotechnologies. The Transatlantic Economic Council (TEC), the primary plenary forum for economic dialogue between the United States and the European Union, first considered nanotechnology in 2008, under its High Level Regulatory Cooperation Forum, and since 2011 as the focus of an independent dialogue on regulatory, scientific, and legislative developments. The U.S. Emerging Technologies Interagency Policy Coordination Committee (ETIPC) nanotechnology working group and the European Commission's Interservice Group on Nanotechnology regularly share information on the developments of regulations and guidance in relation to specific sectors; views and approaches regarding hazard, risk and benefit assessment; views on underpinning research needs and how they inform regulatory developments; and approaches to enhance responsible development and accelerate innovation.

This presentation will review current and future cooperative activities under the TEC, as well as those under the auspices of the US-EU Joint Consultative Group on Science and Technology such as the US-EU Bridging NanoEHS Dialogue (www.us-eu.org). Linkages between these activities and the needs of the nanomedicine community will be drawn, and further expanded upon in the context of the development and commercialization of nanotechnology applications and nanotechnology-enabled industries.

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

BOB CARR, Pauline Carnell, Patrick Hole, Jonathan Smith, Katy Pottage
Malvern Limited, Amesbury, SP4 7RT UK
e-mail: patrick.hole@nanosight.com

A new sizing and characterization technique, Nanoparticle Tracking and Analysis (NTA) is described which allows nanoparticles in a suspension to be individually but simultaneously detected and analysed in real time using a laser-based microscope system. NTA has been commercially developed over the past eight years and now, with over 800 systems installed, is becoming a key characterisation technique in many fields of nanoparticle characterisation particularly in the application of nanomedicines, nanopharmaceuticals and exosomes research and development. Here we discuss the technique, its application to these fields and how recent developments are further enabling advancements in these fields.
In this method a laser beam passes through a suspension at a low angle. The particles scatter light which is collected onto a CCD (or sCMOS) camera by a microscope-type configuration (Fig 1a). Particles between 10-2000nm are tracked individually (Fig 1b) and their diffusion coefficient, and therefore size, calculated directly from their speed (Fig 1c).

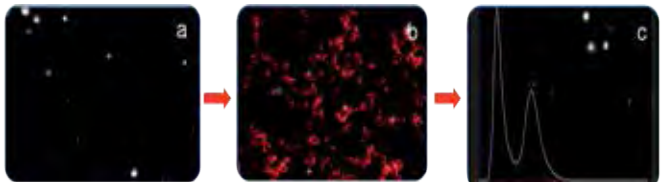


Figure 1: a) Scattered light from particles collected on CCD, b) particles tracked and c) size distribution calculated.

This technique provides significant advantages for sizing over traditional light scatter techniques (e.g. DLS) as the individual tracking of particles results in a greater ability to cope with polydispersed suspensions providing higher resolution.
The technique is also capable of measuring particles in a range of media (not requiring a particular media, or being restricted to pure water). This is relevant across all biological applications, as it is well known that colloidal properties are strongly influenced by their environment.
In addition to its simplistic but powerful sizing approach the technique generates directly a measure of absolute particle concentration, it can be integrated with fluorescence filters to allow fluorescently labelled/loaded particles to be selectively analysed, the application of an electric field across the sample allows the measurement of zeta potential, and the measure of light scatter gives a relative indication of sample material density (or refractive index). Crucially these measurements are not independent but instead give a particle's individual fingerprint with the capability to measure the size, intensity, fluorescence and zeta potential of the same particle. An example of this is shown below (Fig 2) where not only is a tri-modal distribution evident, but it is discernible from the measurement that the 30 and 60nm material (gold, in this case) is of an higher refractive index than the 100nm material (polystyrene here).

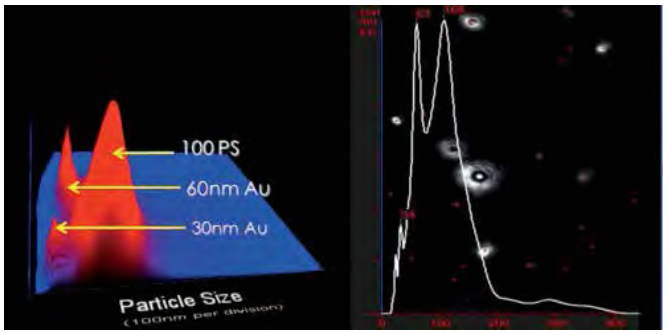
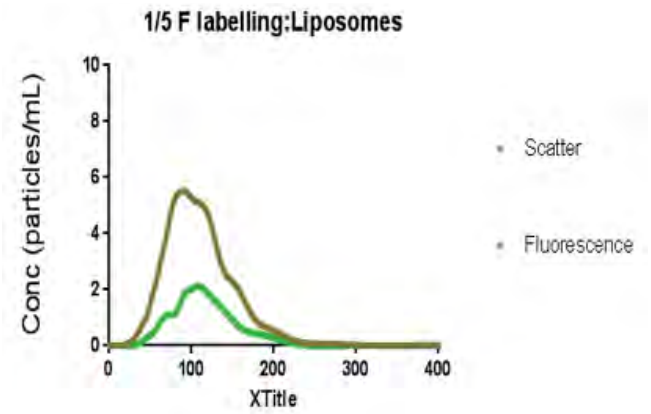


Figure 2: Trimodal measurement of a mixture of 30 and 60nm gold and 100nm latex polystyrene particles.

Also shown below is an example of a fluorescently labelled lipid binding protein tagged with AlexaFluor488 bound to freshly extruded liposomes of size ~100nm as analysed by a NanoSight system operating under fluorescence mode with a 488nm laser fitted.

In the field of pharmaceuticals, a crucial question under inspection is that of protein aggregation and its measurement thereof [1]. In this field NTA has been identified as a technique for characterizing this potentially over previously available techniques [2].



This technique has variously been applied to drug delivery particles such as liposomes, viral vaccines, bacteriophage, VLPs and controlled release drugs. In viral vaccines, for example, where the standard technique for titering a sample (to measure the concentration of virus) takes several days and could not take into account aggregation, the NTA technique is identified as a rapid and accurate alternative [3]. As the technique can also be integrated with fluorescence filters this gives the potential for fluorescently labeled/loaded particles to be selectively analysed. This can be of particular import where a suspension is not purified.
The technique is also being extensively used for characterisation of nanovesicular bodies (also called exosomes) which are shed from cells for amongst other things cell signalling. These bodies are typically in the 50-130nm size range within which NTA is the ideal technique for quantifying these bodies [4]. In this case using NTA in conjunction with fluorescently labelled antibodies, it then becomes possible to speciate the exosomes, which, it is generally believed could lead to significant diagnostic applications.
The technique, several novel developments and their application to the above fields will be described, explaining the importance of obtaining a complete particle characterisation, and in a relevant media. The technique will be compared and contrasted to other techniques. Several examples exploiting both light scattering and fluorescent labelling of samples will be shown.

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A BROAD-SPECTRUM ANTIVIRAL PEPTIDE WITH HIGHLY DISCRIMINATE SUB 100-NM MEMBRANE CURVATURE TARGETING

NAM-JOON CHO[1,2]
[1]School of Materials Science and Engineering, Nanyang Technological University, Singapore
[2]School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore

A recently discovered amphipathic, α-helical (AH) peptide represents a breakthrough, broad-spectrum antiviral drug candidate. It ruptures the lipid envelope of virus particles in a size-dependent manner. The size range of virus particles susceptible to treatment with AH peptide encompasses a wide range of deadly viral pathogens including dengue, hepatitis C virus, and HIV. Uniquely compared to other antiviral medicines in development or in the clinic, the virocidal activity of the AH peptide was originally discovered by surface science techniques probing model biological interfaces—namely lipid vesicles serving as surrogates for lipid-enveloped virus particles. Quartz crystal microbalance with dissipation (QCM-D) monitoring identified that addition of the AH peptide ruptures a layer of intact lipid vesicles to promote structural transformation to a planar lipid bilayer on gold and titanium oxide. Based on this in situ structural transformation, we have used simultaneous QCM-D monitoring and optical reflectometry to determine the antiviral mode of action of the AH peptide, including a vesicle destabilization process which occurs during the binding interaction. Using a newly developed total internal reflection fluorescence microscopy (TIRF)-based single vesicle assay, we have also investigated how AH peptide interacts with lipid membranes to induce pore formation and membrane destabilization. Importantly, the preference of the AH peptide to selectively rupture virus particles of small size appears to be related to highly discriminate sub-100 nm membrane curvature-dependent pore formation. Compared to other known proteins and peptides, AH peptide is unique due to its combination of high potency and specificity for curved membranes. Collectively, these results lay the groundwork for the broader application of surface science techniques to nanomedicine and antiviral drug development.

THE IMPACT OF SIZE AND SURFACE CHARGE ON THE KINETICS OF GOLD NANOPARTICLES

WAN-SEOB CHO[1], Bong Hyun Chung[2], Jayoung Jeong[3]
[1]Department of Medicinal Biotechnology, College of Natural Resources and Life Science, Dong-A University, Busan 604-714, Republic of Korea
[2]Biotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejon 305-700, Republic of Korea
[3]Toxicological Research Division, National Institute of Food and Drug Safety Evaluation, Ministry of Food and Drug Safety, Osong 363-700, Republic of Korea

The behavior and fate of intravenously injected nanoparticles (NPs) can be controlled by several physicochemical factors including size, shape, and surface charge. In this study, gold nanoparticles (AuNPs) were selected as a model NPs for evaluation of impact of size or surface charge on the kinetics of AuNPs. For evaluation of the impact of size, PEG-coated AuNPs (4, 13, and 100 nm) were synthesized and intravenously injected into the mice at a dose of 1 mg/kg body weight and the concentration of Au were measured in several organs by inductively coupled plasma-mass spectroscopy (ICP-MS). Small AuNPs (4 or 13 nm) showed high levels in blood for 24 h and were cleared by 7 days, whereas large (100 nm) AuNPs were completely cleared by 24 h. Levels of small AuNPs were peaked at 7 days in the liver and spleen and at 1 month in the mesenteric lymph node, and remained high until 6 months, with slow elimination. In contrast, large AuNPs were taken up rapidly (~ 30 min) into the liver, spleen, and mesenteric lymph nodes with less elimination phase. To evaluate the impact of surface charge, we used neutral-charged 15 nm-sized PEG-coated AuNPs (AuNPPEG) as a core NP and carboxyl or amine groups were conjugated to AuNPPEG to generate negative (AuNPCOOH) or positive AuNP (AuNPNH2), respectively. Each type of AuNP was intravenously injected into mice at a dose of 1 mg/kg body weight and the concentration of Au was measured in different organs by ICP-MS. The levels of AuNPCOOH and AuNPNH2 in the blood were minimal even at 30 min post-injection whilst AuNPPEG showed high levels at 30 min and 4 h post-injection. The organ distribution also showed the higher deposition rate depending on their functional groups: AuNPPEG for mesenteric lymph node, kidney, brain, and testis; AuNPCOOH for liver; AuNPNH2 for spleen, lung, and heart. All AuNPs had a low elimination rate but the major route of elimination was different depending on their functional groups: AuNPCOOH for bile; AuNPPEG and AuNPNH2 for urine. In conclusion, both size and surface charge of AuNPs produce differences in blood kinetics, organ distribution, and elimination pattern which can be important information for directing NPs to specific organs or improving the kinetic properties.

POLYSACCHARIDE NANOPARTICLES FOR ANTICANCER DRUG DELIVERY

TOMASZ CIACH
Warsaw University of Technology, Faculty of Chemical and Process Engineering, Biomedical Engineering Laboratory, Warynskiego Street 1, 00-645 Warsaw, Poland; and NanoVelos, Trakt Lubelski Street 40A, 04-870 Warsaw, Poland; t.ciach@ichip.pw.edu.pl

INTRODUCTION

Cancer is one of the biggest challenges of contemporary medicine. Despite the enormous scientific and financial effort undertaken in recent years cancer remains one of the major death causes in western civilization. Thanks to this effort various new drugs have been developed but still many types of cancer in metastasis are virtually untreatable. A lot of hope for new treatment methods is in nanotechnology. This is because all the cellular machinery and transport phenomena are happening exactly at this size range. Nanoparticles seem to be a perfect drug carrier for cancer treatment. They offer passive and active cancer area targeting, what promises an effective treatment without the main problem of contemporary cancer chemotherapy – toxic side effects. Passive targeting comes from the size; freshly formed vasculature is permeable for small objects. Active targeting can be achieved by decoration of the nanoparticles surface with metabolites or antibodies. Nanoparticles may carry various anticancer drugs, even multiple drugs with synergistic effect. Such combinatorial therapy is more efficient and prevents development of drug resistance in quickly mutating cancer calls.

First scientific description of nanoparticles synthesis was presented by Michel Faraday in the 19-th century. Since that time many new types of nanoparticles were synthesized. Fate of nanoparticles in our body strongly depends on their surface chemistry. Every foreign body present in the circulatory system, even of nanometric size, can be quickly detected by the complement immunological system and removed. To prevent these phenomenon nanoparticles should not induce small blood plasma protein adsorption. Low surface energy is a way to avoid it and hydrogels, moieties that are highly hydrophilic and contain a lot of water, are the material of choice. Example of this solution is polyethylene glycol currently applied to coat various silica nanoparticles.

Nanoparticles can be biodegradable and nonbiodegradable. Unfortunately nonbiodegradable nanoparticles, the majority of currently manufactured nanoparticles, are dangerous for most types of human cells. Nanoparticles usually get into the cell by endocytosis and are located in endosomes. Endosomes change into lysosomes – digesting vesicles. If the content of such vesicles cannot be hydrolyzed or absorbed, it stays inside the cell interior forever. Accumulation of such residual lysosomes leads to lysosomal storage disease, and the Alzheimer's disease is an example of such.

Perfect materials that are both hydrophilic and biodegradable are polysaccharides. Polysaccharides are susceptible to chemical modification and highly biocompatible; they also prevent small protein adsorption. Additional advantage of polysaccharide structure is the

possibility of cancer cells targeting by the presence of glucose moieties at the surface. Preparation of nanoparticles from polysaccharide gives enhanced targeting properties thanks to increased demand of cancer cells for glucose. As Otto Warburg proved, glucose demand of cancerous cells is about 200 times higher than of primary ones. Following this thought, nanoparticles composed of polysaccharides have a better chance to be up taken by malignant cells. This phenomenon has been already employed in PET cancer diagnosis.

METHODS

Polysaccharide nanoparticles were prepared from Dextrane (poly α-1,6 α-1,3 glucose, 40 and 70 kDa), which is already widely used in medical field to prepare blood expanding solutions. Due to its polysaccharide structure dextran can be easily oxidized to polyaldehydedextran in the reaction with sodium periodate in aqueous solution. In this reaction glucose rings are open and oxidized without breaking of the polysaccharide backbone. Obtained aldehyde groups are covalently linked with hydrophobic side groups like hydrophobic amino acids (leucine) or aliphatic amines. This leads to the self assembly process, which results in nanoparticles formation. Same mechanism of covalent bonding can be applied to various drugs, targeting molecules or fluorescent markers. Nanoparticles production process is performed in water at room temperature without the use of any surfactants or organic solvents.
A series of Doxorubicin and Daunorubicin containing nanoparticles, of various sizes and drug content were prepared. Nanoparticles size distribution was investigated using Malvern Z-Sizer and Nano Sight devices. Drug release rate measurements were performed in the specially designed setup with semi permeable membranes. Stability of obtained nanoparticles in various storage conditions was also investigated. Toxicity of drug containing nanoparticles and empty carriers for various types of mammalian cells, normal and cancerous, were investigated in vitro. Toxicity of drug containing nanoparticles on animals was investigated on nude and white mice. Therapeutic efficacies against various types of human and animal cancers, including multidrug resistant cancers, were also investigated on nude and white mice, life expectancy and tumor sizes were recorded. Toxic side effects were investigated by body weight measurement and also by weighting of selected organs: liver, heart and spleen. Targeting properties of polysaccharide nanoparticles were performed with fluorescence test, drug concentration in tumor and in various organs were estimated.

RESULTS

Dextran, depending on the molecular weight, degree of functionalization and the size of hydrophobic molecule attached, forms stable nanoparticles of a narrow size distribution of 30 – 120 nm in diameter. Nanoparticles are stable, after freeze-drying and room temperature storage; nanoparticles reassemble in water after a short time. Nanoparticles are efficiently transported into the cell interior by endocytosis and are able to carry various drugs and fluorescent markers. It was investigated that nanoparticles containing Doxorubicin, Daunorubicin, 9-aminoacridine undergo quick endocytosis and can be found in endosomes. Trypan Blue, which never gets into the living cells and is used as a live/dead stain, encapsulated in polysaccharide nanoparticles was efficiently transported inside. Experiments on tumor bearing mice revealed targeting properties of polysaccharide nanoparticles. Concentration of nanoparticles in the tumor was about 5 times higher than in other organs. No accumulation of nanoparticles in the spleen, liver, kidney or heart was detected. Due to that phenomenon toxic side effects of the therapy were much lower as compared to the same dose of pure drug. Recorded tumor grow rate suppression depends on the dose administered, drug type and tumor type. Life length expectancy of tumor bearing mice was always higher in the case of polysaccharide nanoparticles administration.

THE UBIQUITIN PROTEOLYTIC SYSTEM - FROM BASIC MECHANISMS THRU HUMAN DISEASES AND ON TO DRUG DEVELOPMENT

AARON CIECHANOVER
Cancer and Vascular Biology Research Center, Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel

Between the 50s and 80s, most studies in biomedicine focused on the central dogma - the translation of the information coded by DNA to RNA and proteins. Protein degradation was a neglected area, considered to be a non-specific, dead-end process. While it was known that proteins do turn over, the high specificity of the process - where distinct proteins are degraded only at certain time points, or when they are not needed any more, or following denaturation/misfolding when their normal and active counterparts are spared - was not appreciated. The discovery of the lysosome by Christian de Duve did not significantly change this view, as it was clear that this organelle is involved mostly in the degradation of extracellular proteins, and their proteases cannot be substrate-specific. The discovery of the complex cascade of the ubiquitin solved the enigma. It is clear now that degradation of cellular proteins is a highly complex, temporally controlled, and tightly regulated process that plays major roles in a variety of basic cellular processes such as cell cycle and differentiation, communication of the cell with the extracellular environment and maintenance of the cellular quality control. With the multitude of substrates targeted and the myriad processes involved, it is not surprising that aberrations in the pathway have been implicated in the pathogenesis of many diseases, certain malignancies and neurodegeneration among them, and that the system has become a major platform for drug targeting.

UNRESOLVED ISSUES IN CHRONIC INFLAMMATION

ANDY CLARK

The inflammatory response is an indispensible defence against injury and infection, necessary for our survival in the face of countless pathogens and other environmental hazards. Yet inflammation is also an underlying cause of many pathologies that create enormous economic burdens in both developed and developing countries. These include conditions in which the inflammatory component is obvious, such as asthma, sepsis or rheumatoid arthritis, as well as others in which the contribution of inflammation is absolutely crucial but less obvious. The latter class includes cancers, cardiovascular disease, metabolic syndrome and age-related decline of immune function. Much of the research of the last few decades has focused on how inflammatory processes are initiated. Enormous progress has been made in identifying triggers of inflammation and understanding the molecular events that they set in motion. Many quite successful new drugs have emerged from this focus on the initiation of inflammation. Yet there is a growing realization that it provides at best an incomplete picture of when, where and how chronic inflammation becomes established. Furthermore, drugs that are designed to block the initiation of inflammation run a significant risk of impairing our defences against infectious disease. We are equipped with many endogenous control mechanisms, whose normal function is to ensure that inflammatory responses are appropriate to the challenges that evoke them: in other words that inflammation is restricted in anatomical location, and proportional in strength and duration. These mechanisms represent considerable barriers against the establishment of chronic inflammation. It follows that chronic inflammatory diseases must involve some defect or breakdown of the endogenous mechanisms of restraint. A greater understanding of how inflammation is normally switched off or resolved will provide new insights into the aetiology of chronic disease. In future, safer and more effective treatments may be based on reinforcement or repair of endogenous mechanisms for the restraint of inflammation, or therapeutic use of key molecules that participate in these processes.

"SQUALENOYLATION/TERPENOYLATION": AN ORIGINAL CONCEPT FOR THE DISCOVERY OF NEW NANOMEDICINES

PATRICK COUVREUR
UMR CNRS 8612, Institut Galien, Université Paris-Sud (France)

The introduction of nanotechnology into pharmacology ("nanomedicine") has importantly influenced the drug delivery field, allowing the appearance of new targeted treatments, more specific and with improved efficacy. So far, current nanotechnologies have serious limitations due to:
(i) Poor drug loading which is usually < 10 % (weight % of the transported drug with respect to the carrier material). As a consequence, either the quantity of the drug administered is not sufficient to reach a pharmacologically active concentration in the body, or the amount of the carrier material to be administered is too high, engendering toxicity or side effects and
(ii) Uncontrolled rapid release (known as "burst release") of the fraction of the drug molecules when adsorbed at the surface of the nanocarrier.
This explains the limited number of marketed nanomedicines, despite the large number of publications in the field. There is, therefore, an urgent need for new ideas to revolutionize drug delivery
Terpenoids are a group of natural compounds that are extraordinary diverse in chemistry, structure and function. Most of the natural terpenoids are flexible and biocompatible biopolymers, having physico-chemical characteristics able to adapt to a wide variety of biologically active compounds. Among them, squalene (SQ) is a natural triterpene which has the unique property of being transformed into the cyclic derivative lanosterol (a precursor of the cholesterol) by spontaneously passing through a highly coiled, compact molecular conformation. Surprisingly, although squalene is a natural and biocompatible lipid known for its dietary benefits, it was never used in the drug delivery and nanomedicine field. In the context of the ERC Advanced Grant TERNANOMED, we have developed the concept of "squalenoylation/terpenoylation" which consists in the chemical linkage of anticancer or antimicrobial compounds to the squalene or to other terpenes. The resulting bioconjugates spontaneously self-assemble as nanoparticles in aqueous medium, displaying various supramolecular organizations, depending on the nature of the drug/terpene pair. Noteworthy, the nature of the polyterpenoid (ie. number of isoprenoid units) may be adapted to the hydrophilic/lipophilic character of the drug molecule to be transported, whereas the nature of the linkage (ester, amide, disulfide bonds etc.) may be selected according to the enzymatic content of the targeted diseased area. From the ratio between drug's and polyterpene's molecular weights, it is deduced that the drug loading may be dramatically improved as compared to the currently available nanomedicines. In other words, the prodrug forms the nanomedicine by self-aggregation without the need of any other transporter material.
The lecture will show how this breakthrough approach allows to design more efficient and less toxic nanomedicines for the treatment of cancer (1-3) and infectious diseases (4,5). Special attention is given to the ability of those squalene-based nanomedicines to overcome mechanisms of resistance (6). Noteworthy, by combining various drugs together or a pharmacologically active compound with an imaging agent, it is possible to design multi-functional nanomedicines in a lego-like approach (7).

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HOW FOCUSSING ON THE PATIENT TRANSFORMS CLINICAL SUCCESS INTO MARKET SUCCESS

TOM CRABBE
BSc., Director, External Discovery Solutions, UCB, Slough (UK)

Considerable attention has been given to increasing the chances of Phase 3 clinical success when attempting to bring novel medicines to patients. It is perhaps underappreciated that this does not guarantee a successful conclusion to the journey so in this brief presentation I will highlight the hurdles that will still need to be overcome. More than ever it is true that keeping in focus the needs of the patient will help to complete this final stage.

BIOLOGICAL INTERACTIONS BETWEEN NANOSCALE LIVING ORGANISMS AND NOVEL METHODS TO INVESTIGATE THEM

KENNETH DAWSON

Nanoscale materials can interact with living organisms in a qualitatively different manner than small molecules. Crucially, biological phenomena such as immune clearance, cellular uptake and biological barrier crossing are all determined by processes on the nanometer scale.
Whilst nanoparticle size is important, the detailed nature of the nanoparticle interface is key to understanding interactions with living organisms. This interface may be quite complex, involving also adsorbed proteins from the biological fluid (blood, or other), leading to a 'protein corona' on the nanoparticle surface that determines its "biological identity". We report that this arena is now beginning to develop rapidly, and we believe that it may soon be possible to show applicable impacts ranging from nanomedicine safety, to nano-systems biology.

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ALBUMIN BOUND SIROLIMUS NANOPARTICLES (ABI-009) – EARLY CLINICAL STUDIES

NEIL P. DESAI
PhD, President and CEO, AADi LLC, Pacific Palisades, California, USA

Albumin-bound sirolimus nanoparticles (nab-rapamycin, ABI-009) is a novel albumin-based nanoparticle version of sirolimus (rapamycin) with a mean particle size of approximately 100 nm, that can be delivered by intravenous (IV) or intravesical administration. This technology has previously achieved significant commercial success with marketing approval for several oncology indications in the case of albumin-bound paclitaxel.
In nonclinical studies, ABI-009 was well tolerated and demonstrated remarkable antitumor activity both as single agent and in combination against various solid tumors. In a phase I dose-finding study in 26 patients with untreatable advanced nonhematologic malignancies, the maximum tolerated dose (MTD) of ABI-009 administered IV weekly (3 of 4 weeks) was determined to be 100 mg/m2, which was well tolerated with evidence of response and stable disease, and produced a fairly dose-proportional pharmacokinetic (PK) profile. Pharmacodynamic (PD) analysis showed that mTOR targets S6K and 4EBP1 were significantly inhibited by ABI-009 treatment. Of 18 evaluable patients, 2 (11%) patients with adenocarcinoma of the kidney had >30% decrease in the target lesion, and 13 (73%) patients had a target lesion objective response evaluation of stable disease. These patients had cancer of the bladder, colorectal, esophagus, head and neck, prostate, retroperitoneal, or uterus. In addition, 3 (17%) patients had target lesion objective response of progressive disease.
Hyperactivation of mTOR pathway has been found in human bladder cancer and is associated with reduced survival. Intravesical rapamycin treatment was effective in suppressing the growth of carcinoma in situ in a genetically engineered animal model of bladder cancer. Based on these findings, a new multi-center phase 1/2 study to investigate the intravesical use of ABI-009 to treat early stage (non-muscle invasive) bladder cancer has recently been initiated.
In addition to human cancers, abnormal activation the mTOR pathway occurs in a number of proliferative conditions, e.g., following endovascular interventions in atherosclerosis where the proliferation of medial smooth muscle cells (SMCs) and inflammation can lead to restenosis. Indeed, in a porcine femoral
artery balloon angioplasty model, ABI-009 intralesional (adventitial) injection via a microinfusion catheter was safe and significantly reduced luminal stenosis, reduced early adventitial leukocyte infiltration, and reduced late medial cell proliferation and fibrosis, suggesting the usefulness of this as an alternative to stent- or balloon-based local drug delivery.

UPDATE ON NEW CLINICAL STUDIES WITH ABRAXANE

NEIL P. DESAI
PhD, VP, Strategic Platforms at Celgene Corporation /Abraxis BioScience LLC, Los Angeles CA (USA)

nab-Paclitaxel (ABRAXANE®) is an albumin-bound nanoparticle form of paclitaxel. Albumin is a key plasmic carrier of hydrophobic molecules, such as paclitaxel, and is highly accumulated in tumors, which may play a key role in the more efficient accumulation of nab-paclitaxel in tumor tissues versus organs. To date, there have been 4 large completed global phase 3 trials with nab-paclitaxel in patients with metastatic disease and all 4 trials successfully met their endpoints. As a result, nab-paclitaxel was approved for the treatment of metastatic breast cancer (MBC) in over 40 other countries globally, and more recently, it was approved in the US for the first-line treatment of locally advanced or metastatic nonsmall-cell lung cancer (NSCLC), in combination with carboplatin and in US and Europe for the first-line treatment of metastatic pancreatic ductal adenocarcinoma (mPDA), in combination with gemcitabine. The recently completed phase 3 trial in chemotherapy-naïve patients with metastatic melanoma has also met its primary endpoint of progression-free survival (PFS), produced an improved trend in overall survival (OS), and an improved safety profile versus standard dacarbazine therapy (Hersh, SMR 2012).
Specifically, in the randomized open-label international study in patients with mPDA (MPACT study), the updated OS with a cutoff of May 2013 showed that the benefit continued to improve with nab-paclitaxel in combination with gemcitabine as first-line therapy, with 8.7 vs 6.6 median months, respectively (Goldstein, ASCO GI 2014). The updated survival rates also significantly favored nab-paclitaxel plus gemcitabine at year 1 (35% vs 22%), year 2 (10% vs 5%), and year 3 (4% vs 0%) as compared with gemcitabine alone. To date the survival monitoring is continuing in a separate follow-up study on the 3% patients who are still alive in the nab-paclitaxel arm (NCT02021500). Since the success of MPACT, this regimen had become the backbone of therapeutic combinations and there are over 30 recruiting trials listed on clinicaltrials.gov that investigate the utility of nab-paclitaxel in combination with various agents in patients with pancreatic cancer in the neoadjuvant, adjuvant, and metastatic setting.
Phase 1-2 studies have shown promising efficacy with tolerable safety profile of nab-paclitaxel plus other agents in various solid tumors, including triple negative (TN) MBC, bladder cancer, advanced gastric cancer, advanced head and neck cancer, metastatic melanoma, and advanced ovarian cancer. In a phase 2 study of TN MBC, 28% response rate was achieved with nab-paclitaxel combined with tigatuzumab and 32% in the nab-paclitaxel alone arm (Vaklavas, ASCO 2013). In another phase 2 study, patients treated with operable TN breast cancer treated with nab-paclitaxel plus carboplatin plus bevacizumab achieved 85% response rate and a median PFS of 9.2 months (Hamilton, 2013). In a phase 2 study of Japanese patients with unresectable gastric cancer, nab-paclitaxel with S-1, an oral fluoropyrimidine, achieved 28% response rate and a promising safety profile (Takiuchi, ASCO GI 2012). In phase 1 studies promising activity has been observed in patients with head and neck cancer receiving combination regimens with nab-paclitaxel. nab-Paclitaxel combined with cisplatin and 5-FU achieved 77% complete response rates in 23 patients (Ley, ASCO 2014). Similarly, in another trial nab-paclitaxel with cisplatin, 5-FU produced 63% response rate and 31% stable disease (Loong ECCO 2013). In a phase 2 multicenter study in patients with metastatic melanoma, nab-paclitaxel combined with bevacizumab had significant activity, and was well tolerated. Of 50 patients 36% had a response rate, with 7.6 months of PFS and 16.8 months of median OS, which is among the highest reported in phase 2 studies (Spitler, AJCO, 2013). In phase 2, nab-paclitaxel with bevacizumab demonstrates substantial antitumor activity and manageable toxicity profile in patients with platinum-resistant recurrent ovarian carcinoma, with 50% response rate and 29% stable disease, and median OS of 17.2 months (Tillmanns, 2013).
Currently, there are also 3 phase 3 clinical studies by Celgene to investigate the efficacy and safety of nab-paclitaxel in triple negative MBC, in the adjuvant treatment of nonmetastatic pancreatic cancer, and squamous cell NSCLC – all 3 trials have recently begun enrollment and results are expected in the next few years.

IMMUNOTOXICITY OF NANOMEDICINES - CLINICAL AND REGULATORY ASPECTS

JACQUES DESCOTES
Prof. Dr. med., PharmD, PhD, fellow ATS
Head, Poison Center and Pharmacovigilance Department
Lyon University Hospitals, Lyon (France)

The applications of nanomedicines as drug delivery systems, targeted nanotherapeutics, imaging nanotools, or diagnostic nanochips and nanosensors are expanding. The properties of materials at the nanoscale (i.e. <100 nm) markedly differ compared to larger-

scale materials of the same substance, and this is suspected to potentially result in somewhat different or unexpected toxic effects. When assessing the potential of nanomedicines/nanomaterials to induce adverse effects on the immune system, i.e. immunotoxic effects, several issues need to be addressed. Cells of the immune system recognize nanoparticles by their surface properties and core composition, and this may trigger an inflammatory response depending on their physiochemical properties, for instance via the release of cytokines. Nanoparticles can also interact with blood constituents and hence lead to activation of the complement cascade. Immunotoxic effects in the clinic as well as in the preclinical setting should be best subdivided into 4 categories, namely immunosuppression, immunostimulation, hypersensitivity and autoimmunity. It is unknown to what extent data derived from nanoparticles in general do apply to nanomedicines that are typically more complex. Because very few nanomedicines have reached the clinical setting, the available information on the immunotoxic effects of nanomedicines in humans is very limited. However, based on the experience gained on the clinical immunotoxicity of other medicinal products, such as biopharmaceuticals, and the growing understanding of immunotoxic effects observed in preclinical studies, a reasonable prediction on the clinical immunotoxicity of nanomedicines can be proposed. That nanomedicines can prove to suppress and/or stimulate immune responses, as intended or unintended effects, is confirmed with the current development of nanoparticles engineered to generate immunosuppressive nanomedicines. Similarly, nanomedicines can be used as vaccine adjuvants, and asthma aggravation and more frequent allergic manifestations in human subjects exposed to potentially immunostimulatory nanoparticles have been reported. Nanoparticles can induce inflammatory reactions and this may result in either immunosuppressive or immunostimulatory effects. Immune-mediated hypersensitivity reactions similar to “drug allergies” are assumed to be unlikely. However, depending on their chemical structure, nanoparticles can be immunogenic and thus trigger antigen-specific hypersensitivity reactions similar to reactions reported with biotherapeutics. In addition, non-immune hypersensitivity reactions due to direct histamine release or complement activation may occur and this potential should be investigated at the preclinical stage. The potential for inducing autoimmunity is ill elucidated, either with nanomedicines or conventional medicinal products. Because of nanomedicine novelty, only limited knowledge is available and there is currently no agreed-upon guideline or even general consensus on the most appropriate preclinical approach for assessing the immunotoxic potential of nanomedicines. However, the majority of current standard immunotoxicological methods is deemed to be applicable, even though modifications of these methods as well as novel approaches may be required.

DYNAMIC NANO-IMAGING OF COMPOUND DELIVERY BY MULTISPECTRAL OPTOACOUSTIC TOMOGRAPHY (MSOT)

WOUTER H.P. DRIESSEN[1,2],*, N. C. Burton[1,2], J. Claussen[1,2], T. Sardella[1], S. Morscher[1,2,3], D. Razansky[2,3], V. Ntziachristos[2,3]
[1] iThera Medical, Zielstattstr. 13, 81379, Munich; wouter.driessen@ithera-medical.com
[2] Helmholtz Zentrum München
[3] Technische Universität München
* Corresponding author

A wide range of nanoparticles differing in size, shape and surface characteristics are available for therapeutic and diagnostic applications in oncology. To ensure optimal accumulation of nanoparticles in solid tumors, it is crucial to obtain quantitative data for their rational design. We employed a sensitive and specific imaging modality, termed Multispectral Optoacoustic Tomography (MSOT), to dynamically evaluate nanoparticle pharmacokinetics and biodistribution in mice with orthotopic tumors. MSOT is a biomedical imaging modality based on the photoacoustic effect. In the used setup, mice are illuminated at multiple wavelengths in the NIR range (680 – 980nm) and by detecting the acoustic waves with a 5MHz tomographic ultrasound array, cross-sectional images can be obtained at a resolution of 150µm in less than a second. Imaging multiple cross-sections allows for volumetric reconstruction of the data and multispectral data analysis enables the specific identification of endogenous absorbers and/or multiple injected contrast agents. Absorption by intrinsic tissue chromophores (e.g. oxygenated and deoxygenated hemoglobin, melanin) provides rich anatomical contrast, while signal from exogenously administered contrast agents can be multispectrally resolved simultaneously allowing for functional and molecular imaging.

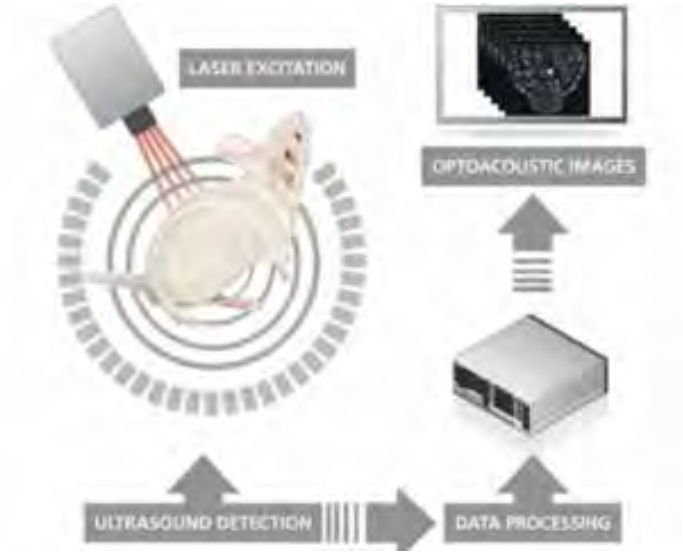

Figure 1. When a sort laser pulse irradiates an absorbing medium there is local absorption that, in turn, leads to ultrasonic pressure waves. By using an array of ultrasound sensors the temporal delay of these pressure wave fronts can be combined into a photoacoustic image. Images are collected at multiple wavelengths, allowing multispectral unmixing of specific absorbers.

In this work, the ability of MSOT to track nanoparticle whole-body biodistribution and pharmacokinetics is demonstrated. Nanoparticles containing near-infrared (NIR) fluorescent dyes were injected intravenously into mice and the accumulation and clearance of the nanoparticles over time were observed by MSOT. Regions of interest included liver, spleen, kidneys, brain, heart and vasculature. The acquisition of data at 10 Hz allowed the visualization of the fast uptake kinetics, while longitudinal data acquisition allowed the determination of the differential pharmacokinetic properties of each compound. Especially by combining MSOT imaging with Dynamic Contrast Enhancement techniques (DCE-MSOT), detailed differential uptake in various organs and tumors could be evaluated at sub-tissue resolution. Moreover, the arterial input function for DCE-MSOT kinetic models could be determined by MSOT as well, leading to more accurate parametric imaging (Figure 2).

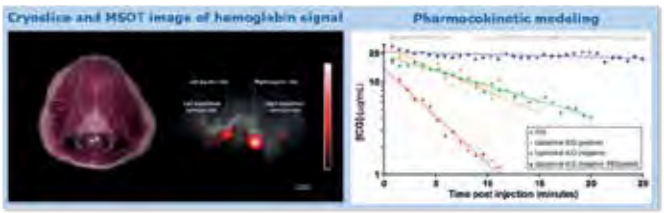

Figure 2. By employing ROI-analysis on a major blood vessel, in this case the jugular vein, the blood pharmacokinetics of nano-formulations can be determined non-invasively. The derived pharmacokinetic parameters are then used to define the arterial input function for DCE-MSOT.

When employing DCE-MSOT to assess nano-material delivery to orthotopic breast tumors, heterogeneous patterns of maximum delivered dose and Tmax are observed, due to the high degree of heterogeneity of vascular supply within tumors. Areas of relative hypoxia can be visualized by MSOT imaging and correlate with delayed particle delivery (Figure 3).

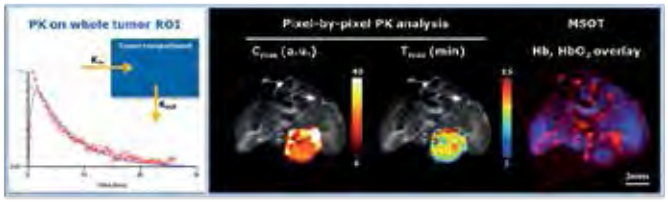

Figure 3. Parametric maps reveal heterogeneity with regard to Cmax and Tmax. Tumor areas with a lower Cmax and longer Tmax correlate with areas of relative hypoxia within the tumor.

In summary, MSOT offers a new and unique imaging modality that (1) has a resolution at least ten-fold higher than nuclear and optical imaging, (2) allows for real-time imaging with molecular specificity through several centimeters of tissue and (3) is safe (i.e. no ionizing radiation) and cost-efficient. With the ability to visualize and quantify fast kinetics and organ specificity of injected NIR-absorbing agents of interest, MSOT is poised to become an invaluable tool in the rational design process of nano-materials by enabling whole-body in vivo visualization of nano-formulation biodistribution.

MULTIFUNCTIONAL NANOMEDICINES FOR INFECTIOUS DISEASES: ENHANCING CELLULAR INNATE IMMUNE RESPONSES AND INTRACELLULAR DRUG DELIVERY USING NANOPARTICLES

ADMIRE DUBE[1], Jessica L. Reynolds[2], Wing-Cheung Law[3], Charles C. Maponga[4], Paras N. Prasad[3], Gene D. Morse[5]
[1]Council for Scientific and Industrial Research, South Africa
[2]Department of Medicine, University at Buffalo, Buffalo NY
[3]The Institute for Lasers, Photonics and Biophotonics, University at Buffalo, Buffalo, NY
[4]School of Pharmacy, University of Zimbabwe
[5]Translational Pharmacology Research Core, School of Pharmacy and Pharmaceutical Sciences Center of Excellence in Bioinformatics and Life Sciences, University at Buffalo, Buffalo, NY

Infectious diseases are a worldwide health concern. Human immunodeficiency virus/ acquired immunodeficiency syndrome (HIV/ AIDS), tuberculosis (TB) and malaria rank among the most deadly of infectious diseases. The World Health Organization reported over 1.7 million deaths from HIV in 2011, 1.4 million deaths from TB in 2012 and 660,000 deaths from malaria in 2010. One feature of infectious disease pathogens is their residence in the intracellular space, where they are able to evade the immune system, persist and multiply to infect other cells. In the case of HIV, TB and malaria, the pathogens are present in CD4+ T cells, macrophages and red blood cells, respectively, at some stage of the infection. Infectious disease pathogens have developed complex mechanisms to evade clearance by the host. For example, Mycobacterium tuberculosis (M.tb), the causative pathogen of TB, is able to persist within macrophages by suppressing the antimicrobial response of the macrophage, through mechanisms which include suppression of intracellular generation of bactericidal reactive oxygen and nitrogen species (ROS/RNS), and secretion of pro-inflammatory cytokines such as Interleukin-12 (IL-12) and Interferon-gamma (IFN-γ). With such knowledge of intracellular events which pathogens exploit for their survival, nanomedicines could be designed to impact the immunological aspects of the diseases in addition to delivering drug pay-load. We view this as a new generation of "intelligent" nanomedicines for infectious diseases, functionalized through carefully selected pharmacologically active ligands carried on the surface of nanoparticles, to modulate the immune response at the cellular level, and concurrently deliver therapeutic drug concentrations inside the cell (Figure 1).

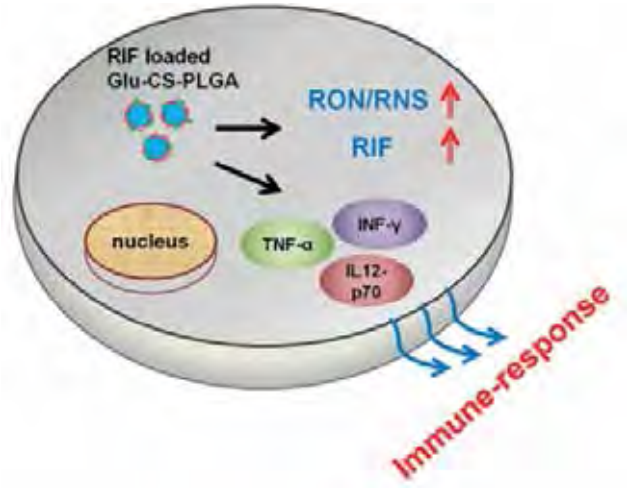

Figure 1: Schematic illustration of the application of multifunctional nanoparticles on cellular immune response (and drug delivery). In the above example the multifunctional nanoparticles for tuberculosis therapy, i.e. rifampicin loaded 1-3-β-glucan –Chitosan-PLGA nanoparticles (RIF loaded Glu-CS-PLGA) interact with surface reeptors (i.e. Dectin-1) and downstream signalling pathways result in increased pro-inflammatory cutokine production (TNF-α, INF-γ, IL-12p70) and reactive oxygen and nitrogen species (ROS/RNS). Phagocytosis of the nanoparticles also results in increased intracellular concentrations of the ant-tubercular rifampicin (RIF). This represents a multimodal approach to eradication of intracellular pathogens as the induced immune response could act synergistically with the action of the drugs to eradicate the pathogen.

To demonstrate proof of concept of this approach, we synthesized 1,3-β-glucan functionalized chitosan poly(lactide)co-glycolide nanoparticles (Glu-CS-PLGA) loaded with the anti-tubercular drug rifampicin, and determined the impact of the particles on macrophage immune response, intracellular drug concentrations and pharmacokinetics of rifampicin. 1.1-3-β-glucan is a molecule known to have immunomodulatory activity. 1,3-β-glucan, activates Dectin-1 on macrophage surfaces, subsequently activating various downstream signal transduction pathways which promote intracellular ROS/RNS generation as well as pro-inflammatory gene expression. Pro-inflammatory cytokines produced through Dectin-1 activation include IL-12 and IFN-γ. We observed that the nanoparticles could significantly enhance macrophage secretion of the pro-inflammatory cytokines IL-12p70 (2.9-fold), TNF-α (16-fold) and INF-γ (23-fold) compared to controls over 24 h (Figure 2).

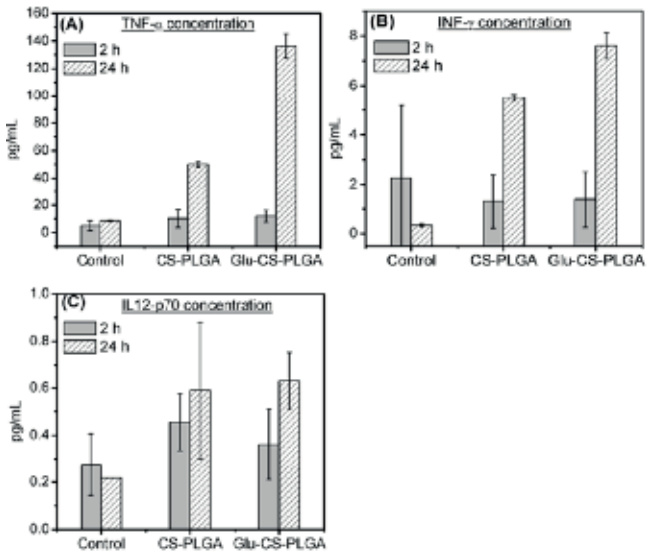

Figure 2: Concentrations of (A) TNF-α (B) IFN-γ and (C) IL-12p70 in macrophages supernatants in control macrophages (absence of nanoparticles) and macrophages incubated with 100 µl of 0.1% v/v aqueous CS-PLGA and Glu-CS-PLGA Nanoparticle suspensions for 2 h and 24 h. Data shown is normalized to 5 × 105 cells.

No significant difference in concentrations of IL-4 and IL-10 at 2 and 24 h ($P > 0.05$), respectively, following uptake of the nanoparticles, compared to control (absence of nanoparticles) was observed. The nanoparticles doubled ROS/RNS generation by the macrophages over 6 h compared to nanoparticles without ligand and RIF solution. The nanoparticles could deliver 4-fold greater rifampicin into ALM compared to rifampicin solution, and intracellular levels were maintained over a 24 h period. These results provide proof-of-concept of multimodal nanoparticles able to stimulate the cellular innate immune response and concurrently deliver drug into cells. Such an approach therefore has application in developing nanomedicines to combat infectious diseases in which pathogens reside within cells.

THE UNDERSTANDING OF CELL MORPHOLOGY AND NANOSCALE STRUCTURAL CHANGE CAN LEAD TO A FRAMEWORK, OBJECTIVE MEASURES AND TREATMENTS THAT CAN HELP RESTORE THE BODY TO A PRE-INJURY STATE: MOLECULAR MEDICAL DEVICES TO STABILIZE AND REPAIR TISSUE

RUTLEDGE ELLIS-BEHNKE[1,2,3,4,5], Yu-Xiang Liang[5], Sunny Cheung[5], David KC Tay[5]
[1]Dept. of Ophthalmology, Medical Faculty Mannheim of the University of Heidelberg, Germany
[2]Dept. of Brain & Cognitive Sciences, MIT
[3]Dept. of Neurosurgery, University of South Florida Medical School
[4]Institute for Regenerative Medicine, Wake Forest University Medical School
[5]Dept. of Anatomy, University of Hong Kong Faculty of Medicine

Regeneration is dependent upon three things: (1) an environment that will permit the body to heal itself; (2) stabilizing the injury site: by immediate hemostasis; by preserving tissue; stopping the invasion of bacteria, and foreign bodies, while also controlling inflammation; and (3) an objective measure that can be used to monitor the progress non-invasively.

AN UPDATE ON WOUND AND TISSUE STABILIZATION

Control of the healing process is critical for recovery. (1) A barrier needs to be created to stop bleeding and exclude bacteria and dirt; and (2) hydration control is critical for the preservation of organ function: too little, the kidneys fail; too much and the lungs fill, causing pneumonia and/or death.

- Stopping bleeding in less than 15 seconds without clotting: We have shown that hemostasis can be achieved in less than 15 seconds in multiple tissues, as well as a variety of different wounds, using a self-assembling peptide that establishes a nanofiber barrier incorporating it into the surrounding tissue to form an extracellular matrix in both large and small animal models. Hemostasis that does not rely on heat, pressure, platelet activation, adhesion, or desiccation.
- Creating an environment that enables healing to progress in much the same as organogenesis with reduced inflammation along with matching modulus of the tissue.
- Preserving cells: We have demonstrated that when cells are placed in a defined system it is possible to delay their proliferation, differentiation and maturation depending on the density of the cell population, density of the matrix, and the local environment, and possibly eliminating the need for immuno-suppressants.

TRANSLATIONAL DEVELOPMENT

There have been some recent breakthroughs in nanomedicine research in both animals and humans: using combination devices for detecting and identifying infectious agents. Several challenges are slowing the movement of nanomedicines to the bedside:

- The misconception that many small molecules are therapeutics
- Multiple technologies are being combined to create drug delivery devices or even theranostics
- New technologies are being used to evaluate efficacy and safety on materials that are orders of magnitude smaller in concentration.

SYSTEMS BIOLOGY APPROACHES IN NANOSAFETY ASSESSMENT

BENGT FADEEL, Neus Feliu, Pekka Kohonen, Lena Palmberg and Andreas Nyström
Nanosafety & Nanomedicine Laboratory, Division of Molecular Toxicology, Institute of Environmental Medicine, Nobels väg 13, Karolinska Institutet, 171 77 Stockholm, Sweden

Transcriptomics, or gene expression profiling, could aid in the prediction of mechanisms of toxicity. Indeed, the use of global "omics" technologies coupled with computational approaches to determine statistically significant perturbations of genes or pathways represents an attractive method to identity the potential hazards and mechanisms of action of chemicals and nanomaterials. Several groups have applied cDNA microarray-based approaches to assess the toxicity of nanoparticles. However, few studies have focused on the effects of low or non-cytotoxic doses of nanomaterials on gene expression. We investigated the transcriptional changes in human primary bronchial epithelial cells exposed to low doses of poly(amidoamine) (PAMAM) dendrimers with neutral (OH) or positive (NH2) surface groups using whole transcriptome sequencing. Bronchial epithelial cells were selected as a model system as these cells play an important role in the innate immune defence against inhaled pathogens and particulates. Our study demonstrates the feasibility of applying systems biology tools to assess cellular responses to nanomaterials, not least at low doses.

FUNDING

Work in the author's laboratory is funded, in part, by the Swedish Research Council, Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning, Swedish Cancer and Allergy Foundation, and by the European Commission.

NANOMEDICINE IN 2014: FROM ACADEMIC DISCOVERIES TO SOCIETAL IMPACT

OMID C. FAROKHZAD
Laboratory of Nanomedicine and Biomaterials, Department of Anesthesiology Brigham and Women's Hospital and Harvard Medical School 75 Francis St, Boston, MA 02115
Fax: +617-730-2801, E-mail: ofarokhzad@zeus.bwh.harvard.edu

A variety of organic and inorganic materials have been utilized to generate nanoparticles for drug delivery applications, including polymeric nanoparticles, dendrimers, nanoshells, liposomes, nucleic acid based nanoparticles, magnetic nanoparticles, and virus nanoparticles. The two most commonly used systems are polymeric nanoparticles and liposomes [1, 2]. Controlled release polymer technology has impacted virtually every branch of medicine, including ophthalmology, pulmonary, pain medicine, endocrinology, cardiology, orthopedics, immunology, neurology and dentistry, with several of these systems in clinical practice today such as Atridox, Lupron Depot, Gliadel, Zoladex, Trelstart Depot, Risperidol Consta and Sandostatin LAR. The annual worldwide market of controlled release polymer systems which extends beyond drug delivery is now estimated at $100 billion and these systems are used by over 100 million people each year. Polymeric nanoparticles can deliver drugs in the optimum dosage over time, thus increasing the efficacy of the drug, maximizing patient compliance and enhancing the ability to use highly toxic, poorly soluble, or relatively unstable drugs. These systems can also be used to co¬deliver two or more drugs for combination therapy [3]. The surface engineering of these nanoparticles may yield them "stealth" to prolong their residence in blood [4] and the functionalization of these particles with targeting ligands can differentially target their delivery or uptake by a subset of cells [5], further increasing their specificity and efficacy [6]. The successful clinical translation of therapeutic nanoparticles requires optimization of many distinct parameters including: variation in the composition of the carrier system, drug loading efficiency, surface hydrophilicity, surface charge, particle size, density of possible ligands for targeting, etc., resulting in a large number of potential

variables for optimization which is impractical to achieve using a low throughput approach. More recently combinatorial approaches have been developed to precisely engineer nanoparticles and screen multiple nanoparticle characteristics simultaneously with the goal of identifying formulations with the desired physical and biochemical properties for each specific application [7]. The goal of this talk is to review our efforts in the design and optimization of polymeric nanoparticles for medical applications, which formed the foundation for the clinical translation of the first-in-human targeted and controlled-release nanoparticles, BIND-014 and SEL-068 [8, 9].

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THE TOY KIT AGAINST MALARIA: RUSSIAN DOLLS, MAGIC BULLETS, LEGO, AND TROJAN HORSES

XAVIER FERNÀNDEZ-BUSQUETS, Ernest Moles, Joana Marques and Patricia Urbán
xfernandez_busquets@ub.edu
Nanomalaria Joint Unit, Institute for Bioengineering of Catalonia, Barcelona
Nanomalaria Joint Unit, Barcelona Centre for International Health Research, Barcelona
Nanoscience and Nanotechnology Institute, University of Barcelona

The medical history of malaria tells us that defeating Plasmodium is not an easy task, and will require a combined effort in bringing together our most developed research skills. As an old African proverb advices, “If you want to go fast, go alone. If you want to go far, go together”, a saying that can be also applied to nanomedicine, particularly with reference to targeted drug delivery. Different encapsulating structures (lipid-based, polymers, dendrimers), drugs, and targeting molecules (antibodies, polysaccharides, DNA aptamers), can be combined as LEGO parts to assemble multifunctional nanovectors that can be modulable, adaptable and versatile. With progressive small modifications, a nanovessel can be modulated to fine-tune it for a better performance: changing the lipid formulation in a liposome or modifying the chemical structure of a polymer for longer blood residence time, improving targeting efficacy through single-base modifications in the sequence of an aptamer or simply switching to encapsulating a new and better drug are strategies that should make feasible modulating a first prototype to ameliorate its activity. This tuning capacity is particularly required for malaria to adapt targeting molecules to the continuously changing repertoire of markers exposed on Plasmodium-infected red blood cells (pRBCs) as a consequence of the elevated antigenic variability of the parasite.

Through more profound changes in its structure, any nanovector design should be also susceptible of adaptation to new targets such as different Plasmodium species or new stages in the parasite life cycle. Currently developed pRBC-targeted nanocapsules will likely require major remodeling such as significant modifications in the nanocapsule itself or in the type of targeting elements to adapt them for drug delivery to new cellular forms in the life cycle of the parasite. Future strategies will even have to be highly imaginative to target stages in the mosquito vector, like gametes, ookinetes and oocysts, in what might be the first nanomedicine designed to cure an insect with the highest prize in mind of, simultaneously, curing humans.

Finally, in the very nature of nanovectors resides their versatility that enables assembing different elements to obtain chimeric nanovessels tailored to fit the requirements for different administration routes, particular intracellular targets, or combinations of different drugs. As it has been discussed above, the simultaneous administration of more than one drug significantly improves the antimalarial effect of the individual compounds. Liposomes are particularly adept structures in this regard because they allow the encapsulation of hydrophobic drugs in their lipid bilayer and of water-soluble compounds in their lumen, thus being a potentially interesting platform for ACTs where lipophilic and hydrophilic drugs are delivered together. On the other hand, liposomes are not adequate for oral delivery, which is a necessary administration route for malaria therapies currently needed in endemic regions to reduce disease prevalence, and polymeric nanovectors are more suited to this need. The ideal magic bullet against malaria is far from being a reality because of the complexity of the parasite’s life cycle and the tricks of its pathophysiology. Even if the right cell type could be targeted with complete precision, two final steps might pose phenomenal obstacles to eliminating Plasmodium: delivering sufficiently high drug amounts and making them reach the parasite. Once a drug has been injected into, say, a pRBC, the compound might still have a long way to go through the parasitophorous vacuole membrane, the plasma membrane of Plasmodium, and some additional lipid bilayer(s) if the target is inside a particular organelle as for example in the case of fosmidomycin which hits an enzyme inside the apicoplast. The sought-after Trojan horse against malaria not only has to enter the cell without finding serious opposition; it must also deliver a lethal amount of antimalarial to the right place. Likely, both objectives will not be achieved by a single type of nanocapsule/targeting element/drug. The answer might be in assembling different types of nanocapsules into a composite structure, a kind of Russian doll at the molecular scale. The engineering of multifunctional pharmaceutical nanocarriers combining several characteristics in one particle can significantly enhance the efficacy of many therapeutic and diagnostic protocols. However, a realistic balance between complexity, efficacy, and cost will be necessary to obtain for each situation an efficient antimalarial drug delivery nanosystem as simple and economically affordable as possible. It is wrongly believed that nanomedical products are always expensive and that this limits their use to diseases prominent in the developed world such as cancer, Alzheimer’s, or AIDS. But if a nanomedicine were indeed costly, shall the research community accept the arguments of a significant proportion of pharmaceutical companies that would likely be reluctant to develop expensive antimalarial nanomedicines urgently needed in low per capita income countries? Obviously not, and all currently available tools have to be fully applied to design the best possible diagnostic tests, prophylactic vaccines and therapeutic treatments for any disease regardless of the economic profile of the regions where it will be deployed. Here, public agencies should fill the gaps that private enterprise will not cover, to make sure that monetary profit will not be an issue when deciding where nanomedical research has to orient its efforts.

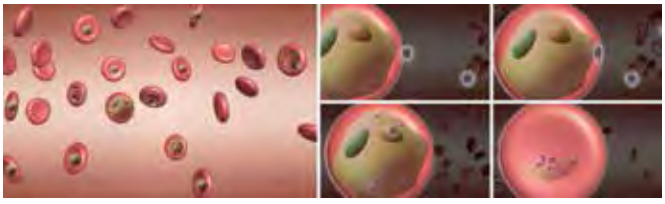

Figure 1. When added to Plasmodium-infected blood (left panel), targeted nanovectors (small panels, clockwise from upper left) (i) bind and (ii) enter parasitized erythrocytes, (iii) releasing drug that (iv) eliminates the pathogen.

This research was supported by grants BIO2011-25039 and 2009SGR-760

REGULATION OF NANOMEDICINES BY THE THERAPEUTIC GOODS ADMINISTRATION

ANNE FIELD

The Therapeutic Goods Administration (TGA) is part of the Australian Government Department of Health, and is responsible for regulating medicines and medical devices. The TGA administers the Therapeutic Goods Act 1989, applying a risk management approach designed to ensure that therapeutic goods supplied in Australia meet acceptable standards of quality, safety and (as appropriate) efficacy. The TGA regulates therapeutic goods through a combination of pre-market assessment and post-market monitoring and enforcement of standards. In addition, the TGA is responsible for the licensing of Australian manufacturers and for verifying overseas manufacturers' compliance with the same standards as their Australian counterparts. The TGA approves and regulates products through established principles of risk/benefit analysis. The TGA's approach to risk management involves identifying, assessing and evaluating the risks posed by therapeutic products, and applying any measures necessary for treating the risks posed.

Nanomedicine may be defined as the application of nanotechnology in the area of healthcare, including diagnosis, treatment and prevention of disease. A number of therapeutic products incorporating nanotechnologies are already approved by the TGA and are included in the Australian Register of Therapeutic Goods (ARTG). Some of these products were assessed and approved several decades ago, but were not formally identified as nanomedicines at the time of approval. Examples of therapeutic products that incorporate nanotechnologies and are currently available in Australia include medicines composed of nanosized particles, specialised drug delivery systems (including liposomal and polymeric substances), metals and metal oxides used as diagnostic agents as well as sunscreens and excipients used in therapeutic products.

Therapeutic applications of nanotechnology offer significant advances in the diagnosis and treatment of disease. One of the primary goals is to reduce toxicity while maintaining or enhancing therapeutic effects, allowing better tissue targeting, and resulting in lower systemic exposures and increased tolerability of a medicine. Reformulation of medicines into nanosized particles can make drug delivery within the body more efficient, helping to overcome problems of poor bioavailability and erratic absorption associated with low water solubility. Drug delivery systems utilising nanotechnology include drugs encapsulated in liposomes and polymers, as well as albumin bound drugs.

Theoretical concerns about the safety of nanomedicines include those relating to nanoparticle pharmacology, including the physiochemical properties of nanomaterial, and possible changes to pharmacokinetics and pharmacodynamics. Concerns of nanoparticle toxic effects (nanotoxicology) include the potential to cause adverse effects (possibly associated with changes in pharmacokinetics), as well as any new unintended toxicity. It is also possible that nanomedicines may exhibit decreased toxicity.In 2009 the TGA participated in a whole of government programme known as the National Nanotechnology Strategy (NNS; now replaced by the National Enabling Technologies Strategy or NETS). Under this programme, the TGA has undertaken the following activities:

- Conducted a review of the capacity of existing regulatory arrangements to assess and manage issues arising from the use of nanotechnology in the therapeutic arena. This review included the establishment of databases for both existing and in-line therapeutic products;
- Undertook an analysis of the scientific literature related primarily to therapeutic goods that incorporate nanomaterials; and
- Coordinated a regulator's training program in nanotechnology, held in May 2009.

The TGA has thus engaged in scientific capacity building of its nanotechnology assessment ability, and through NNS (NET) has supported the coordinated whole-of-government response to the issue.

The NNS concluded that the TGA is well placed to regulate products incorporating nanomaterials since it generally operates in a data rich environment, and has a high level of expertise to bring to bear on the assessment of new technologies. It has the legislated authority to require additional data in support of the safety assessment of new materials where this is warranted, and, in the most part, deals with sponsors who have the technical expertise to adequately address key safety issues. It was recognised that we need to maintain capacity building and engage internationally to ensure development of appropriate guidelines.

In conclusion, the TGA continues to support the success of nanomedicine through its regulatory activities, ongoing review of the scientific literature, engagement with NETS, and participation in international fora, such as the Nanomedicines International Working Group.

JANUS MAGNETIC LIPOSOMES FOR CANCER THERAPY

ALKE PETRI-FINK, Christophe A. Monnier, Cécile Bonnaud, Calum Kinnear, Dimitri Vanhecke, Barbara Rothen-Rutishauser
Adolphe Merkle Institute, Rte de l'Ancienne Papeterie, P.O. Box 209, 1723 Marly 1, Switzerland
Department of Chemistry, University of Fribourg, Chemin du Musée 9, 1700 Fribourg, Switzerland; alke.fink@unifr.ch

Liposomes have been increasingly developped as drug carriers to overcome poor pharmacokinetics and inappropriate biodistribution. To reduce their phagocytic uptake and minimize non-specific interaction with other proteins, liposomes are tyically coated with hydrophilic compounds, e.g. Polyethylene glycol (PEG). Active targeting is then achieved by conjugating the drug loaded liposomes with molecules that bind to overexpressed antigens or receptors on the target cells. Releasing drugs at their target in a controlled fashion remains a key determinant of successful treatment. First and second generation nanovectors include responsive systems, for example, pH-sensitive polymers or those activated by enzymes specific to the disease site, while third generation nanovectors are capable of more complex functions, such as time-controlled deployment of multiple waves of active nanoparticles across different biological barriers and different subcellular targets.

In this context, we have recently integrated monodispersed superparamagnetic iron oxide NPs into the membrane of liposomes1,2. The magnetic nanoparticles provide extra functionality that allows controlled contents release via magnetic hyperthermia.

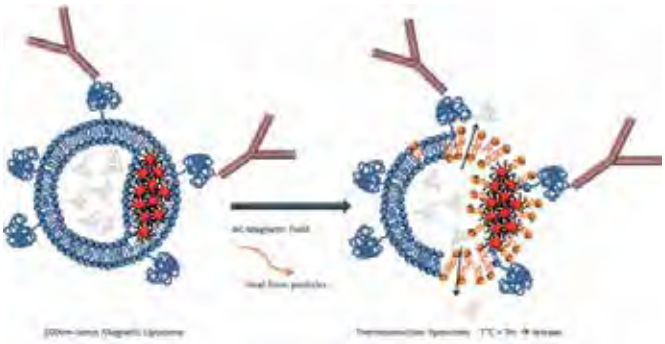

Fig 1: Concept of triggered drug release using magnetic liposomes

LUMINESCENT BIOMATERIALS FOR DIAGNOSTIC AND THERAPEUTIC APPLICATIONS

GINA L. FIORE
Adolphe Merkle Institute, University of Fribourg,
Route de l'Ancienne Papeterie, CH-1723 Marly, Switzerland,
e-mail: gina.fiore@unifr.ch

Luminescent lanthanide-based biomaterials were prepared as a new class of molecular probes that are resistant to photobleaching and offer other optical advantages over conventional organic dyes and quantum dots. Organic dyes typically used in this context often photobleach, have short lifetimes, as well as excitation wavelengths in the ultraviolet (UV) range, which can cause damage to

cells and absorbance by proteins and surrounding media. Quantum dots are a good alternative due to their typically narrow emission spectra, resistance to photobleaching, longer lifetimes, and longer excitation wavelengths; however, the heavy metals used to create quantum dots are generally highly toxic. Lanthanide-based biomaterials can serve as viable alternative as they possess similar properties to quantum dots (e.g., resistant to photobleaching, sharp emission lines, high quantum yield in aqueous environment, long excited state lifetimes, possibility for excitation by two photon absorption), but when coordinated with ligands they lack the toxicity associated with quantum dots.

Combining the advantages of polymeric self-assembly and unique optical properties of lanthanide complexes, a single component system comprised of biodegradable, amphiphilic polymers of poly(ethylene glycol)-b-poly(ε-caprolactone) (PEG-b-PCL) and non-toxic metal ions (europium, terbium) were prepared (Figure 1). These materials were then self-assembled into nanoparticles using solvent displacement methods. The size, morphology and optical properties of the nanoparticles self-assembly were systematically tuned. The overall properties of lanthanide-containing biomaterials for the potential use as the next generation of drug delivery vectors and molecular probes will be presented.

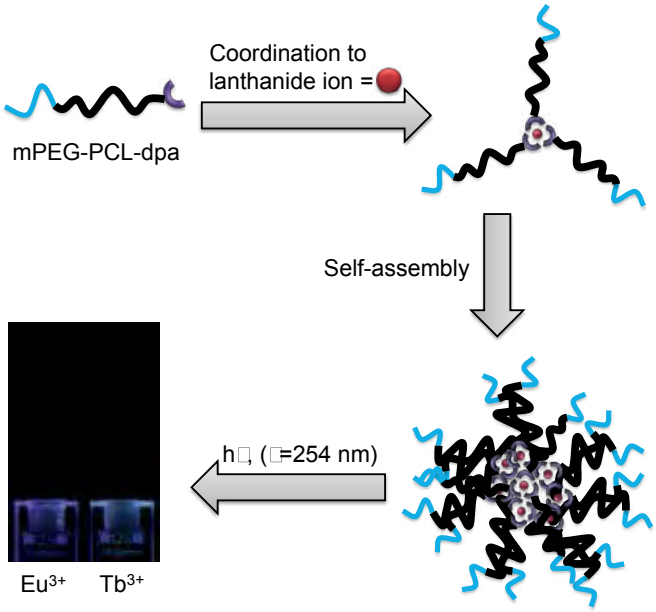

Figure 1. Schematic representation of luminescent biomaterials.

BIO-RELEVANT IN VITRO RELEASE ASSAY FOR LIPOSOMAL FORMULATION

ANDREAS FISCH

Fellow Pharmaceutical Development Parenterals, Novartis, Basel (CH)

During the screening and development of nano-carrier formulations there is a need for a robust in vitro screening system for drug release which should be as predictive as possible for the in vivo situation (PK profile). Ideally, an appropriate release system should reflect the most important factors affecting drug release from the carrier effective in vivo, including the huge dilution effect of the formulation after intravenous injection or infusion and the interaction with plasma proteins, like albumin or lipoproteins and membranes of blood and endothelial cells. On the other hand, the release system should be as simple as possible, avoiding the use of animal or human cells for safety and convenience reasons and enable reliable, quick and cost effective screening of different nano-formulation variants in parallel.

This lecture will discuss the strength and weaknesses of different release test options reflecting the different physiological factors potentially influencing drug release from liposomes in vivo. The results of different in vitro release test strategies performed in the laboratories of Novartis will be discussed and compared with PK data from animal studies.

EFFICIENT TARGETING OF NON-CODING RNAS IN ANIMAL MODELS WITH LNA ANTISENSE OLIGONUCLEOTIDES

NIELS MONTANO FRANDSEN

The ENCODE project has revealed that ~80% of the human genome is actively transcribed while only ~2% of the transcriptome encodes protein. Understanding the role of non-coding RNA is therefore one of the most exiting frontiers in biology today. Development of tools for efficient knock down ncRNA in cell cultures and in live animals are therefore of crucial importance.

Single stranded high affinity LNA oligonucleotides are versatile tools for functional analysis of miRNAs, lncRNAs and other ncRNAs as well as proteins. These tools are routinely used in cell cultures, but are now increasingly also being successfully applied in vivo. Potent activity with RNaseH catalyzing antisense oligonucleotides against mRNA targets has traditionally been restricted to tissues that accumulate high amounts of oligonucleotide such as liver and kidney. However in the past 5 years many high profile publications have reported efficient inhibition of miRNA activity by systemic administration of saline solutions of short LNA enhanced oligonucleotides in a broad range of tissues and even in organs that accumulate little oligonucleotide such as the heart and lungs. As a result important discoveries about miRNA function are being made that could not have been achieved with cell cultures alone and several miRNA inhibitors are currently being investigated for their potential as pharmaceutical agents. These miRNA antisense inhibitors sequester their miRNA target in a stable and inactive complex but do not recruit RNaseH activity. Recently Exiqon has developed a highly performant empirically derived algorithm for the design of short RNaseH catalyzing gapmer oligonucleotides. RNaseH is primarily localized to the nucleus and we and others have found that nuclear retained RNAs are supersensitive gapmer targets as opposed to mRNA that is rapidly exported to the cytoplasm after maturation. In an effort to explore in which tissues potent KD can be achieved with such targets we have identified a potent gapmer against the nuclear retained and ubiquitously expressed lncRNA Malat1. I will present preliminary data showing broad range activity in mice with potent KD even in challenging tissue such as muscle and heart. This has important implications for the functional analysis of important classes of lncRNAs that reside in the nucleus for extended periods of time.

EU HORIZON 2020 – A PATH TOWARDS THE FUTURE OF MEDICINE[1]

HEICO FRIMA

European Commission, Directorate-General for Research & Innovation

The first Call for Proposals of the new European Union Horizon 2020 Framework Programme for Research & Innovation (2014 – 2020) was published in December 2013. Horizon 2020 has a budget of nearly € 80 Billion and it has three main priorities: 'Excellent Science', 'Industrial Leadership' and 'Societal Challenges', each with several specific objectives. Together, the funding opportunities in Horizon 2020 span the entire innovation cycle, from frontier research to rather close to market activities.

Horizon 2020 offers many opportunities for research and development in the medical and pharmaceutical field, notably in the field of nanomedicine. The presentation will give an overview of Horizon 2020 and discuss the important role of the 'Key Enabling Technologies' (nanotechnology, advanced materials, industrial biotechnology, nano-electronics, nano-photonics and advanced manufacturing), the Health Programme, the Innovative Medicines Initiative, the Infrastructure Programme, the European Research Council, and the opportunities for closer to market research in the 'Fast Track to Innovation'.

The nanomedicine community with the European Technology Platform for Nanomedicine has been very active and successful in the preceding Framework Programme 7. The FP7 NMP (Nanotechnology, Materials and Production Technologies) Programme supports about 85 nanomedicine projects with more than € 400 million EU

funding. These projects develop novel biomaterials for implants and regenerative medicine, novel nano-enabled diagnostics and therapy of diseases, and smart implants using bio-nano-info convergence. A strong pre-clinical nanotechnology competence for applications in medicine has meanwhile been established in European R&D laboratories.

The FP7 nano-medicine developments will be continued in the first call of Horizon 2020, but putting emphasis on the translation of preclinical results into therapies and products that can be used by the clinicians, for the benefit of the patients. This includes the setting-up of pilot manufacturing facilities for scaling-up the production of nano-pharmaceuticals from lab-scale to the quantities that are needed for clinical testing. The physical, chemical, structural and biological characterisation of nanopharmaceuticals is still a difficult issue and Horizon 2020 therefore calls for a new infrastructure, a NanoCharacterisation Laboratory. Horizon 2020 will provide opportunities for clinical testing of new nano-medicine therapies and products, especially in the Health Programme. There is also a call for a Support Action to provide networking and advice on translation for SME's in the nano-biomedical sector.

Finally, the complementarity will be highlighted between Horizon 2020 and the EU Regional and Structural Funds Programme that has € 80 Billion available for funding innovation in Europe.

[1]Disclaimer: All views expressed are entirely of the author, do not reflect the position of the European Institutions or bodies and do not in any way engage any of them.

CHRONIC CYTOTOXICITY: CELLULAR EFFECTS OF NANOPARTICLE ACCUMULATION

FRÖHLICH, E.[1], C. Meindl[1], M. Mrakovcic[1], R. Baumgartner[2] and E. Roblegg[2]

[1]Center for Medical Research, Medical University of Graz, Stiftingtalstraße 24, 8010 Graz, Austria; emails: Claudia.meindl@klinikum-graz.at maria_mrakovcic@hotmail.com; eleonore.froehlich@medunigraz.at

[2]Institute of Pharmaceutical Sciences/Pharmaceutical Technology, Karl-Franzens University, Universitätsplatz 4, 8010 Graz, Austria. emails: eva.roblegg@uni-graz.at; ramona.baumgartner@edu.uni-graz.at

Humans can be exposed to nanoparticles in the environment, in consumer products, at the workplace, and in medical products. In most cases exposure is for prolonged time at rather low doses [1]. This compares to in-vitro screening which is generally performed for short time at rather high concentrations. According to ISO 10993-5 and other guidelines for cytotoxicity testing, compounds are added to cells in the sub-confluent state and can be assessed until the control cells reach the confluent state. In the confluent state proliferation decreases by contact inhibition and these cultures are no more a valid control for non-inhibited growth [2]. This testing is suitable to identify acute effects on membrane disintegration and on apoptosis but less suitable to identify effects by chronic exposure, which could arise by intracellular persistence of the particles.

In this study, time-dependent changes in particle size, cellular particle uptake, as well as effects on lysosomal function, cell number, apoptosis, and membrane integrity were studied. EAhy926 endothelial cells and THP-1 monocytes were exposed to carboxylated and plain polystyrene particles of 20-500 nm and 50 nm silica particles (Aerosil®OX50) were assessed in. Changes in particle size were determined by photon correlation spectroscopy and cellular uptake by the use of fluorescently labeled particles. Long-term exposures were performed in microreactors (BioLevitator™ for EAhy926 cells and CELLine CL350 for THP-1 cells) and by sub-culturing of cells. For the longer exposures cells were exposed to non-acutely cytotoxic particle concentrations . Lysosomal accumulation and lysosome function were assessed in growth-retarded cells to prevent dilution of particles by cell division. For these experiments, carboxylated polystyrene particles were used because they were ingested to higher extent by the cells.

Sizes of silica particles, present as primary particles and as aggregates in the suspension, remained constant during the incubation time while the size of 20 nm plain polystyrene particles increased by a factor of two [3]. The fraction of this aggregate formation by silica particles was concentration dependent. For instance, at 12.5 µg/ml Aerosil®OX50 roughly 40% of the particles were detected as single particles and a smaller fraction was present as aggregates, at 100 µg/ml only 10% of the particles were present as single particles and the majority as aggregates. Both endothelial cells and monocytes ingested carboxylated polystyrene particles to a higher degree than plain polystyrene particles, but plain polystyrene particles acted more cytotoxic. After one dose of particles they persisted in lysosomes for 3d without obvious interference with lysosome function ([4]; Fig. 1).

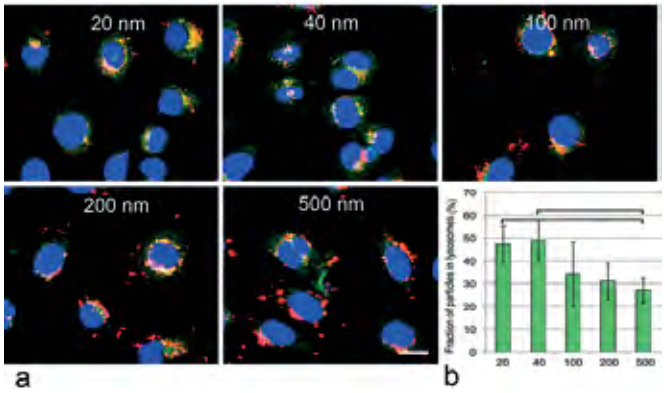

Figure 1: Co-localization (yellow) of fluorescently labeled 20-500 nm carboxylated polystyrene particles (red) and lysosomes identified by staining with LysoSensor (green) in EAhy926 cells 24h after addition of the particles. a: 20 nm-500 nm FS co-localize with lysosomes. Scale bar: 10 µm. b: significant higher lysosomal localization of 20 nm and 40 nm compared to 500 nm particles.

Testing in the bioreactor BioLevitator™ was more sensitive for endothelial cells than assessment in sub-cultures [5], while testing in sub-culture produced more reliable results than CELLine CL350 for monocytes. Upon long-term exposure, 20 nm plain polystyrene particles reacted cytotoxic at much lower concentrations than in the short-term exposure, while differences in cytotoxic concentrations between short-term and long-term exposure were smaller for silica nanoparticles. 200 nm plain polystyrene particles and 20 nm carboxylated polystyrene particles (that aggregated in medium with 10% serum) did not act cytotoxic in short-term and in long-term exposure. 20 nm plain polystyrene particles caused membrane damage, induced interleukin 8 secretion in monocytes, and disruption of membrane integrity, and apoptosis in endothelial cells. Exposed to the same concentration of 20 nm plain polystyrene particles, uptake rates were higher (7% compared to 4%) in monocytes than in endothelial cells. After 24h, a concentration of 50 µg/ml did not cause acute cytotoxicity, but after 14d relative cell numbers were lower (8% compared to 56%) in monocyte than in endothelial cell cultures (Fig. 2).

Based on the obtained data, the following conclusions can be drawn: i) accumulation in lysosomes per se does not lead to organelle dysfunction, ii) long-term cytotoxicity appears to be mainly caused by single particles (not by aggregates), and iii) the degree of cellular uptake potentially can indicate more sensitive cell types.

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THE ENHANCED PERMEABILITY AND RETENTION (EPR) EFFECT AND ITS RELEVANCE TO NANO-MEDICINE AND CANCER THERAPEUTICS

ALBERTO GABIZON
MD, PhD
Shaare Zedek Medical Center and Hebrew University-School of Medicine, Jerusalem, Israel

Enhanced permeability of the tumor vasculature allows macromolecules to enter the tumor interstitial space, whereas the suppressed lymphatic filtration allows them to stay there. This phenomenon, enhanced permeability and retention (EPR), has been the basis of nanotechnology platforms to deliver drugs to tumors1. A hallmark of the long-circulating pegylated liposomal drug carriers is their enhanced accumulation in tumors2. The mechanism underlying this passive targeting effect is the phenomenon known as enhanced permeability and retention or EPR. Initially described by Maeda3 to account for increased deposition of macromolecular drug carriers in tumors, the EPR effect also applies to liposomes and other nanoparticles.

The EPR effect4 has been described in a broad variety of experimental tumor types and appears also to be a relevant phenomenon in human cancer5. The disorganized and tortuous morphology of the tumor microvasculature with its discontinuous and porous endothelium, broken or non-existent basement membrane, blind and dilated loops, irregular flow, and lack of effective lymphatic drainage6-7, can be considered as the Achilles Heel of tumors vis-à-vis the selective accumulation of circulating nanoparticles and macromolecules. This window of opportunity is however time-limited since the tumor interstitial fluid pressure gradually increases with tumor size shutting off vascular supply of large tumor areas and limiting all forms of drug delivery, whether of soluble drugs or nanoparticle-associated drugs6,8.

The occurrence of EPR in tumors coupled with the low permeability of most normal tissues to nanoparticles results in a high tumor:normal tissue ratio for liposomal drug concentration. Passive accumulation of pegylated liposomes in tumors has been demonstrated in a number of studies. Microscopic observations with gold-labeled pegylated liposomes in experimental tumors indicate that their distribution is limited to the extracellular fluid and tumor-infiltrating macrophages9-10. Using fluorescently labeled liposomes in the mouse skin-fold tumor chamber model, most liposomes appear to accumulate in the immediate perivascular area with little or no deep penetration into the tumor cell layers11. This phenomenon of passive targeting can lead to an enhanced therapeutic effect for liposomal drugs. The effect of EPR on pegylated liposomal doxorubicin (PLD) tumor deposition can be clearly visualized in experimental models because of the red–orange color of doxorubicin12. Tissue distribution studies show high PLD concentrations in tumor tissue peaking between 48 and 72 h after injection with a large increase in AUC tumor concentration (~30-fold) over free doxorubicin2,13.

However, progress in developing effective drugs using the EPR approach has been hampered by heterogeneity of EPR effect in different tumors and limited experimental data from patients on effectiveness of this mechanism as related to enhanced drug accumulation. The observed heterogeneity in EPR may be a contributing factor to the limited impact of nanoparticle-based drugs in demonstrating antitumor efficacy as compared with small-molecule anti-cancer agents in clinical studies. Further understanding and predictability of EPR function in primary tumor and its metastatic sites through the use of imaging studies may aid the development of future, effective nanodrugs14. Correlation of EPR activity to clinical responses would likely provide direct clinical data to determine whether tumors with high EPR tumor activity will be more amenable to effective treatment using nanoparticle-based therapies.

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NANO-PSO: A NOVEL ANTIOXIDANT FORMULATION FOR MS TREATMENT

RUTH GABIZON, Orli Binyamin , Liraz Lirush, Kati Frid, Yael Friedman-Levy, Michal Maatuf, Haim Ovadia, Oded Abramsky and Shlomo Magdassi
Department of Neurology, Hadassah University Hospital and Casali Institute of Chemistry, The Hebrew University

INTRODUCTION

Multiple sclerosis is a chronic inflammatory disease of the central nervous system, associated with demyelination and neurodegeneration. While the mechanisms of pathogenesis in MS is not totally understood, recent data suggests that oxidized phospholipids were highly enriched in active multiple sclerosis plaques and that such oxidation damage was central to the pathological mechanism in MS.

QUESTION

Consistent with this knowledge, we asked whether anti-oxidative treatments based on natural safe reagents that can be administrated for long periods of time, could be beneficial for individuals first diagnosed for MS.

METHODS

Mice induced for EAE were treated with novel nanoparticle formulations of Pomegranate seed oil (Nano-PSO) and appropriate controls. PSO comprises a unique conjugated fatty acid, Punicic acid, considered one of the strongest natural antioxidants. Treated and untreated mice were scored for EAE for several weeks. Subsequently, brains and spinal cord from treated and untreated EAE mice were tested for demyelination, infiltration of activated lymphocytes and lipid oxidation.

RESULTS

While EAE mice treated with PSO at high concentrations already presented reduced burden of disease, this effect was significantly elevated when EAE induced mice were treated with Nano-PSO at designated nanoparticle size, and could be achieved at much lower doses that those required for PSO. Non PSO controls such as Nano-soybean formulations had no anti EAE effect. Pathological examination revealed that Nano-PSO formulations dramatically reduced demyelination, infiltration of T-cells and oxidation of lipids in EAE plaques.

CONCLUSIONS

We propose that safe Nano-PSO formulations may be considered for the initial treatment of patients diagnosed for MS either alone or in combination with established drugs.

NANOENCAPSULATION OF SILVER-BASED ANTIMCROBIAL DRUGS

JACINTHE GAGNON[a,b]*, Martin J. D. Clift[b], Alke Petri-Fink[b], Barbara Rothen-Rutishauser[b], Katharina M. Fromm[a]*
[a]Department of Chemistry, University of Fribourg, Fribourg, CH
[b]Adolphe Merkle Institute, University of Fribourg, Marly, CH
*Corresponding authors: Jacinthe.Gagnon@unifr.ch, Katharina.Fromm@unifr.ch

Implant-associated infections still remain an issue in medicine and can cause various medical complications1. In order to ensure proper host-cell integration and biocompatibility to an implant, it is essential to prevent bacterial adhesion during the critical period of 6 hours after surgery2,3. Moreover, as implants are increasingly used in medicine, bacteria are becoming more resistant to antibiotics, in such a way that new developments in preventing and curing infections are imperative.

Silver compounds and nanoparticles are gaining more interest from the scientific society as a replacement to antibiotics. However, silver compounds may be too soluble and even toxic for the host. Encapsulation poses as an advantageous method to increase the stability and biocompatibility of silver drugs. In addition, it allows a more controllable release of antimicrobial agents.

In this study, ceria capsules with integrated silver nanoparticles (AgNP/CeO2 capsules) were synthesized according to the method depicted in Figure 1. The synthesis could be followed using transmission electron microscopy (TEM) as presented in Figure 2. The capsules were then further characterized using X-ray diffraction (XRD), and scanning electron microscopy (SEM) (Figure 3). After calcination, the AgNP/CeO2 capsules appear as hollow spheres. The complete removal of polystyrene (PS) after calcination was confirmed by infrared spectroscopy (data not shown). Ag NPs are visible on the surface as well as in the cavity of AgNP/CeO2 capsules on the SEM image (Figure 3), suggesting that they are integrated within the ceria shell.

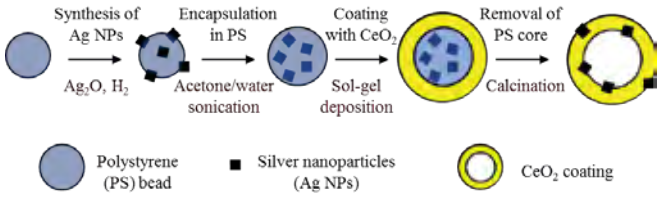

Figure 1: Schematic representation of the synthesis of AgNP/CeO2 capsules.

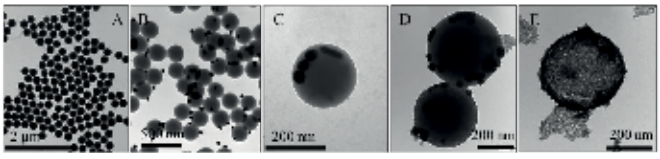

Figure 2: TEM images at each step of AgNP/CeO2 capsule synthesis: A) PS beads, B) Ag NPs synthesized on the surface of PS beads, C) Ag NPs encapsulated into the PS beads, D) coating with CeO2, and E) hollow AgNP/CeO2 capsules after PS removal.

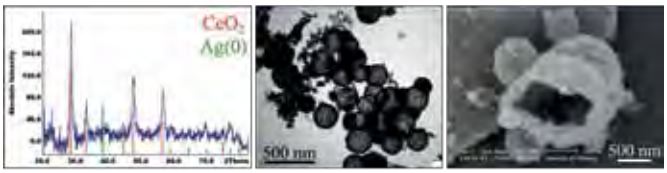

Figure 3: From left to right, PXRD, TEM and SEM images of AgNP/CeO2 capsules. The red and green lines on the PXRD indicate the theoretical position of the XRD peaks of cerium oxide and silver respectively. Silver nanoparticles appear as white spots on the SEM image.

AgNP/CeO2 capsules can release silver during a period exceeding three months (Figure 4), which demonstrates a good release control of the antimicrobial agent. This was confirmed by the antibacterial tests, which demonstrate an efficient antimicrobial activity against E. coli (Figure 5). The AgNP/CeO2 capsules also have been shown to exert limited cytotoxicity towards human alveolar epithelial cells in vitro, as determined by the lactate dehydrogenase (LDH) assay (Figure 6).

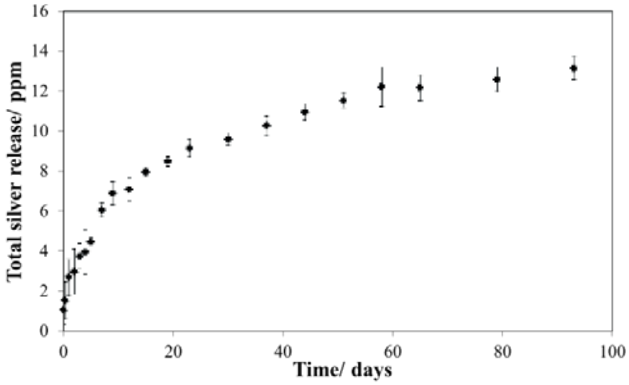

Figure 4: Silver release as a function of the immersion time in water at room temperature for AgNP/CeO2 capsules.

Figure 5: Determination of the antibacterial activity of AgNP/CeO2 capsules against E. coli by macrodilution method (A-C) and by agar diffusion method (D).

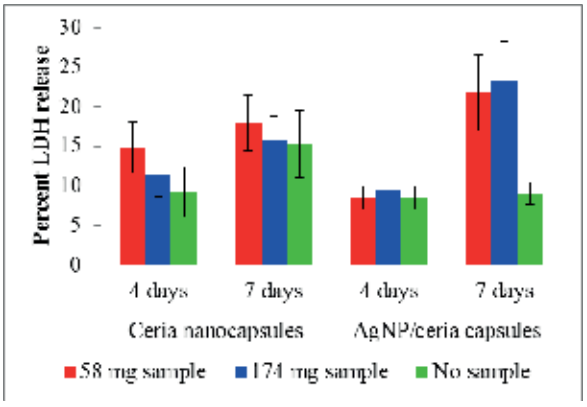

Figure 6: LDH assay for 58 mg/well (blue) and 174 mg/well (red) of CeO2 nanocapsules and AgNP/CeO2 capsules after 4 and 7 days exposure to A549 epithelial cells. Data is the % mean ¥ standard error of the mean compared to the positive control (0.2% TritonX-100). Data can be compared to the negative control (green), which corresponds to A549 cells growing on a glass culture chamber.

In conclusion, AgNP/CeO2 capsules were successfully synthesized. Silver nanoparticles are integrated within the ceria shell, which enables a remarkably slow silver release. These capsules also demonstrated a good antibacterial activity against E. coli. Cytotoxicity tests suggest that both CeO2 nanocapsules and AgNP/CeO2 cap-

sules have a low cytotoxicity. An increase in LDH release was observed after 7 days of incubation for AgNP/CeO2 capsules. These capsules therefore offer an advantageous strategy towards preventing implant-related infections.

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ACKNOWLEDGEMENTS

We are grateful to the Swiss National Science Foundation (NRP-62), the University of Fribourg, FriMat and the Adolphe Merkle Foundation for generously supporting this project.

BMP-7 LOADED MICROSPHERES CAN SUPPRESS GLIOBLASTOMA INITIATING CELLS IN HUMAN PRIMARY TUMOR MODELS

M.GARCIA-FUENTES[1]*, P. Gonzalez-Gomez[2], J. Crecente-Campo[1], C. Zahonero[2], M. de la Fuente[1], H. Mira[2] and P. Sanchez-Gomez[2]
[1]CIMUS, Universidad de Santiago de Compostela, Santiago de Compostela, A Coruña, 15782, Spain
[2]UFIEC, Instituto de Salud Carlos III, Majalahonda, Madrid, 28220, Spain
*presenting author: marcos.garcia@usc.es

INTRODUCTION

Glioblastoma initiating cells (GICs) are believed to be responsible for high-rates of tumor recurrence, since they present high resistance to conventional chemotherapy and capacity to initiate and sustain tumor growth [1]. Bone morphogenetic proteins (BMPs) have been identified as signaling proteins capable of transforming GICs in cells without tumor initiation properties, a therapeutic strategy that suppress tumor recurrence [2]. We have recently reported the design of BMP-7 loaded controlled release microspheres as potential implants for GIC suppression [3]. These devices are intended for intracraneal implantation upon primary tumor resection to address the major limitations of BMP-7 therapy: short in vivo half-lives and negligible penetration through the blood-brain-barrier. In this previous work, we reported a GIC suppressive effect evaluated on a U87MG cell line in vitro. Herein we report a validation of this strategy based on in vivo experiments performed on xenografts of primary cultures from human glioblastomas. We have taken advantage of this advanced tumor model to investigate the mechanism of action behind the tumor suppressive effect of this nanomedicine.

EXPERIMENTAL METHODS

Microsphere preparation

BMP-7 was encapsulated following an optimized procedure to enhance its encapsulation and to preserve its bioactivity during encapsulation and release [3, 4]. Briefly, BMP-7 was nanocomplexed sequentially by heparin and Tetronic®. The nanocomplexes were freeze-dried, and redispersed in a PLGA organic solution. Microspheres were prepared from this solution following an O/O emulsification method. Microspheres were characterized for size and morphology by scanning electron microscopy (SEM). Microspheres were characterized for BMP-7 encapsulation efficiency by an extraction method, and for controlled release properties in PBS with 1% BSA. BMP-7 was quantified by ELISA.

In vivo bioactivity assays

Primary culture of a human glioma (12O12, Hospital Doce de Octubre) were used for in vitro experiments. Sphere formation assays were carried out plating 12O12 cells under clonogenic dilution in the presence or absence of BMP7 at 50 ng/uL. Results were analyzed after 7 days by counting the number of spheres and by measuring their diameters from pictures of random fields captured using a x10 objective. In self-renewal assays, spheres from the previous experiment were dissociated and seeded again under clonogenic dilution, both in the absence of BMP-7 this time. After 7 days, the number of spheres was counted.

Bioactivity assays in vivo

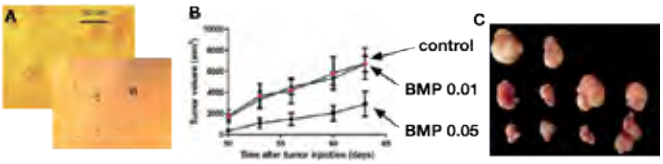
Microspheres were co-injected together with 2-3 million cells from primary culture of a human glioma (12O12, Hospital Doce de Octubre) in nude mice. Three preparations of microspheres were tested: blanks, and 0.01% and 0.05% (w/w) loading. Tumor growth was quantified for over 2 months. After this time, the tumor were surgically removed for observation and direct measurement.

Mechanistic assays

Samples from in vitro and in vivo testing were used for analyzing the mechanism of action. Activation of cell signaling pathways was studied by immunoblot analysis. Effect on cell proliferation were studied by generating cell growth curves. Cell phenotype was characterized by quantitative RT-PCR and immunocytochemistry. In vivo tumor formation was further studied by histopathological analysis.

RESULTS AND DISCUSSION

We have previously reported the preparation of biomaterials specifically optimized for the encapsulation of heparin-binding proteins and their release in a bioactive state [3, 4]. This method was adapted for the preparation of microspheres of 20-80 µm of mean particle size, with production yields above 90%. It was found by scanning electron microscopy that these microspheres are often hollow; FTIR experiments indicated some non-covalent interaction between the polyester matrices and the heparin-Tetronic nanocomplex used for BMP-7 incorporation. BMP-7 was efficiently encapsulated (>85%) and released in a controlled way for more than two months, a process that can be correlated with the degradation of the polymer matrix.



Studies in cell culture showed that BMP-7 can suppress tumor formation in primary cultures, and that this therapeutic effect was dependent on the expression of BMP receptors in the specific primary culture. Studies in cell cultures showed that BMP-7, even that obtained from 90 days release samples, maintained its bioactivity, and was able to suppress neurosphere formation (Fig. 1A, lower image). The absence of neurosphere formation in this experimental group contrasted with the presence of many cell aggregates in the control group (Fig. 1A, upper image).

In vivo data validated the therapeutic potential of the microspheres loaded with BMP-7 in a human primary culture xenograft model (Fig. 1B, C). The tumors formed in the back of the mice in the presence of microspheres loaded with 10 µg of BMP-7 (0.05% loading) were significantly smaller than those observed for the same microspheres loaded just with 2 µg (0.01% loading), or for the blank formulation. This therapeutic effect is related to BMP signaling cascade activation, to the induction of cancer stem cell differentiation, and to upregulation of p21. BMP-7 loaded microspheres showed no cytotoxic effect, since tumor histopathology was similar to that observed for control animals.

CONCLUSION

In this work we present a new composition for the controlled delivery of BMP-7, intended for its implantation in the brain as a glioblastoma suppressor. The data obtained in vitro and in vivo validates the potential of microspheres loaded with BMP-7 as a potential treatment against glioblastoma initiating cells. This is to the best of our knowledge, the first validation of BMP controlled release implants in human glioblastoma primary cultures.

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ACKNOWLEDGMENTS

This work has been financed by Fundación Ramón Areces (XVI Concurso Nacional CIVP16A1832) "Nanomedicinas reprogramadas nanotecnológicamente", Xunta de Galicia, programa de Proyectos de Investigadores Emerxentes (EM2013/042) and Comunidad de Madrid, Consorcio NEUROSTEM CM S2010/BMD-2336.

MOLECULAR MANIPULATION AT THE NANO-SCALE FOR THE DEVELOPMENT OF NOVEL DRUGS, DIAGNOSTICS AND BIOMATERIALS

EHUD GAZIT

Department of Molecular Microbiology and Biotechnology, Department of Materials Science and Engineering, Tel Aviv University, Tel Aviv 69978, Israel

The formation of ordered amyloid fibrils is the hallmark of several diseases of unrelated origin. In spite of 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. Significant part of the activity in our lab is related to development of new therapeutic agents based on this notion. Our works on the mechanism of aromatic peptide self-assembly, lead to the discovery that the diphenylalanine recognition motif self-assembles into 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 aramides, semi-conductive, piezoelectric and non-linear optic properties. We also demonstrated the ability to use these peptide nanostructures as casting mould for the fabrication of metallic nano-wires and coaxial nano-cables. The application of the nanostructures was demonstrated in various fields including electrochemical biosensors, tissue engineering, and molecular imaging. 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 "nanoforests". We recently demonstrated that even a single phenylalanine amino-acid can form well-ordered fibrilar assemblies of distinct electron diffraction pattern and toxic properties.

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MAGNETIC NANOPARTICLES AS INTRAOCULAR DRUG DELIVERY SYSTEM TO TARGET RETINAL PIGMENTED EPITHELIUM (RPE)

MARTINA GIANNACCINI[1], Marianna Giannini[1], M. Pilar Calatayud[2], Gerardo F. Goya[2], Alfred Cuschieri[1], Luciana Dente[3], Vittoria Raffa[3]
[1]Institute of Life Science, Scuola Superiore Sant'Anna, Pisa, Italy
[2]Institute of Nanoscience and Matter Physics Department, University of Zaragoza, Spain
[3]Department of Biology, University of Pisa, Italy

Disorders of the retina and RPE tissues, located in the posterior eye chamber, are responsible for the majority of blindness both in childhood and adulthood. Long-term delivery of biologically active molecules to the RPE is problematic and remains a challenge. The cornea/sclera constitutes a static barrier severely limiting ocular bioavailability of surface instilled drugs and retinal-blood barrier prevent ocular drug diffusion after systemic administration. Intravitreal (IVT) and subretinal injections are considered as the most effective ways of delivering material to the back of the eye. In particular, subretinal injection seems to be the only effective option to target RPE but are very invasive with reduced patient compliance compared to IVT injections. IVT injection involves injection of drug in solution directly into the vitreous which is far from optimal for three reasons: short term complications caused by the initial high drug concentration, very short retention time and lack of tissue specificity.

In the present study, we provide a method for exclusive and fast localization of drugs to RPE, with the use of magnetic nanoparticles (MNPs), by IVT injection. MNPs form a powerful drug delivery system because their reactive surface can be easily functionalized with biocompatible coatings and bioactive molecules to prevent interaction with healthy tissues and increase their target specificity. In addition to their established role as molecular carriers, MNPs have two other advantages. They can be controlled by non-contact forces and tracked by magnetic resonance imaging (MRI). Furthermore, different MNPs have FDA approval for clinical use e.g., Endorem® (MRI contrast agent for diagnosis of liver tumors). Although the MNPs have not yet been tested on humans for ocular applications, there are evidences from studies in rats, that the iron oxide MNPs are non-toxic to the ocular structures.

We have investigated the ability of MNPs to target RPE by IVT injection, using wild type Xenopus laevis as model system. Xenopus offers favorable features, such as external development, large supply of embryos with each fecundation, a very short early development time (3 days to reach tadpole) and close homology with human genes. A remarkable similarity in the molecular signaling processes, cellular structure, anatomy, and physiology of eye has been observed among Xenopus and other high-order vertebrates, including humans. Their relatively large size (from 1-1.2 mm (zygote) to 1 cm (5 days old)) enables an easy manipulation and IVT microinjection. Finally, the use of this model generates minor ethical issue compared to mammals because these embryos are considered to not be sufficiently sentient or experience nociceptive sensations when subjected to experimental procedures.

We used commercial fluorescent MNPs with a negative surface charge and a hydrodynamic size of 252 nm to analyze their biodistribution after intravitreal injection, i.e., in the anterior part of the eye and, specifically, in a region behind the lens surrounded by the vitreous humor, into the left eye of Xenopus embryo. 24 h after the injection, we observed, by Prussian blue staining of paraffin sections, that MNPs are specifically retained in the ocular tissues without any diffusion to the other tissues, including the contralateral eye (Fig 1a-e).

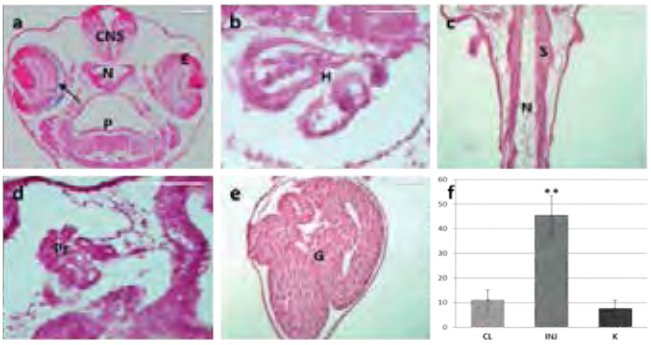

*Figure 1. Distribution of MNPs 24h after IVT injection in the left eye of X. laevis. a-e) Prussian Blue staining on paraffin section. The arrow points to blue labeled MNPs. CNS: central nervous system; E: eye; N: notochord; P: pharynx; H: heart; S: somites; Pr: pronephros; G: gut. f) Iron content assay on explanted eyes. CL: contralateral eye (non-injected); INJ: injected eyes; K: wild type eyes. Scale bar: 50µm. n=3, **p<0.001, t-test.*

Quantitative analysis of the iron content by thiocyanate colorimetric assay was used to confirm that all the MNPs were retained in the injected eye. The average ferric iron content in the injected eyes was significantly higher than in the control eyes (Fig 1f). These results, together with the histochemical observations, demonstrate that the MNPs are retained exclusively inside the injected eye.
We did not observe any toxic effects on the ocular structures caused by MNPs: no death or embryonic malformations were observed and the injected eye exhibited completely normal development (Fig 1).
Even if the particles were injected in the anterior part of the eye they localized preferentially in the posterior segment, in a region corresponding to the RPE. The RPE is a single layer of pigmented cuboidal epithelial cells adjacent to the neural retina. In order to define precisely the MNP localization after one day from the injection, we studied the fluorescence of MNPs on cryostat sections without pigment bleaching, to highlight RPE (Fig 2a). In this way we established the precise localization of the MNPs, as red spots, in RPE. The moderate red background is most likely due to a partially degradation of the MNPs linked fluorophore, as confirmed by the histological staining (Prussian Blue) of particles which were found to co-localize only with RPE layer (Fig 2b).

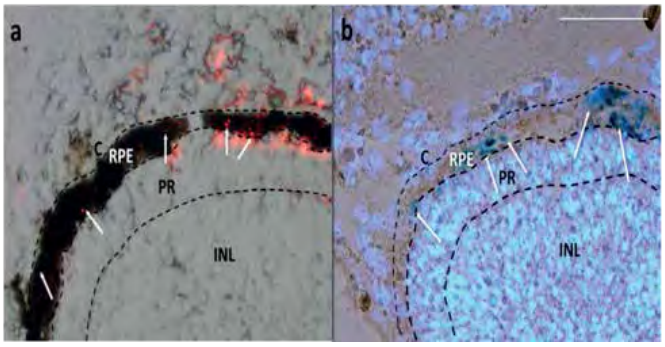

Figure 2. Cryostat sections of Xenopus embryos 1 day after injection. a) Merged image of bright field (showing the pigmented RPE) and red fluorescence field (red spots are MNPs). b) Image from a bleached section merging the bright field (MNPs are dark blue spots by Prussian Blue staining) and fluorescence field (nuclei are fluorescent blue by Hoechst staining). White arrows point some MNPs. C: choroid; RPE: retinal pigmented epithelium; PR: photoreceptors; INL: inner nuclear cell layer. Scale bar: 50µm.

In order to characterize the kinetics of the migration process, we monitored the localization of MNPs at different time points starting from 5 min to 24 h after injection. Just 5 min after injection, the particles started to spread out from the vitreous chamber (VC), adhering to the neural retina (NR) and only few were in RPE (Figure 3a). After the MNPs continue to progressively migrate at RPE from the vitreous chamber and the migration process is completed within 24 h (Figure 3a).

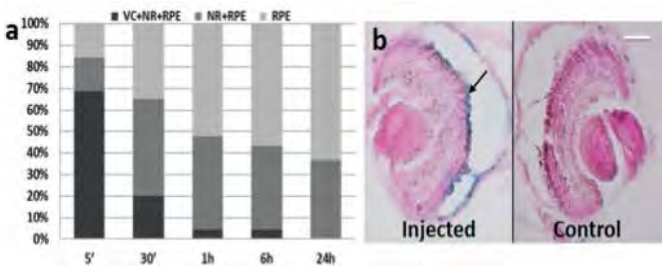

Figure 3. a) Graphical representation of MNP localization in eye regions of the embryo population. VC: vitreous chamber; NR: neural retina; RPE: retinal pigmented epithelium. b) Prussian Blue staining on paraffin section of Xenopus embryos at 20 days from MNPs injection. MNPs are blue labeled. Arrows point to MNPs derived from RPE microvilli. Scale bar: 50µm.

Another crucial factor for efficient drug delivery is permanence at the intended target site. For this reason, we monitored the retention of MNPs in embryos for periods up to 20 days. The MNP localization follows RPE during its development, included in the later stages (20 days) where RPE microvilli interdigit with the outer segment of photoreceptors (arrow in fig 3b).
It is known that NPs size and surface charge influence the movement of nanoparticle-based ocular drug delivery systems. We investigated the effect of size and charge on MNP movement by comparing the localization of our MNPs (250 nm, -17 mV) with the localization of particles of similar size but more negatively charged (MNP-), or positively charged (MNP+), or particles with neutral charge but small size (MNPs). Surprisingly, the results were the same with all kinds of MNPs, i.e., they localized in RPE one day after injection with only a small fraction in NR and no particles diffusion to extra-ocular tissues. For the first time we demonstrated that charge surface, beyond the size, does not influence the localization of nanoparticles in RPE. We speculate that MNPs, with different charge and size, can diffuse in the vitreous, infiltrate among retinal neurons without cells engulfing until their reach RPE. These cells have a strong phagocytic activity, required for maintaining constant renewal process of the photoreceptor outer segments.
In order to understand if the capability of MNPs to localize in RPE is species specific, we injected MNP in the left eye of zebrafish embryos at 48 hour post fertilization. We found that after 1 day from injection the MNPs localize specifically in RPE also in zebrafish (Fig 4). This datum suggests that the localization of MNPs in RPE is not species-specific.

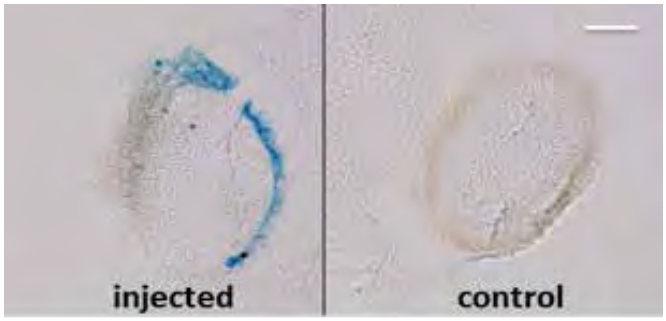

Figure 4. Prussian Blue staining (without pararosaniline counterstaining) on paraffin section of zebrafish embryos after 1 day from injection. MNPs are blue labeled. Scale bar: 50µm. n=45.

In conclusion, we have developed a protocol for fast and specific localization of magnetic nanocarriers in RPE layer, in an embryo model for the study of vertebrate diseases as a first step for therapeutic proof-of-concept studies, replacing or drastically reducing the use of mammals. We demonstrated that MNPs localize autonomously and specifically in RPE after IVT injection independently by particle size and surface charge. Moreover, this process seems to be not specie-specific. The MNPs have the potential for development as an ocular drug delivery, capable of targeting RPE with sustained controlled drug release providing MRI tracking for a variety of retinopathies. Moreover, the MNPs could be exploited also for magnetic hyperthermia treatments of ocular iper-proliferative dis-

eases. Additionally there are other challenging applications which could be explored on the use of MNPs, such as magnetic targeting of RPE in the treatment of retinal detachment by applying external magnetic forces.

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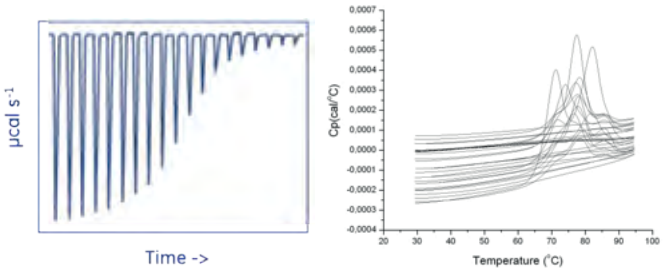
MANAGING HEAT AND DISORDER, CALORIMETRIC ASSAYS IN LIFE SCIENCES

PETER GIMESON
GE Healthcare, Life Sciences

This seminar will discuss the use and application of calorimetric assays in determining affinities and stability parameters in life science. It will highlight the added value given by thermodynamic profiling in order to differentiate interaction systems and monitor effects of modifications seen in enthalpy/entropy changes. We will also discuss high resolution thermal stability assay for domain de-convolution and how modifications and composition influences domain/global thermal stability. The seminar will cover instrumentation, application examples and sample preparation.



BIOPHYSICAL MARKERS FOR PERSONALIZATION OF NANOMEDICINE-BASED TUMOR THERAPY

BIANA GODIN, Kenji Yokoi, Tomonori Tanei, Fransisca Leonard, Anne Van de Ven, Jenolyn F. Alexander and Mauro Ferrari
Department of Nanomedicine, Houston Methodist Research Institute, 6670 Bertner St., Houston, Texas 77030, USA

Apart from genomic or proteomic factors at the nano-scale, such as upregulation of anti-apoptotic proteins or overexpression of efflux mechanisms, tumor therapeutic response may be profoundly influenced by elements at coarser physiological scales in the tumor microenvironment, such as diffusion gradients of the drug. These bio-barriers reside at multiple physical scales, spanning an enormous range of ten orders of magnitude from the subcellular nano- to the tumor tissue and whole body scales. Yet the coarser tissue- and the finer nano-scales are intricately linked, with effects in one influencing the other in unexpected ways. Solid tumors typically consist of stromal components, fibrous and connective tissues, and even multiple tumor cell genetic clones. The functionally and anatomically irregular tumor vasculature is layered atop this intrinsic heterogeneity, and is characterized by diminished oxygen tension, collapsed vessels, intermittent flow, and an abnormally large mean tissue-to-vessel distance. As a result, the tumor microenvironment can be highly heterogeneous.

The bio-barriers are highly dependent on the nature of the organ and thus, for the same tumor, differ drastically between primary tumor and metastatic lesion's loci. Systemically administered molecules and nanovectors must first flow through the vasculature towards the tumor site, and then either attach to the tumor vascular endothelium or extravasate and diffuse through the tumor tissue. Alternatively, the nanovectors could be designed to be uptaken by the cells and to release drug intracellularly. There is a need to find the factors that may prescribe parameters for rational design of nanotherapeutics to efficiently localize in the tumor tissue. Since in the case of tumors the majority of nanotherapeutics are intended for intravenous administration, the main bio-barriers that affect homing of nanotherapeutics to the disease site are the blood flow dynamics in the lesion and the intactness of the blood-tumor barrier (including fenestrations in the blood vessel walls and basal membrane). The aim of this presentation is to describe a few markers, which can be useful in personalization of nanotherapeutics design, based on the nature of the lesion.

To evaluate the nature of the lesions produced by the same cell population in different organs of BALB/C mice, we are using orthotopic models of primary and metastatic tumors using breast cancer (4T1) and lung cancer (3LL) cell lines. In the case of primary tumor, the cells are implanted in the primary location, as an example breast cancer cells in the mammary fat pad. Experimental liver metastasis are produced by injecting the cells into the spleen from where they naturally disseminate into the liver. Brain metastasis are based on the injection of cells into the right carotid artery and for lung metastasis intravenous injection of the cells into the tail vein of the animal is used.

The blood flow and accumulation of nanovectors in the different organs were evaluated by intravital microscopy and histological analysis. It was shown, for example, that while, primary breast tumor is enriched with blood neovasculature, the same tumor in the liver has insufficient or non-functional blood vessels, as presented in Figure 1. Thus, delivery of macromolecules and nanotherapeutcs to the center of the lesion significantly differs. These transport variables affect the efficacy of therapeutics which are unable to reach the intended site in the case of liver metastasis. As a result, our studies show that in the case of liver metastasis it is beneficial to enhance the accumulation of the nanotherapeutics into the tumor associate macrophages, thus retaining the drug in the lesion for a prolonged time and enabling higher therapeutic response.

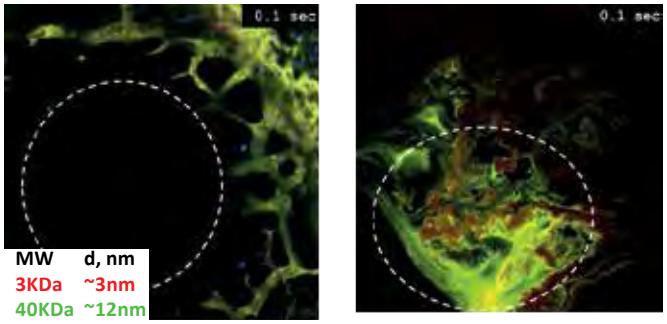


Figure 1: Intravital micrographs showing differences in the functional vasculature of 4T1 liver metastasis (left) and primary tumor in the mammary fat pad (right). Dextran tracers having MW of 3KDa (red) and 40KDa (green) were injected intravenously. Marked area represent tumor sites.

Another parameter that can affect the accumulation of the nanotherapeutics into the tumor is the intactness of the basement membrane. We correlated the extravasation of nanotherapeutics with tissue levels of collagen type IV and plasma levels of MMP-9 and TIMP-1 and found that the accumulation of doxorubicin liposomes in various organs and various tumors is governed by the balance between MMP-9 and TIMP-1, which can be detected in the plasma. These factors could serve as surrogate biomarkers for selecting patients who are likely to be more susceptible to nanotherapy by accumulating higher concentrations of nanoparticles in their tumors.

Based on our data, we propose to use biophysical markers, such as blood flow and serum factors that can be evaluated prior to therapy to personalize nanotherapy and to find the population of patients which will benefit from it the most.

INTEGRATIVE NANO-COMPOSITES AND REGENERATION OF THE EYE (I-CARE)

MAY GRIFFITH
Integrative Regenerative Medicine Centre, Linköping University, Linköping, Sweden

Our aim was to develop a regenerative medicine-based treatment for corneal Herpes Simplex Keratitis (HSK), to replace the current ineffective donor cornea transplantation used to treat the vision loss. We designed an approach to simultaneously treat the disease while regenerating the damaged cornea, drawing heavily on nano-biotechnology (tissue reconstruction) and nanolaser surgery. We developed nano-composite, hydrogel-based implants with multi-scale 3D architectures that stimulated the body's own stem cells to regeneration the damaged cornea. We also developed a method for patterning the hydrogels to modulate cell behavior. We also developed implants with nanoparticulate carriers loaded with drugs or components from the innate immune system, e.g. cationic antiviral peptide, LL37, to prevent viral reactivation and circumvent further inflammation mediated damage to the eye. As HSK can deplete the healthy stem cell pool, while remaining latent within the cornea and nerves, we also developed techniques to confer "viral resistance" to therapeutic replacement cells by transfer of LL37 genes to the cells. Overall, these technologies will help bring to the clinic some future regenerative treatments for corneal HSK and other high risk transplantation.

WHAT DO NANOMED ENTREPRENEURS NEED FOR DEVELOPING THEIR BUSINESS?

CHRIS GUIFFRE

INTRODUCTION TO CERULEAN

- Winning the fight against cancer with dynamically tumor targeted nanopharmaceuticals
- Dynamic Tumor Targeting™ platform
  - Differentiated -- more drug in tumors for prolonged periods with less systemic exposure
  - Attractive -- new chemical entities with composition of matter IP
  - Broad -- small molecule and RNAi payloads
- Lead candidate, CRLX101, being developed in three indications
  - Combinable topo 1 and HIF-1a inhibitor
  - Multi-indication potential
- Platform-generated pipeline
  - 2nd candidate, CRLX301, expected to enter clinic by end of 2014
  - Extensive additional product opportunities
- Potential near-term value creation with robust news flow in 2014 and 2015

CERULEAN HISTORY

- Founded with MIT technology
- Advanced with CalTech technology
- Focus on internally developed products v. being a CRO to Big Pharma
- Initial clinical success
- Subsequent clinical setback
- Clinical rebirth of CRLX101

PREPARING FOR THE POSSIBILITY OF SUCCESS

- Innovative technology
- Strong venture syndicate
- Talented and experienced management team

PREPARING FOR THE INEVITABLE SETBACKS

- Optionality
- Committed venture syndicate
- Tenacious management team

SHORT-TERM AND LONG-TERM PLAN

- Capital-efficiency and optionality
- Race to POC
- Multi-product platform oncology company
- Self-commercialization in the US
- Partnered platform

THE PROS AND CONS OF GRANT FUNDING

- Non-dilutive funding
- Small dollars, large time investment, low POS

THE PROS AND CONS OF ISTS

- Incredibly capital efficient way to create options
- Hard to control pace of enrollment

THE PROS AND CONS OF PARTNERING WITH BIG PHARMA

- Non-dilutive funding
- Validation
- Invaluable expertise and resources
- Additional value creation stream(s)
- Time sink with limited early rewards
- More or less relevant once public?

BUILDING A PUBLIC NANOPHARMACEUTICAL COMPANY

- Promising lead asset with strong clinical data
- Attractive clinical and regulatory plan with near-term milestones
- Large market opportunities
- Platform-generated pipeline

CREATING VALUE BY HELPING PATIENTS

- Randomized trials against standard of care
- Better efficacy AND better safety . . . the promise of nanotechnology

ENABLE PROCESSES OF CO-CREATION DURING RESEARCH AND INNOVATION- HOW CAN SOCIETY BENEFIT FROM NANOTECHNOLOGY?

S. HARTL[1], P.v. Broekhuizen[2], A. Grobe[3], A. Porovic[4], D. Schuurbiers[5], A. Falk[1]
[1]BioNanoNet Forschungsgesellschaft mbH, Elisabethstraße 9-11, 8010 Graz, email: sonja.hartl@bionanonet.at; andreas.falk@bionanonet.at
[2]IVAM UvA – Nanotechnology and chemical Risks, Amsterdam, NL
[3]University of Stuttgart, Stuttgart, Germany
[4]Nanotechnology Industries Association, London, United Kingdom
[5]Responsible Innovation Collective, Delft, Netherlands

The public perception on nanotechnologies has been diminishing over the past years. Regarding society-relevant solutions and applications close to the consumer, it is evident that the public discussion on nanotechnologies lacks vision and inspiration. For this reason it is of utmost importance to engage public society into nanotechnologies development on production and development level in order to encourage the beneficial effects (e.g. in nano-medicine). Furthermore stakeholder engagement and dialogue are essential to the responsible development of nanotechnologies in Europe.
The FP7 project NanoDiode integrates vital engagement activities along the innovation value chain: at the level of research policy, research & development (R&D), and the diffusion of nanotechnology innovations in society. The project combines 'upstream' public engagement (by way of dialogues that integrate societal needs, ideas and expectations into the policy debate) with 'midstream' engagement (by organising innovation workshops at the level of the R&D practices that are at the heart of the research and innovation enterprise) and 'downstream' strategies for communication, outreach, education and training.
In order to enable the process of co-creation, user committees will be established representing a platform enabling the transfer of knowledge exchange of information about new scientific developments that are expected to enter the market in the form of innovative products or processes. The aim of UCs is not only to give a voice to stakeholders and citizens, but to also give them an opportunity to participate in the innovation process. This means they are involved in Research and Innovation (R&I) projects where they can provide their input (i.e., their preferences, wishes, (dis-)approval, fears, presumptions, etc.) on the envisaged products or processes, and to discuss these with the researchers. Amongst others, a topic for UC is Nanomedicine –connecting fundamental research with industrial applications and pharmaceutical developments to solve the question: "How can society benefit from nanotechnology?

As such, the UC allows the researchers to assess their ideas and expected results against the opinions of societal stakeholders (i.e., those who will become the "users" of any eventual products). This gives the researchers the opportunity to identify and resolve possible barriers to innovation. Adaptations to the research or product design could avoid or counteract identified dilemmas and attune R&I to societal values and needs.

Finally, dialogue workshops bring together lay Europeans and the European nano-community to discuss nanotechnologies' contribution in addressing important societal challenges. The aim is to draft recommendations for preferred innovation areas, with respect to ethical, social and environmental questions

DEVELOPMENT OF NANOPARTICULATE NUCLEIC-ACID BASED THERAPEUTICS, SNARES™, FOR THE TREATMENT OF CLOSTRIDIUM DIFFICILE AND PREVENTION OF RECURRENCE

KOSTAS HATZIXANTHIS[1], Teresa Díaz Calvo[1], Rahul Choudhary[3], Doroty Codoni[1], Davide Carta[1], Alejandro Marin[1], Clara Franch[1], Peter Warn[2], Cristina Freire[3], Mark Saunders[3] and Michael McArthur[1]
1Procarta Biosystems, Norwich, UK, [2]Euprotec, Manchester, UK, [3]Kuecept, Potters Bar, UK

OBJECTIVES

To provide a lasting solution to the treatment of bacterial infections, using a proprietary technology based on nanoparticulate oligonucleotide therapeutics. These nucleic acid-based, Transcription Factor Decoys (TFDs), act on novel genomic targets by capturing key regulatory proteins to block essential bacterial genes and defeat infection. This is a platform technology allowing rapid development of novel antibacterials and has been validated in vitro and in vivo against MRSA. Here we report our latest work to develop an antibacterial to prevent recurrence in C. difficile associated disease (CDAD).
Our aim is to develop a new type of oligonucleotide antibacterial capable of tackling both the infection and the symptoms of the disease. Ideally this would have four properties: Narrow spectrum activity, activity against resistant strains, ease inflammatory response and prevent recurrence.
A summary of advancements against Gram positive bacteria including preliminary in vitro data against Gram negative bacteria will also be presented.

METHODS

To assess the ability of TFD-loaded nanoparticles to deliver to C. difficile cells, an exponentially growing culture was incubated with nanoparticles loaded with fluorescently labelled TFDs and microscopy slides were prepared and examined by confocal microscopy. In vitro studies were performed in which stationary phase C. difficile cultures were incubated with nanoparticles loaded with an oligonucleotide which effectively blocks sporulation. After 24 hours, number of spores and vegetative cells present in the treated sample and in an un-treated control were assessed and compared.
TFDs were encapsulated in a nanoparticulate suspension (Loaded NanoParticles LNP) using a proprietary delivery lipid (CM2) and given orally by gavage. Golden Syrian Hamsters were pre-conditioned with clindamycin and were infected after 24h with C. difficile, BI1, spores. 4h post infection, hamsters received 3 x daily, for seven days, vehicle, scrambled TFD (LNP), active TFD (LNP) or 25 mg/kg vancomycin. Vegetative cells, spores and C. difficile toxins were determined.

RESULTS

Confocal microscopy images shown efficient transfection of C. difficile, with a strong fluorescent signal from labelled TFDs present in the inside of the bacterial cells (Fig. 1). The in vitro sporulation data showed that the TFD rendered C. difficile asporulate in vitro when compared with an un-treated control sample. Oral administration of novel TFDs in a hamster model of severe CDAD gave significant protection compared to vehicles. Data confirming TFD potency including survival rates, spore counts and toxin levels will be presented (Fig. 2).

CONCLUSION

During therapy, antibiotics act on pathogenic bacteria but also disturb the host microbiome by inhibiting non-pathogens. Suppression of resident gut flora allows dormant spores of C. difficile to colonize the gut and generate cytopathic toxins which cause an inflammatory response leading to Pseudomembranous colitis (PMC). In the later stages of the infection bacterial endospores are shed in the faeces and greatly increase the chance of re-infection or spread to fellow patients. The recurrence rate of the disease is estimated to be between 20 and 45% following resolution of initial treatment and the ability to break the cycle of re-infection is a major unmet medical need. This work contributes to the development of a new treatment that can uniquely counter the problem of recurrence and alleviate the symptoms of CDAD (ease PMC, minimise damage to commensal bacteria).

TFD TARGETS

The antibacterial under development is a nanoparticle containing a transcription factor decoy designed to kill C. difficile in the gut and prevent sporulation, to create a narrow-spectrum antibacterial to better treat CDI. The TFD targets Spo0A, a highly conserved transcription factor that controls sporulation in all strains of C. difficile as well as other clostridial and bacilli species (Escobar &Castano 2009 In Silico Biol. 9: 142-162). A C. difficile strain with a deletion of Spo0A shows markedly lower levels of re-infection and transmission than the wild-type control in mouse models (Deakin et al. 2012 Infect. Immun. 80: 2704-2711). Spo0A also has a role in toxin production and the severity of other virulence factors, including biofilm formation (Underwood et al. 2009 J. Bacteriology 191: 7296-7305). The ability of C. difficile to produce resistant and highly infectious spores is likely one of the major reasons for the high rate of recurrence of C. difficile infections.

DELIVERY MECHANISM

Delivery of large and charged oligonucleotides to C. difficile present a substantial challenge as the therapeutic must cross a proteinaceous outer layer (S-layer) and a phospholipid membrane to enter the cytoplasm. Procarta has developed a proprietary lipid that self-assembles with the TFD to form a nanoparticle that is capable of delivering to all bacteria tested (both Gram-positive and Gram-negative pathogens). The lipids used to form the nanoparticles are in a class referred to as bolaamphiphiles as they consist of two polar head groups joined by a hydrocarbon chain, a dequilinium analogue). Close to 100% TFD encapsulation was achieved and the resultant lipoplexes were stable in a wide range of biological fluids and conditions. To demonstrate the delivery of oligonucleotides to C. difficile the bacteria were counter-stained with a dye to label the cell wall red, and the bacteria treated with nanoparticles loaded with fluorescently labelled TFD (green) and visualized by confocal microscopy (Fig. 1). This study demonstrated that the TFD was successfully & efficiently internalized. It is worthy of note that similar studies showed internalization to MRSA and E. coli, emphasizing that the delivery system the company has developed is 'one size fits all'. This means that advances in the understanding of the nanoparticles properties (expected from EU grant DNA-TRAP) and advances on their formulation (expected from the TSB grant Formulation Products – meeting the product & process design challenge (240223)) will be tested in the near future and will be able to be translated to other products in the company's pipeline. The Nanoparticles have a controlled average size and are cationic in nature. The nature of the bacterial membrane (thickened cell walls and changes to the charge), a potential resistance mechanism, does not affect the uptake, as no change in the MIC was seen across a panel of Gram positive and negative bacteria. The working hypothesis of nanoparticle degradation and cargo release upon entry into the bacterial cytoplasm will be discussed.

IMPROVEMENTS TO FORMULATION

A combination of biophysical analysis of the nanoparticles and biological activity assays has been used to improve the stability and efficacy of preparations by using a combination of off the shelf excipients. Further, work has been initiated investigating methods of spray- and freeze-drying the preparations for longer term storage.

In vitro POC studies, in which Procarta is currently developing a solid formulation that will allow its nanoparticulate oligonucleotides to be formulated for oral delivery to the gut to treat C. difficile, will be completed in Q3 2014. Project partner Kuecept has developed several novel drug delivery technologies that can facilitate the targeting of therapeutic compounds to specific regions of the gastro-intestinal tract, either to improve lumenal stability and systemic absorption or facilitate local release for topical applications. By Q3 2014 Procarta will also have access to futher information on the impact on formulation development through the FP7 IAPP project DNA-TRAP. This will lead to significant advances in formulation and therefore be instrumental to Procarta to drive further this programme to Toxicology studies and Phase I clinical trials.

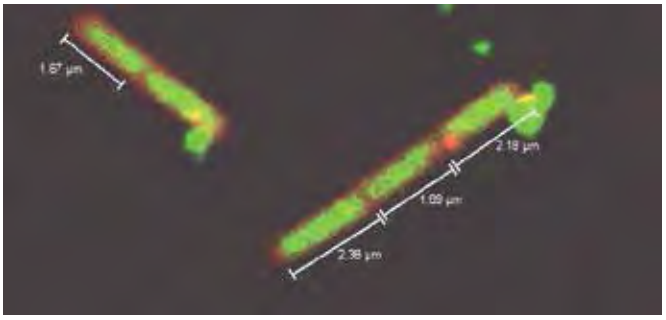


Fig 1: Confocal microscopy confirms nanoparticulate delivery of TFD oligonucleotides to C. difficile.

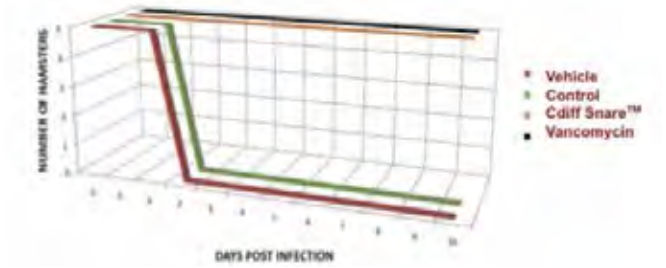


Fig 2: Survival data following infection with C. difficile SpoOA nanoparticle. Golden Hamsters were treated with Clindamycin to sterilize their guts 24h before infection with C. difficile spores. A further 24 h later animals were treated by oral gavage with 25 mg/ml Vancomycin (Vancomycin), 0.2 mg/ml nanoparticulate C. diff TFD, Vehicle control (PBS) or an empty nanoparticles (Control) all treatments were given every 8 h for 7 days and the health of the animals and bacterial load monitored for 10 days in total.

BIOENGINEERING AND NANOTECHNOLOGY: PREVENTING ANTIBIOTICS RESISTANT MICROBES

JAMES L. HEDRICK

With the increased prevalence of antibiotic-resistant infections, there is an urgent need for development of innovative antimicrobials. Macromolecular antimicrobial agents such as cationic polymers and peptides have recently received increasing attention because they can selectively target and disintegrate bacterial membranes via electrostatic interaction and insertion into the membrane lipid domains, avoiding potential bacterial resistance. Despite their efficacious antimicrobial activity, both peptides and synthetic polymers have seen limited clinical applications because of several inherent problems. For example, antimicrobial peptides (the first host defense for many organisms against environmental parasitic infections) are generally sensitive to enzymatic degradation, suffer from expensive large-scale production, and their pharmacokinetics are inadequately studied. Regardless of promising clinical trial results, no antimicrobial peptide has received FDA approval for general public use. On the other hand, a plethora of bio-inspired synthetic polymers have been proposed and are achieving considerable success in overcoming many drawbacks found in using peptides. These polymers often have comparable if not better antimicrobial activities than peptides. Unfortunately, biocompatibility and/or biodegradability have presented significant problems during in vivo administration.

In this talk, a new class of antimicrobial polymers will be discussed. These antimicrobials are based on biodegradable cationic polycarbonates, which are synthesized by our organocatalytic living ring-opening polymerization approach. This synthetic platform yields polymers with well-defined molecular weight and structure, which is crucial in the future clinical applications as individual molecular weight fractions of a polydisperse system are expected to exhibit distinct pharmacological activities in vivo. Polymers with various molecular configurations (e.g. linear, branched, star-like, random and block) have been designed and synthesized. The polymers with optimal hydrophilicity/hydrophobicity balance have strong activities against multidrug-resistant Gram-positive and Gram-negative bacteria as well as fungi without inducing significant toxicity both in vitro and in vivo. Therefore, these antimicrobial polymers hold potential for use in the prevention and treatment of multidrug-resistant infections.

THE ROLE OF IT AS ENABLER OF TRANSLATIONAL NANOMEDICINE

MICHAEL HEHENBERGER

The ultimate goal of Clinical Nanomedicine will have been achieved when deep Life Sciences knowledge and understanding have been fully translated into medical, clinical practice. Only then patients will benefit from the nanomedical breakthroughs conceived by biomedical scientists.

Information Technology plays a role throughout this process, by

- complementing laboratory experiments via data management and analysis
- assisting scientists in their literature search and hypothesis generation activities
- predicting outcomes via modeling and simulation of biochemical processes, applied e.g. to disease target identification in drug discovery and targeted nanomedical drug delivery
- integrating various diagnostic sources of information to support clinical decision support
- connecting healthcare stakeholders to enable the transformation of clinical care towards targeted and personalized delivery of "precision medicine"

To illustrate the points made above we will provide concrete examples such as cancer diagnostics and treatment, discuss the significant progress that was made during the past decade, but also point to the inhibitors of change.

MEGAOPPORTUNITIES WITH A NANORISK? INTRAVASCULAR APPLICATION OF MAGNETIC METAL NANOPARTICLES

INGE K. HERRMANN[1-2], Andrea A. Schlegel[1-2], Lukas Bircher[1-2], Melanie Jacobsen[1-2], Christoph M. Schumacher[3], Judith Peters[1-2], Urs Ziegler[1], Rolf Graf[1-2], Pierre-Alain Clavien[1-2], Wendelin J. Stark[3], Beatrice Beck-Schimmer[1-2]

[1]University of Zurich, Zurich, Switzerland
[2]University Hospital Zurich, Zurich, Switzerland
[3]ETH Zurich, Zurich, Switzerland

Magnetic particles hold great promise for a number of applications including targeted destruction of malignant cells by magnetic drug delivery or hyperthermia. However, commonly used magnetic oxide particles, although they show potential in clinical studies, suffer from low saturation magnetization and consequently weak response to magnetic field gradients. Recent technological developments have allowed production of magnetic nanoparticles with a metallic iron core, increasing the saturation magnetization by a factor of three. While these particles show greatly improved performance in magnetic separation efficiency, side effects of these nanoparticles remain unknown.

In this project, we investigated two of the most promising future applications of carbon-coated nanoparticles, being 1) magnetic blood purification and 2) magnetic drug targeting. Importantly, we performed a detailed risk evaluation in parallel by studying effects on the vascular compartment, including blood coagulation, interactions with endothelial cells under stationary as well as flow conditions, uptake by phagocytes and complement activation. In order to gain insight into relevant exposure concentrations, we looked at the separation efficiency of magnetic nanoparticles in an extracorporeal setting, defining worst-case exposure scenarios. We then performed an in vivo long-term study in mice, focusing on possible particle-induced side effects such as tumors, fibrosis or inflammation.
While carbon coated magnetic nanoparticle-based applications show great promise for rapid detoxification and pathogen removal, the particles are of high chemical stability calling for caution when translating the therapeutic concepts towards clinical application. However, absence of any significant adverse effects suggests further investigations of both chances and risks of carbon coated metal particles in nanomedicine with the aim of identifying therapeutic applications that outweigh potentially associated risks.

THE IMPORTANCE OF THE COMPOSITION OF THE SHELL TO INFLUENCE THE IN VIVO BEHAVIOUR OF INORGANIC NANOPARTICLES

HEINRICH HOFMANN

Powder Technology Laboratory, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland; heinrich.hofmann@epfl.ch

Superparamagnetic iron oxide nanoparticles (SPIONs) are recognised as promising advanced materials for various biomedical applications, such as targeted drug delivery, contrast agent for imaging, cell tracking, and transfections 1-6. Iron oxide, γ-Fe2O3, is of special interest because of the approved biocompatibility of these nanoparticles (NPs), including the well-understood metabolism of the NPs in the liver 7. For the majority of the aforementioned biomedical applications, the NPs are in contact or taken up by various cell types of the living tissue; in this case, the physicochemical properties of the NPs and of their surface in particular play a crucial role in their interaction with cells. Studies conducted in the last few decades on the interaction of engineered nanomaterials with biologically relevant molecules have improved our understanding of the behaviour of these materials in human and animal bodies and have helped to identify in vitro assays that are predictive of in vivo biodistribution/toxicities. However, there are still valid concerns regarding in vitro methods for determining the biocompatibility of NPs or toxicity tests for engineered NPs 8. After NPs have been in contact with biological media, their surfaces are covered by various biomolecules (e.g., proteins), which is known as a “protein corona” 9-14. Here, using the unique magnetic properties of the SPIONs, the NPs were extracted from rat sera after in vivo interaction with the rat’s physiological system; thus, one of the very few studies on in vivo protein-NP complexes was performed. The SPION core of PVA coated NPs does not (or minimally) have an influence particle biodistribution and, most likely, the core particles are protected by PVA molecules against direct interaction with plasma proteins and cells. The information from ex vivo protein adsorption and biodistribution bring us more accuracy and understanding of the overall picture of NPs fate in vivo initially from the NP-protein interaction to physiological aspect. The evaluation of soft corona would be a next interesting step for further studies to understand more the detail of protein adsorption and biological process relationship which will be useful not only for diagnostic, drug delivery, but also nano-safety of the NPs for bio-application in the future.

ACKNOWLEDGEMENTS

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

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SCIENTIFIC INTRODUCTION: FUTURE CONTEXTS OF CLINICAL NANOMEDICINE AND TARGETING MEDICINE

PATRICK HUNZIKER

Prof. Dr. med., University Hospital Basel

This introductory talk reflects on the current needs of patients and society for a more effective, more innocuous, sustainable and economic medicine. It defines the scope of this conference as a bridge between fundamental research and clinical application, as a joint step forward by a highly interdisciplinary scientific and clinical community, and as an event that links a broad spectrum of stakeholders from researchers to clinicians, from industrialists to regulatory bodies, and from application orientation to the exploration of implications. It sheds light on the specific characteristics of nanomedicine and targeted medicine that render those fields particularly promising for advances in medical prevention, diagnosis and therapy, and gives examples where significant contributions to the state of the art and to healthcare systems in developing and developed countries can be made.

ERADICATION OF ATHEROSCLEROSIS SYNOPSIS AND OUTLOOK

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

This talk explains the goal of atherosclerosis eradication in terms of patient benefit, societal benefit, and cost control for healthcare. It gives an concise overview of the current state in clinical work focusing on control of atherosclerosis progression and the induction of atherosclerosis regression and discusses the strengths and weaknesses of such approaches. It considers a range of potential strategies and the challenges to be overcome to reach the goal of eradication of atherosclerosis.

INNOVATION IS DRIVEN BY THOSE WHO CARE AND THEREFORE FIND WAYS TO CHANGE THE STATUS QUO

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

This statement reflects on the value of motivation and of personal involvement in paving the path of scientific progress and of societal improvements.
Highly dedicated and motivated individuals have played important roles in steps forward in patient care.
In an era where strategic planning of research is increasingly focused on enabling product development on beaten paths of research and development, the value of the individual caregiver and scientist for major advances in healthcare, science and society merits its emphasis.
Therefore a sound balance in academic institutions, enterprises and funding policy between bottom-up and top-down approaches needs to be re-established.

PROTEIN-GOLD NANOPARTICLE ADSORPTION ASSAY FOR PROSTATE CANCER DETECTION AND DIAGNOSIS

QUN HUO[1], Shiyun Zhang, Nickisha Pierre-Pierre
[1]NanoScience Technology Center, Department of Chemistry in College of Science, Burnett School of Biomedical Science in College of Medicine, University of Central Florida, 12424 Research Parkway Suite 400, Orlando, FL 32826. Email: Qun.Huo@ucf.edu
Tel: 407-882-2845

INTRODUCTION

Prostate cancer (PCa) is the most common malignancy and the second leading cause of cancer-related death in Western male population. The American Cancer Society estimates that about 233,000 new cases of prostate cancer will be diagnosed and 29,480 men will die of prostate cancer in the United State in 2014.1 Current prostate cancer diagnostic tests have difficulty to distinguish aggressive cancer from indolent tumor, resulting in a significant number of unnecessary drug treatments and surgical procedures. These overtreatments can bring urinary, bowel, and/or sexual side effects that negatively impact the quality of life for many patients. Although a number of new tests for prostate cancer have become available in the last few years, these newest tests have limited clinical applications. There is a lack of low cost, non-invasive screening tests that can detect and identify high risk prostate cancer at early stages. Such tests are essential for reducing the number of over-diagnosis and overtreatment, and reducing cancer-related death by enabling the patients with aggressive cancer to receive appropriate treatment as early as possible.
Dynamic light scattering is an analytical tool used routinely to determine nanoparticles size in solution. Gold nanoparticles (GNPs) scatter light intensely at or near their surface plasmon resonance wavelength. By introducing GNP as a light scattering probe, and using dynamic light scattering as a read-out system, our group has developed a nanoparticle-enabled dynamic light scattering assay (NanoDLSay) for chemical and biological detection, and for biomolecule-nanoparticle interaction study.2,3 NanoDLSay detects target analyte molecules by measuring the average particle size change of gold nanoparticle probes upon binding with the target analyte molecules (Figure 1A). Proteins are macromolecules with a typical diameter ranging from 2-3 nm to 10s of nm. For example, the diameter of a bovine serum albumin is 6-7 nm, and the diameter of an IgG is 7-10 nm. Proteins readily adsorb to the negatively charged citrate-protected GNPs to form a “protein corona” on the nanoparticle surface through a combination of electrostatic interactions, van der Waals interactions, Au-N and Au-S bonding. When a layer of protein is adsorbed to the GNPs, the average size of the nanoparticle could increase by twice of the diameter of the protein. DLS not only can detect protein adsorption to GNPs, but also reveal the size and morphological information of proteins such as oligomer and aggregate formation.

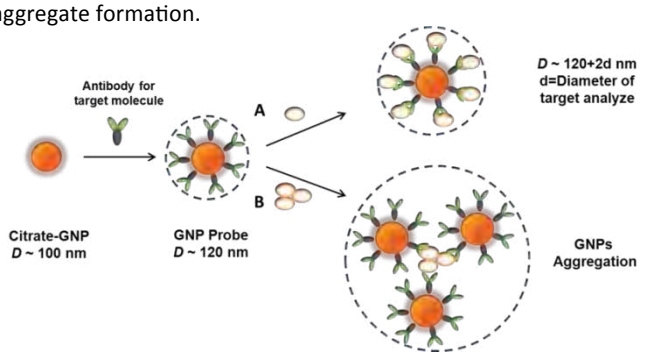

Figure 1. Schematic illustration of NanoDLSay . NanoDLSay detects target molecules by measuring the average particle size change of gold nanoparticle probes upon binding with the target analyte molecules. When a layer of protein is adsorbed to the GNPs, the average size of the nanoparticle could increase by twice of the diameter of the protein (A). When the proteins exist as oligomers, they can crosslink GNPs, resulting in a substantial average particle size increase (B).

The adsorption of protein oligomer and aggregates to GNPs can lead to crosslinking of nanoparticles into clusters, resulting in a substantial average particle size increase that is largely exceeding twice of the diameter of the protein monomer (Figure 1B).

RESULTS AND DISCUSSIONS

In 2011, we first reported a finding from the serum protein-GNP interaction study of mice models with and without prostate tumor using NanoDLSay technique.4 It was discovered from this study that the average particle size of the GNPs upon adsorption of serum proteins from prostate tumor-bearing mice was substantially smaller than mice without tumor. From a follow-up study, we identified that the presence of prostate tumor especially affects the adsorption of circulating human immunoglobulin G (hIgG) to the gold nanoparticles.5 In this study, a small amount of prostate tissue lysate was mixed with hIgG solution. After incubating for 30 min, the treated hIgG solution was mixed with GNP to conduct hIgG-GNP adsorption assay. A reverse correlation was observed from the assay results and the prostate cancer status and tumor grade (Figure 2). The average particle size of the assay solution of prostate tumor tissue lysates is smaller than the assay solution of non-cancerous prostate tissue lysates, which include both normal prostate (p-value<0.0001) and prostate with benign prostate hyperplasia (BPH) (p-value<0.0001). The higher grade tumor (Grade 3) leads to smaller particle size than the lower and intermediate Grade 1 (p-value<0.0001) and 2 (p-value<0.0001) tumor. Statistical analysis show significant difference between cancer and non-cancer group, and tumor with different grades. These results are very encouraging because they suggest that the new nanoparticle assay may be used to detect aggressive prostate cancer.

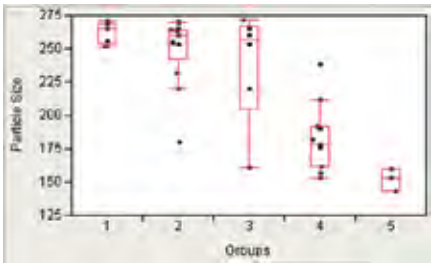

Figure 2. Tissue lysates-spiked hIgG adsorption to gold nanoparticles. To conduct the study, a small amount of prostate tissue lysate was mixed with hIgG solution. After incubating for 30 min, the treated hIgG solution was mixed with GNP to conduct hIgG-GNP adsorption assay. The average particle size of the assay solution was measured. Each group represents: 1: Normal; 2: Benign Conditions; 3: Grade 1; 4: Grade 2; 5: Grade 3. (Data adapted from reference 5)

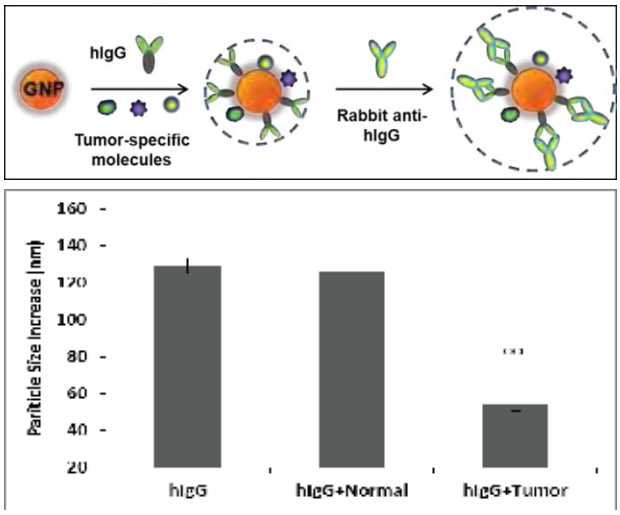

Figure 3. A two-step assay to determine the relative adsorption of hIgG to GNP. (A) Illustration of the assay. In the first step assay, mixture of hIgG and tissue lysates is adsorbed to GNPs. In the second step of the assay, rabbit anti-hIgG is added to determine the relative quantity of hIgG adsorbed to the GNPs. The particle size difference between the second-step assay versus the first-step assay was calculated and expressed as the assay results. (B) The particle size increase after adding rabbit anti-hIgG. The p value for the tumor versus the normal group is < 0.001, suggesting the difference is statistically significant (***). Two normal and two tumor tissue lysates were analyzed and data presented is the average results of multiple assays conducted on each sample.

From our further study, we discovered that the presence of prostate tumor caused a reduced adsorption of hIgG to GNP. A two-step assay as illustrated in Figure 3A was conducted to determine the relative quantity of hIgG adsorbed to GNPs. Following the adsorption of tissue lysate-treated hIgG adsorption to GNPs, a rabbit anti-hIgG was added to the assay solution. After a 6 min incubation time, the average particle size of the assay solution was analyzed again. If hIgG is present on the GNP surface, the binding of rabbit anti-hIgG to the surface-bound hIgG on GNPs will lead to a further particle size increase, and vice versa. Figure 3B is the assay results of pure hIgG, normal and tumor tissue lysates-treated hIgG. When rabbit anti-hIgG was added to the pure hIgG-GNP adsorption solution, an average particle size increase of ~130 nm was detected from both pure hIgG and normal lysate-treated hIgG solution. In contrast, a particle size increase of ~50 nm was observed from tumor lysate-treated hIgG. These data provided strong support to a reduced hIgG adsorption to GNPs at the presence of prostate tumor.

We conducted the same hIgG-GNP adsorption assay on several other cancer types, including lung, breast and colon cancer. Figure 4 is the summary of the results. The p-value for the normal and tumor group is 0.91, 0.18, and 0.16 for lung, breast and colon cancer respectively, indicating that these is no statistically significant difference between normal and tumor groups for these three cancer types. This limited study suggests that the competitive hIgG-GNP adsorption assay may be specific to prostate cancer.

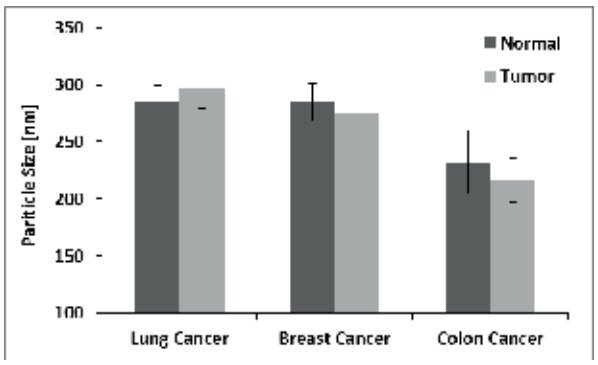

Figure 4. Tissue lysate-treated hIgG-GNP adsorption assay conducted on lung, breast and colon cancer. The normal and tumor tissue lysates for this study were matched samples from the same patients that were diagnosed with cancer. Normal tissue was taken from the non-cancerous region of the organ. Sample size for the three cancer groups are n=8, 12, and 5 for lung, breast and colon cancer respectively.

In summary, we discovered and developed a simple protein-GNP adsorption assay for prostate cancer detection and diagnosis. Results obtained from our most recent study along with the data published previously by our group4,5 confirm that the competitive hIgG-GNP adsorption assay has the potential to detect aggressive prostate cancer. The test is now placed under clinical validation study. Our final goal is to develop a non-invasive molecular test for early stage aggressive prostate cancer screening using urine or blood serum samples.

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DISCORDANCE BETWEEN FREE PACLITAXEL PLASMA LEVELS AND CLINICAL EFFICACY – LESSONS FROM TOCOSOL®-PACLITAXEL IN BREAST CANCER

LARN HWANG, Monica Choi, Kouros Motamed and Vuong Trieu
Sorrento Therapeutics Inc., Irvine, CA

STUDY PURPOSE

Nab-paclitaxel is a Cremophor-free formulation of paclitaxel with increased systemic level of free (unbound) paclitaxel. It has been hypothesized that the increased exposure to free paclitaxel accounted for the increased response rate observed for nab-paclitaxel (Abraxane®). This hypothesis was challenged by examining the phase III data of IG-002- Paclitaxel (formerly Tocosol®-Paclitaxel), where free paclitaxel levels were 4X higher than Cremophor-EL Paclitaxel (Taxol®) at Cmax.

METHODS

PK Clinical Study: The primary objective of this clinical pharmacology study was to compare the pharmacokinetics of free paclitaxel in plasma ultrafiltrates of patients receiving equivalent paclitaxel doses administered as IG-002 Paclitaxel and as Cremophor-EL Paclitaxel. Eligible patients were required to have an advanced non-hematological malignancy for which there was no curative therapy and for which treatment with a single agent taxane was appropriate.

Phase 3 trial data: MBC patients (1050) were screened for the study and a total of 821 were randomized to receive either IG-002 (100

mg/m2 weekly, IV) or Cremophor-EL Paclitaxel (80 mg/m2 weekly, IV) until disease progression.

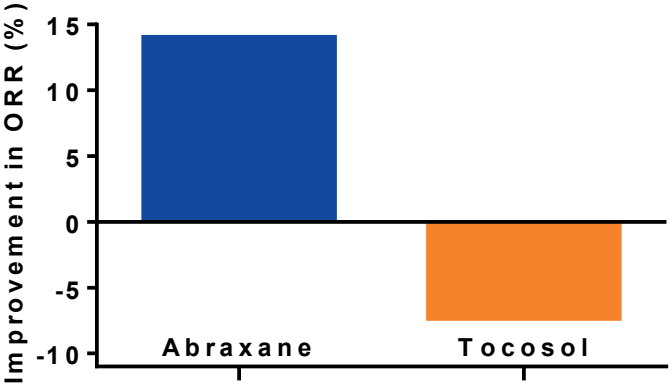


Fig. 1. Summary of Phase III Efficacy Between Abraxane® and Tocosol®-Paclitaxel (IG-002). Improvement in ORR observed for Abraxane® but not for Tocosol®-Paclitaxel compared to Cremophor-EL Paclitaxel

RESULTS

The PK clinical study showed a single 175 mg/m² dose of IG-002 Paclitaxel produces a mean 67% higher exposure to unbound paclitaxel, and a mean 108% higher exposure to total paclitaxel, than an equivalent single dose of Cremophor-EL Paclitaxel. Phase 3 data showed IG-002 Paclitaxel 100 mg/m2 weekly, results in greater myelosuppression compared to Cremophor-EL Paclitaxel 80 mg/m2 weekly, assessed by duration of ANC <1500, <1000 or <500 cells/mm³. There was significantly more overall neutropenia ($p<0.001$) and shift towards higher grades of neutropenia ($p<0.001$) for IG-002 Paclitaxel compared to Cremophor-EL Paclitaxel (Fig. 2). These differences were also seen with leukopenia ($p <0.002$). However, the primary endpoint of adjudicated ORR demonstrated an ORR of 44.7% for Cremophor-EL Paclitaxel and 37.4 %for IG-002 Paclitaxel.

Neutropenia	Grade	Taxol® (N=409)	Tocosol®-Pac (N=408)
Tocosol	0	130 (32%)	63 (15%)
	1	81 (20%)	40 (10%)
	2	102 (25%)	79 (19%)
	3	82 (20%)	161 (40%)
	4	12 (3%)	59 (15%)
	Any	277 (68%)	339 (83%)
Abraxane	Any	Taxol® (N=225) 185 (82%)	Abraxane® (N=229) 183 (80%)

Fig. 2. Comparison of Neutropenia in Phase III Breast Cancer Trial of Taxol® vs. Tocosol®-Pac. Higher unbound paclitaxel levels in Tocosol®-Paclitaxel treated patients did not result in an expected improved tumor response.

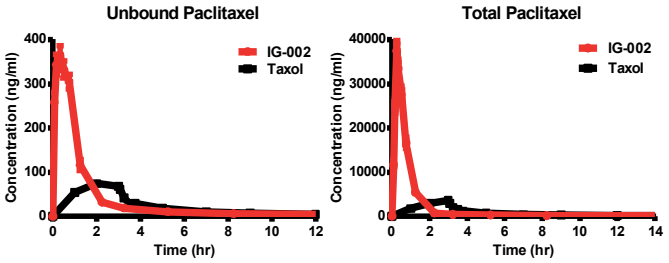
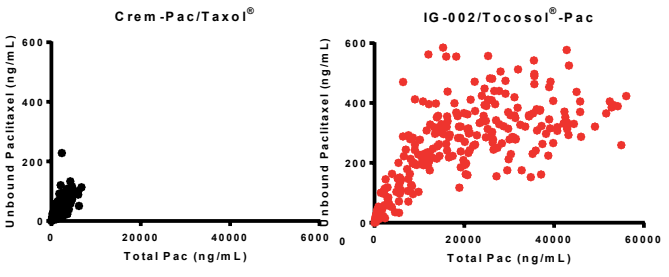


Fig. 3. Paclitaxel Pharmacokinetics for IG-002/Tocosol®-Pac



Unbound Paclitaxel	Taxol	Abraxane®	Taxol	IG-002
Unbound/Total (% AUCinf)	1.96%	5.70%	2.48%	1.99%

Unbound Paclitaxel	Taxol N=14	Abraxane® N=14	Taxol N=31	IG-002 N=31
Infusion Time (h)	3	0.5	3	0.25
Dose (mg/m²)	175	260	175	175
AUC_{inf} (μg·h/mL)	0.409 0.143	1.158 0.337	0.369 0.083	0.617 0.135
AUC_{inf}/dose (ng·h/mL per mg/m²)	2.3 0.82	4.5 1.30	2.11 0.47	3.53 0.77
C_{max} (ng/mL)	121.8 39.62	1,283 532.17	86 34	447 146
Unbound/Total (% AUCinf)	1.96%	5.70%	2.48%	1.99%

Total Paclitaxel	Taxol[1] N=14	Abraxane N=14	Taxol N=31	Tocosol N=31
Infusion Time (h)	3	0.5	3	0.25
Dose (mg/m²)	175	260	175	175
AUC_{inf} (μg·h/mL)	20.82 5.39	20.32 3.97	14.9 4.0	31.0 8.5
AUC_{inf}/dose (ng·h/mL per mg/m²)	119.0 30.8	78.2 15.3	85.1 22.85	177.14 48.57
C_{max} (μg/mL)	5.13 1.68	19.56 7.07	3.8 1.1	38.7 10.9

	Tocosol/Taxol Ratio (100mg/m²/80 mg/m²)	ABX/Taxol Ratio (260 mg/m²/175 mg/m²)
$AUC_{total\ paclitaxel}$	(31.0/14.9)*1.25 = 2.60	(20.32/20.82) = 0.976
$AUC_{unbound\ paclitaxel}$	(0.617/0.369)*1.25 = 2.09	(1.158/0.409) = 2.83

Fig. 4. Unbound Paclitaxel Exposure of Tocosol®-Pac vs. Crem-Pac

CONCLUSIONS

Higher observed levels of unbound paclitaxel in Abraxane®-treated patients vs. Taxol®-treated patients has been hypothesized to be responsible for the observed improved tumor response. Reexamination of phase III data of IG-002 Paclitaxel versus Cremophor-EL Paclitaxel revealed that higher systemic exposure to free paclitaxel resulted in greater bone marrow suppression manifested as neutropenia without concomitant increase in tumor response. The deeper tissue penetration required for tumor response involving albumin-mediated transport of paclitaxel could explain why free paclitaxel was not a predictor of tumor response.

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INVESTIGATING THE RELATIONSHIP BETWEEN NANOMATERIAL BEHAVIOUR AND THEIR PHYSICO-CHEMICAL PROPERTIES

HELINOR JOHNSTON and Vicki Stone
Nano-Safety Research Group, Heriot-Watt University, Edinburgh (UK)

A diversity of nanomaterials (NMs) may be used in a clinical setting to improve the diagnosis and treatment of disease including for example carbon nanotubes, gold, silver, iron oxide, dendrimers and polymer based nanomaterials. The use of NMs for such purposes has been termed nanomedicine. Nanomedicines have the commonality that they are all typically <500nm in size, however their other physico-chemical properties can be vastly different (e.g. size, surface area, composition, morphology, and agglomeration/aggregation status). The physico-chemical properties of nanomedicines will inevitably influence their behaviour following administration by dictating biodistribution (pharmacokinetics), cellular uptake, efficacy and hazard. In fact, over recent years a 'safe by design' approach to the generation nanomedicines has been adopted which involves manipulation of the physico-chemical properties of NMs to try to control their targeting, efficacy and hazard. There are very few studies which have been able to systematically alter NMs or nanomedicines in order to evaluate the influence of a wide range of physicochemical properties on their behavior. In the absence of such studies information can be exploited from more diverse forms of nanomaterials (such as titanium dioxide, carbon black and polystyrene) where an extensive amount of historical information

exists on how the properties of these materials relate to their behavior. The combination of historical particle toxicology and more recent nanotoxicology has helped to enhance understanding of how these different factors contribute to NM behaviour in the body, however further information is required to improve the predictive nature of safety-by-design and to reduce the amount of laboratory testing required.
Studies which have assessed the biodistribution, cellular uptake and hazards posed by engineered nanomaterials to human health have increased over recent years using NM types likely to be exploited as nanomedicines or diagnostic tools (e.g. metals, metal oxides, polymers and carbon based structures). In addition, the variety of target sites studied has also increased (e.g. lung, liver, immune system), and such studies have used a range of in vitro and in vivo models. However the relevance and usefulness of in vitro tools for NM testing have been questioned. Our recent work suggests that simple hepatocyte cell line (C3A) culture with medium containing serum is very good at predicting the livers response to injected NMs in a rodent model. Following administration via the lung, NMs that were highly agglomerated (e.g. carbon nanotubes) or highly charged generated a liver response in the animal model that did not reflect the in vitro response in terms of oxidative stress and pro-inflammatory mediator production. However, spherical metal and metal oxide particles with low charge behaved similarly in the in vitro hepatocyte model compared to the livers of the animals exposed via the lung. Following oral exposure via gavage the liver responses to a range of NMs were not reflected in the in vitro assays.
This suggests that the route of exposure influences the biological behavior of NMs possibly via impacting upon their physico-chemical properties. As NMs enter the body they become coated with biological molecules (e.g. protein), which modifies the surface properties and behavior of NMs. Therefore, evaluation of the relationship between the route of exposure, physico-chemical properties and subsequent behaviour in the body will be useful in enhancing nanomedicine safety-by-design, but also targeting and efficacy. Since it is necessary to use in vitro studies to screen the toxicity of NMs, work is required to enhance how they mimic in vivo conditions following different routes of exposure. Accordingly, by carefully considering how NMs are prepared (i.e. what they are dispersed in) in vitro studies could be tailored to better reflect 'real-life' exposure conditions to evaluate how the physico-chemical properties of NMs are modified, and the impact this has on NM behaviour (beneficial and detrimental).
An outline of the relationship between NM physico-chemical properties and their toxicity will be provided. This will initiate discussions that inform how the experimental design of hazard investigations that assess nanomedicine behaviour could be improved in order to better understand how the physico-chemical properties of NMs impact on their toxicity. It is envisioned that this will stimulate discussions in the following areas:
- What physico-chemical properties of NMs influence their behaviour in vivo and in vitro?
- Why is NM physico-chemical characterisation needed?
- What properties should be prioritised within the characterisation of NMs?
- What approaches are used to characterise NMs?
- How can in vitro studies be designed to consider how transformations of NMs during their life cycle affect NM toxicity?

SYNTHETIC VACCINE PARTICLES FOR ANTIGEN-SPECIFIC IMMUNE TOLERANCE

LLOYD JOHNSTON
Ph.D., Chief Operating Officer & Senior Vice President R&D
Selecta Biosciences, Inc., Watertown, MA USA

Current treatments to control pathological or unwanted immune responses often employ broadly immunosuppressive drugs. New approaches to induce antigen-specific immunological tolerance that control both cellular and humoral immune responses are desirable. Here we describe the development of Synthetic Vaccine Particles (SVP) for the antigen-specific inhibition of undesired immune responses. These self-assembling, biodegradable poly(lactide-co-glycolide) (PLGA) nanoparticles containing either protein or peptide antigens and a tolerogenic immune modulator are capable of inducing durable antigen-specific tolerance that control adaptive immune responses and withstand multiple immunogenic challenges with antigen even in the presence of toll-like receptor agonists. We demonstrate that administration of SVPs through multiple routes (e.g. subcutaneous and intravenous) inhibits the activation of antigen-specific T cells and B cells while inducing antigen-specific Tregs. These effects are dependent on the presence of the encapsulated immunomodulator, as an equivalent dose of the free immunomodulator was ineffective. Tolerogenic SVPs effectively prevented formation of anti-drug antibodies to multiple biologics including KLH in wild type mice, Humira in transgenic mice expressing human TNF-a, and FVIII in a mouse model of hemophilia A. Remarkably, despite multiple challenges, antigen-specific immune tolerance was sustained for at least 166 days after the last treatment in the hemophilia A mice. Tolerogenic SVP therapy represents a potential novel approach for the treatment of treatment of allergies, autoimmune diseases, and prevention of anti-drug antibodies (ADA) against biologic therapies.

COGNITIVE COMPUTING APPROACH: "WATSON ONCOLOGY"

DAVID KERR
Director Watson for Healthcare, IBM Corporation, Armonk, NY (USA)

Cognitive Computing systems adapt and learn based on experience, evaluate evidence discovered in unstructured documents and reason to discover new knowledge and establish confidence in results. In this talk we will describe how IBM is helping physicians understand their patients and evaluate possible treatment options in an era of ever increasing knowledge and medical complexity in caring for cancer patients.

APPLICATION OF NANOMEDICINE IN SURGERY FOR THORACIC ONCOLOGY

HYUN KOO KIM
MD, PhD, Associate Professor
Department of Thoracic and Cardiovascular Surgery,
College of Medicine, Korea University Guro Hospital

With advancement of biotechnology, even patients with cancer have wanted to have better quality of life after treatment for cancer. Therefore, use of less invasive treatment modality including surgery must be considered.
The sentinel lymph node (SLN) biopsy is a minimally invasive method of identifying the greater risk patients and facilitating the selective use of more aggressive surgical and systemic therapies to improve outcomes with little additional morbidity. It is currently regarded as the standard treatment method for malignant melanoma and breast cancer and applications are expanding to other malignancies, including lung cancer.
The real-time near infrared (NIR) fluorescence imaging system with appropriate lymphatic tracer has recently attracted considerable interest in the field of SLN identification optical imaging using the NIR fluorescent lymphatic tracer enables real-time visualization of lymphatic channels and SLNs during operation. Therefore, NIR fluorescence imaging could provide an alternative for, or an addition to, conventional techniques used for SLN mapping with several advantages such as better penetration and visibility than blue dyes, and capability of real-time visualization and removing ionizing radiation over radiotracers
Recently, real-time imaging technologies could offer the possibility to put the images right under the hands of the surgeons, warranting intra-operative image-guided surgery during cancer surgery. This technology would make it possible to discriminate between tumor and normal tissue and consequently determine an adequate tumor-free margin during surgery. It would bring new paradigm into the field of oncologic surgery in the future.

STABILIZATION OF VIRAL VECTORS FOR VACCINES AND GENE THERAPY

SILKE KROL[2], Maria Pelliccia[1,2,6], Marco d' Alicarnasso[1,3], Patrizia Andreozzi[2], Andrea Civra[5], Valeria Cagno[5], Randy P. Carney[4], David Lembo[5], Varpu Marjomaki[6], Francesco Stellacci[2,4]
[1]European school of Molecular medicine (SEMM) and Università degli Studi di Milano, Italy
[2]Fondazione I.R.C.C.S. Istituto Neurologico "Carlo Besta", IFOM-IEO-campus, Milan, Italy
[3]CEN-European Centre for nanomedicine, Milan, Italy
[4]EPFL-École polytechnique fédérale de Lausanne, Switzerland
[5]Department of Clinical and Biological Sciences, University of Turin, S. Luigi Gonzaga Hospital, Orbassano (Turin), Italy
[6]Department of Biology and Environmental Science, University of Jyväskylä, Finland

The "cold chain" problem for vaccines consists in the need of keeping a vaccine always cold from the producer to the patient. It is estimated that constant refrigeration is responsible for up to 70% of the vaccine cost. More importantly, it determines a number of transportation challenges in countries where doctors have to reach remote villages. Both these problems are obvious hurdles to global vaccination in Africa and other developing countries. Vaccines are thermally unstable because viral vectors lose their infectivity with time at moderate temperature. In this paper we address the issue of thermal stability in viruses that contain double stranded genetic material. We identify the main reason for the thermal instability in the random fluctuations of the capsids units that constitute the virus shell due to the internal osmotic pressure present in these viruses. We show that these fluctuations can be damped and the internal osmotic pressure compensate when molecules or nanoparticles are introduced in the virus solution, so that these additives increase the solution viscosity and create a compensating force. We present an analytical theory to frame our understanding of the problem, and experimental results that show that with these additives it is possible to stabilize a virus solution up to 21 days at 37°C (or 70 days at room temperature). Importantly, given the relevance of this problem for poor countries, we show that the best results can indeed be achieved by simply adding to the solution an inexpensive molecule as sucrose.

ARE CLINICAL TRIALS OF NANOPHARMACEUTICALS SPECIFIC?

CHRISTINE KUBIAK

As for any new medicinal products, the clinical development of new nanopharmaceuticals is based on the demonstration of safety and efficacy through clinical trials. This is a critical step to obtain a marketing authorisation approval and it is crucial to integrate as soon a possible the regulatory constraints applicable to the nanomedicinal products.
The presentation will give an overview of the regulatory context and the challenges applicable to nanopharmaceuticals.

EFFECTS OF PEGYLATED LIPOSOMAL NANOPARTICLES ON FUNCTIONALITY OF MACROPHAGES AND LYMPHOCYTES IN TUMORS

NINH M. LA-BECK[(1)], Manoj Sabnani*[(1)], Robin Rajan*[(1)], Bradley Rowland[(1)], Vikram Mavinkurve[(1)], Laurence Wood[(1)], Alberto Gabizon[(2)]
*equal contribution
[(1)]Texas Tech University Health Sciences Center, [(2)]Shaare Zedek Medical Center

INTRODUCTION

Nanoparticles (NPs) are known to be internalized by phagocytes such as hepatic Kupffer cells and can activate circulating complement proteins to varying extents depending on their physicochemical characteristics1-4. The clinical significance of interactions between NPs and the immune system is reflected in cancer patients where the extent of NP-induced blood complement activation correlated with development of acute infusion reactions5. Additionally, phagocytic function of circulating monocytes, precursors of macrophages, correlated with increased NP clearance in patients6. However, the mechanisms and consequences of NP-induced immune responses on tumor progression are poorly understood. Importantly, uptake of liposomes by cultured macrophages increased production of TGF-beta7, which is consistent with the cytokine production of immune suppressive macrophages8 found in the tumor microenvironment. These tumor associated macrophages (TAMs) are believed to promote tumor growth through multiple mechanisms including suppression of antitumor functions of cytotoxic T lymphocytes (CTLs)8. Moreover, TAMs can augment or antagonize efficacy of chemotherapy and radiation therapy, depending on the tumor model and the treatment9 which suggest that it is also possible for TAMs to regulate response to NP therapy. Therefore, we hypothesized that NP internalization polarize TAMs towards an immunosuppressive phenotype, inhibiting anti-tumor immune responses, and thereby promoting tumor progression and attenuating therapeutic efficacy. The objective of this study was to determine the effect of pegylated liposomal NPs on macrophage and lymphocyte function in tumors, as well as their impact on tumor growth.

METHODS

Tumor Model. Wild-type C57BL/6 female mice 6-8 weeks old (Jackson Laboratory) were injected with syngeneic TC-1 tumorigenic cells subcutaneously on the hind flank. These cells express HPV-16 E7 oncoprotein10 which enables monitoring of adaptive immune responses to this tumor antigen. Mice were then treated with 3 to 4 weekly intravenous injections of NPs (no drug loaded within) similar in size and composition to the pegylated liposomal carrier used in Doxil/Myocet, or vehicle control. We chose liposomes as a model NP because five (Doxil/Myocet, DaunoXome, Myocet, DepoCyt, and Marqibo) approved anti-neoplastic NPs11-15 utilize similar liposomal platform. Therefore our findings in this NP model will have high clinical relevance. To evaluate the impact on tumor growth, tumor size was monitored biweekly via digital caliper and tumor volume calculated using the equation volume = (D1 x D22)/2; where D1 is the largest diameter and D2 is the perpendicular diameter.
Cell Isolation. At endpoint, mice were sacrificed, and tumors harvested for evaluation of immune cell number and function. To obtain single cell suspensions, tumors were digested in Hank's buffered saline solution (HBSS) containing DNase I and Liberase and passed through 70 um cell strainer. Then red blood cells were lysed and the cell pellet was washed and further purified by centrifugation in 30% Percoll density gradient followed by two additional washes in phosphate buffered saline (PBS). Single cell suspensions were counted and viability assessed using trypan blue exclusion assay (Vi-Cell).
Immunophenotyping. For each FACS assay, two million cells from each sample were stained with fixable viability dye eFluor 506 (eBioscience), Fc-blocked, and stained with fluorophore conjugated antibodies. To evaluate TAM number and polarization, tumor derived cells were stained against CD45 (pan-leukocyte marker), CD11b (myeloid cell marker), F4/80 (macrophage marker), and Gr1 (granulocytic cell marker). Cells were then fixed and permeabilized, and stained intracellularly with antibody against arginase-1 (indicative of immunosuppressive phenotype). To evaluate number of tumor infiltrating T cells, tumor derived cells were stained against CD45, CD8 (a marker of cytotoxic T cells), TCR-beta (T cell receptor), CD4 (a marker of T helper cells), and CD62L (an activation marker). All FACS analyses were performed on a BD LSRFortessa flow cytometer with 4 lasers and up to 14 channels.
Functional Studies. To further evaluate cell function, a separate aliquot of tumor derived cells were stimulated ex vivo. To determine TAM response to inflammatory stimulus, cells were incubated at 37oC in RPMI media containing 10% FBS, CpG 10 ug/ml (to stimulate cytokine production), and monensin 1.3 ug/ml (to inhibit cytokine secretion) for 6 hrs, then harvested and stained with antibodies against myeloid cell surface markers (CD45, CD11b, F4/80, and Gr1), fixed and permeabilized, and intracellularly stained for

pro-inflammatory cytokines (IFN-gamma and IL-12) and immunosuppressive cytokines (TGF beta and IL-10). To evaluate functionality of tumor infiltrating T cells, cells were stimulated ex vivo with plate-bound anti-CD3 (10 ug/ml) and soluble anti-CD28 (2 ug/ml) in the presence of monensin and IL-2 2.5 ng/ml (necessary for cytokine production) for 6 hrs. Then cells were harvested and stained for lymphocytes (CD45, CD8, CD62L and TCR-beta), fixed and permeabilized, and intracellularly stained for IFN-gamma. All samples were analyzed by flow cytometry (BD LSRFortessa).

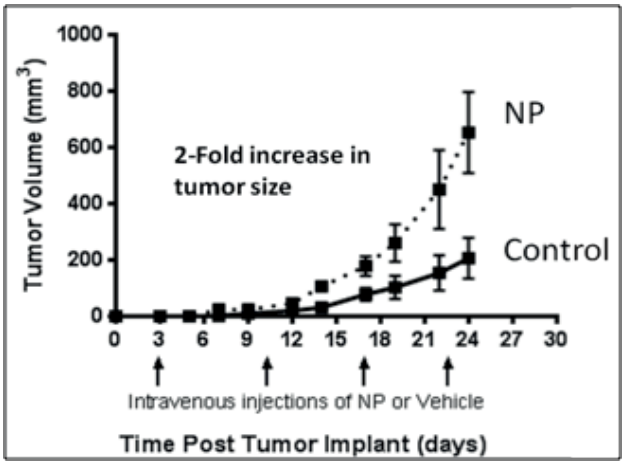

Figure 1. NPs promote tumor growth. C57BL/6 mice bearing grafted TC-1 tumors were treated with weekly doses of NPs (n=5) or vehicle control (n=4). At endpoint, mean tumor volume in NP treated mice was 653 versus 206 mm3 in the vehicle group (p<0.05, unpaired T-test, data are mean + SEM). Results were similar in three independent experiments, total n = 23.

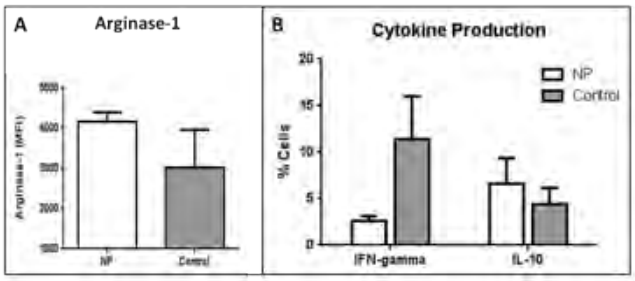

Figure 2. NPs enhanced immunosuppressive functionality in tumor associated macrophages. A) Tumor derived cells from NP or vehicle treated mice were stained with antibodies and analyzed by flow cytometry. Arginase-1 expression (mean fluorescence intensity; MFI) was determined in macrophages (CD45+ CD11b+ F4-80+ Gr1-). B) Cells were also stimulated with CpG in the presence of monensin, then stained with antibodies and analyzed by flow cytometry. Functionality was assessed by determining percent of macropahges producing IFN-gamma and IL-10. Data are mean + SEM, n = 3 each group.

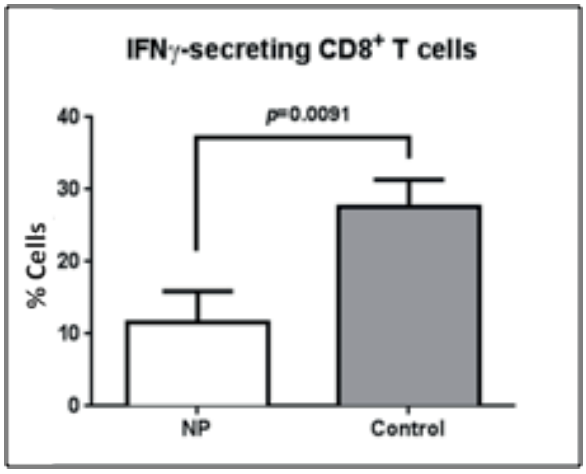

Figure 3. NPs inhibited cytotoxic T lymphocyte (CTL) IFN-gamma production. Tumor derived cells from NP or vehicle treated mice were stimulated with plate-bound anti-CD3 and soluble anti-CD28 in the presence of monensin and IL-2 , then stained with antibodies and analyzed by flow cytometry. CTL (CD45+ CD8-beta+ TCR-beta+) functionality was assessed by determining percent of those producing IFN-gamma. Data are mean + SEM, n = 3 each group; p<0.01 by unpaired T-test.

RESULTS

At endpoint, tumor volume was significantly larger in NP treated mice as compared with controls (Fig. 1). We hypothesized that NP-enhancement of tumor growth was due to NP-induced changes in TAM function since this carrier is readily phagocytosed by macrophages. To ascertain the effect of NPs on TAM infiltration and function, we quantified number of macrophages (CD11b+ F4/80+ Gr1-) in tumors as well as their arginase-1 expression (associated with an immunosuppressive phenotype).

Although there was no difference in TAM infiltration between treatment groups, we found that TAMs in NP treated mice had increased expression of arginase-1(Fig. 2A), indicating that they were functionally polarized towards an immunosuppressive phenotype. Since altered cytokine production may be evident only upon stimulation, we examined cytokine production in tumor derived cells stimulated ex vivo with CpG. We found that cells from NP treated mice had decreased production of IFN-gamma and increased production of IL-10 (Fig. 2B), supporting that they were functionally more immunosuppressive.

Since TAMs have been proposed to enhance tumor progression through inhibition of cytotoxic T lymphocyte (CTL) responses, we next examined the impact of NPs on CTL function. We measured production of IFN-gamma, a pro-inflammatory cytokine associated with antitumor function, by CTLs (CD8+ TCR-beta+) isolated from these tumors. We found that NP treatment resulted in impaired production of IFN-gamma by tumor-infiltrating CTLs, suggesting that they have decreased anti-tumor activity (Fig. 3). Taken together, these results support that NPs promote an immunosuppressive tumor microenvironment.

DISCUSSION & CONCLUSIONS

The mechanisms of NP-induced immune responses and consequences on tumor progression are poorly understood. Importantly, we found that NPs significantly accelerated tumor growth in a syngeneic tumor model in immunocompetent mice. NP-enhancement of tumor growth was associated with changes in the tumor microenvironment characterized by polarization of TAMs towards an immunosuppressive phenotype and decreased IFN-gamma production by CTLs. These results are the first to show that NPs may promote tumor growth through mechanisms that involve enhancement of an immunosuppressive tumor microenvironment. Our findings may partially explain why, except for a small subset of patients, currently approved anticancer drug formulations in liposomal nanoparticles have not produced major improvements in overall survival rates of cancer patients. These results need to be confirmed in other tumor models, particularly since tumors expressing viral antigens, such as the TC-1 model, may be relatively more immunogenic. Our ongoing studies will dissect the mechanisms by which liposomal NPs modulate antitumor immunity and develop innovative therapeutic strategies targeting these mechanisms in order to improve treatment of cancer.

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USPIO-LOADED MICROBUBBLES FOR MR-CONTROLLED BBB PERMEATION

TWAN LAMMERS

In addition to being leaky, tumor blood vessels are also highly disorganized and heterogeneously distributed. The former aspect is extensively used in the nanomedicine field, and forms the basis for the Enhanced Permeability and Retention (EPR) effect. The latter two aspects, however, are generally not taken into account, even though they arguably substantially impact the efficacy of (nano-) chemotherapeutic interventions. In order to 'normalize' the tumor vasculature, and to thereby deliver drugs and drug delivery systems more efficiently and more homogenously to tumors, we are working on the development of nanomedicine formulations targeted to tumor-associated macrophages (TAM). TAM are prominently involved in angiogenesis, and their pro-angiogenic and pro-inflammatory properties promote heterogeneous vessels distribution and improper vessel function. As it is known that i.v. administered nanomedicines strongly accumulate in TAM, we reasoned that by targeting TAM, and by attenuating pro-angiogenic signalizing by TAM, the delivery of subsequently administered (nano-) chemotherapeutic agents can be substantially improved. When used in such a neo-adjuvant setting, pre-treatment of tumors (and metastases) with TAM-modulating nanomedicines might be highly relevant for improving the efficacy of combination therapies. This not only holds true for subsequent treatment with chemotherapeutic drugs, but also with radiotherapy, as an improved perfusion and a more homogenous vessel distribution leads to a better oxygenation of tumors. In the present lecture, the basic principles and some initial results for the use of Neoadjuvant Nanomedicines for vascular Normalization (NeoNaNo) will be presented.

NANOCAPSULES AND THEIR SPECIFIC INTERACTIONS WITH CELLS

KATHARINA LANDFESTER

Max Planck Institute for Polymer Research, Ackermannweg 10, 55128 Mainz, Germany

Control over the nanoscopic scale opens nearly endless opportunities for many scientific areas. In particular polymeric nanoparticles offer the versatility to cover a wide range of mesoscopic properties for sophisticated applications. However, making smart nanoparticles is inevitably linked to a deep understanding of the overall physic-chemical principle of their formation. By means of the miniemulsion process, we can design custom-made nanoparticles and nanocapsules for nearly any purpose. This is facilitated by the enormous versatility of the miniemulsion process that has been developed and conceptually understood by our group. Moreover, the accumulation of understanding the formation process has led to successful and precise control of important nanoparticle parameters such as size, shape, morphology, degradation, release kinetics and surface functionalization. This degree of control is unique and allows us to tune specific properties tailored to particular applications; the successful up-scaling of process is of technical relevance. Furthermore, the encapsulation and release of a great variety of payloads, ranging from hydrophobic to hydrophilic substances has been successfully achieved in a highly controlled manner and with an unmatched high encapsulation efficiency.

Our vision is to construct multipotent drug-loaded nanocapsules of high homogeneity in size and surface functionality, which find their target cells in the desired organs and release the drug in a controlled manner in the cytoplasm of these cells. For the delivery of bioactive compounds such as therapeutic proteins/peptides siRNA or drugs to a specific cell, it is not only vital to improve the stability of the therapeutic agent during passage through the blood stream, but also to extend the circulation time in the body. Consequently the interaction with blood components has to be controlled to limit aggregation processes. Furthermore, uptake in blood cells like macrophages has to be minimized. Only then the drug can reach the target cells. In this field, we have achieved several essential milestones, mentioned in the following sections.
Our developments in the field of miniemulsion have shown that the miniemulsion technique is an extremely powerful and versatile approach for the formation of complex carriers in order to encapsulate different kinds of reporter molecule and drugs, demonstrating a high significance for medical applications. The main advantage clearly lies in the simultaneous encapsulation of relevant hydrophobic or hydrophilic drugs or biomolecules (DNA, siRNA, enzymes or proteins) and/or fluorochromes. The following criteria are important for implementing these highly specialized particles or capsules in therapeutic and diagnostic applications. We have addressed all these criteria in detail:

- Control of capsule homogeneity: Capsules of different polymers can be defined - with respect to size and morphology - highly uniform. New strategies for the formation of homogeneous capsules using bioorthogonal reactions have been established.
- Control of capsule surface: The capsules are defined with respect to their surface according to their spatial (topological) and chemical structure. New surface characterization methods have been developed.
- Control of protein adsorption onto nanocapsules: How the nanoparticles interact with the medium surrounding them is especially important in the biomedical area. We are one of a few research groups worldwide that are capable of performing multi-angle DLS on concentrated human blood serum as a routine measurement to monitor aggregation events between nanocapsules and human blood serum. Mass spectrometry (with the group of K. Müllen and with S. Tenzer from the University Medical Center Mainz) was used for the identification of the adsorbed proteins and for the first time for the the determination of the adsorption kinetics. Using ITC we are first able to monitor binding kinetics between nanocapsules with whole serum as well as with specific proteins.
- Defined targeting structure on the nanocapsules: Addressing the capsules in vivo represents a significant challenge. To target a nanocontainer to a specific type of cell, a naked capsule must first interact as little as possible with unspecific cell types in general; it has to disguise itself. We have now been successful in producing nanocapsules based on hydroxyethyl starch that are not recognised by any cell type unspecifically (additionally, these capsule show an excellent long circulation time which is better than the "gold" standard (PEGylated liposomes). This enabled us to target tumour cells overexpressing the folic-acid receptors and dendritic cells carrying the (tri)mannose receptor. Also more complex protein molecules as interleukin-2 were successfully coupled to nanocapsules in defined

quantities and the specific interaction to T cells could be shown in close collaboration with the University Medical Center of Mainz.

• Control of the release of payloads from nanocapsules by introducing molecular switches in the shell: Nanoapsules are not always meant to permanently protect or shield an encapsulated substance from external influences, because mechanisms need to be employed to selectively release a stored substance again. We have developed nanocapsules which can be opened by different stimuli. For example, enzymatically cleavable nanocapsules were constructed to use specific (e.g. intracellular) enzymes in order to achieve a highly specific and time controlled drug release. The release kinetics in vitro was determined in order to achieve the relevant pharmacokinetic profile. Investigations of the time course of intracellular degradation and release are widely lacking. This has been tackled by approaches using confocal laser scanning microscopy in close collaboration with the group of H.J. Butt. Although differentiation or cellular functions may in some cases not be affected by the presence of nanoparticles, we have shown that the intracellular signaling e.g. the inflammatory response is strongly affected. Also the trafficking of nanoparticles themselves in different compartments inside the cell has been elucidated and hitherto unrecognized compartments have been identified.

• Release of the payload in the cytoplasm: The nanocapsule payload needs to reach the cytoplasm of the cells (and thus penetrate through the endosome that the capsules reached through absorption) in order to be effective. New strategies for overcoming the endosome membrane have been developed.

The platform thus represents a route to develop advanced and highly complex nanoparticles and nanocapsules which is achieved due to the precise regulation of the surface composition, the high degree of uniformity, and the ability to create specific functionality. From the experiments we learn significant steps how to lead nanocapsules containing drugs to specific target cells within the body (in vivo) by virtue of the capsules' surface properties and how to release the drugs at the target site.

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PUBLICATION ETHICS

KARIN LASON

Recent prominent scandals helped to raise awareness of the apparent decadence of sound scientific publishing. Various underlying causes for lax dealing with Publication Ethics shall be outlined in this context, introducing the basics of proper scientific handling of manuscripts and discussing possible ways out of the dilemma.

Ever since the Open Access hype new publishers popped up all over using promising publication fees as basic principle for their business models. Unfortunately, some of them did not seem to include minimum scientific publication standards such as a real peer review to their journals.

Steadily increasing pressure on life-scientists to rapidly publish large quantities of papers as fast as possible and to get cited by as many as feasible obviously makes up for another prime reason for quality decay. Only a slight reversing trend is starting to take place in some sectors within the realms of possibility.

In no case one of the most important factors shall be left out of this discussion. That is the sheer lack of knowledge on proper scientific writing and preparing of research manuscripts. Learning-by-doing-it-yourself seems to be the way young scientists have to start their publishing career. Lack of time or tutor maybe coupled with the lack of attached value to scientific writing are distressing reasons for this.

This contribution is a plea for enhancing the consciousness towards Publication Ethics - to give them the relevance they deserve. Even though it may sound pathetic: the mere credibility of Science is jeopardized if we don´t care.

IN VIVO APPLICATION OF CU-64 LABELED UPCONVERSION NANOPARTICLES

DONG SOO LEE

Seoul National University, Seoul, Korea

The understanding of the in vivo behavior of the nanoparticles will enlighten the usability of novel nanoparticles. Upconversion nanoparticles (UCNP) such as NaYF4:Yb3+, Er3+ nanoparticles which we used here use excitation laser of infrared (980 nm) and collecting emitting light of visual ranges (400-700 nm) for upconversion luminescence imaging (ULI). When we labeled Cu-64 whose half-life is 12.7 hours on the surface of UCNP, Cu-64 UCNP became an optimal nanostructure that enabled us to characterize in vivo behavior including excretion with positron emission tomography (PET) and ULI bimodal imaging for in vivo study and for ex vivo analysis.

NaYF4:Yb3+, Er3+ nanoparticles (UCNPs) were encapsulated and labeled with NOTA-stearyl amine (SA) and 64Cu. In vivo bio-distribution study using microPET showed liver uptake at early period and expedited excretion through the bowel. Percent of injected dose was 40% or more in the feces and the remaining activity was in the liver at 24 hours. Ex vivo biodistribution studies corroborated this quantified PET imaging results in that 20% was in the liver among the remaining 50% of the activity. Intestine (including feces) had 13%. Hepatobiliary excretion of UCNP was documented both with microPET imaging and ex vivo specimen. The possibility that free 64Cu and 64Cu-NOTA-SA is released and excreted was tested and excluded by the microPET studies using free 64Cu and 64Cu-NOTA-SA microPET. ULI of mice in vivo was feasible only with the thousand-fold or more amount of UCNP. To find the mechanism of hepatobiliary excretion, (ULI-capable) mg amount of UCNP was injected and the liver was harvested at 1 hour post-injection for transmission electron microscopy (TEM). UCNP were found in the sinusoid, cytoplasm of hepatocytes, bile cannaliculi as well as Kupffer cells. Collected feces showed radioactivity and luminescence on microPET and ULI. Within the hepatocytes, aggregates were found in addition to the scattered particles.

64Cu-NOTA encapsulated UCNPs showed the characteristics of expedited hepatobiliary excretion as well as Kupffer cell phagocytosis. Long half-life of 64Cu enabled the visualization and quantification at 24 hours after injection. Quantified results of in vivo microPET were well matched with those with biodistribution studies of ex vivo radioactivity. UCNPs in the feces were confirmed by ex vivo PET and ULI imaging. TEM corroborated the entire process of excretion. 64Cu-NOTA-SA UCNPs can be used for bimodal ULI and nuclear imaging in vivo which are to be used for the characterization of their in vivo behavior including desirable rapid excretion via hepatobiliary system.

WHAT ARE THE THREE KEY OPTIONS OF YOUR REGULATORY SYSTEM TO BE PUT ON THE TABLE TO SUPPORT THE SUCCESS OF NANOMEDICINE?

LADA LEYENS

As the Swiss Agency for Therapeutic Products, Swissmedic is responsible for the authorisation and supervision of therapeutic products (medicinal products and medical devices). The wide range of activities is carried out in accordance with both our legal mandate and the needs of the stakeholders. These include patients, the therapeutic products industry, healthcare professionals, authorities and organisations in Switzerland and abroad, and the media.

Our core competencies encompass:
- the authorisation of medicinal products
- licences for manufacturing and wholesale, and inspections
- market surveillance of medicinal products and medical devices
- establishing standards
- authorisation and inspections of clinical trials
- laboratory testing regarding the quality of medicines
- Information to stakeholders
- national and international co-operation.

The three key options of our regulatory system, which from our point of view support the success of Nanomedicine, are:
1. Swissmedic created an **internal Task Force** devoted to nanomedicine in order to address the challenges and specificities inherent to the new field of nanomedicine. This group is formed by members and experts from all relevant departments within the organisation, who meet on regular bi-monthly meetings to discuss current topics on nanomedicine, questions that may arise from within the organisation, queries that come from companies and questions from the general public.
As a Centre of Excellence for all questions related to nanotechnology, the group and its members have the following tasks:
- Swissmedic Centre of Excellence for the monitoring of national and international developments in the area of nanotechnology in medicinal products and medical devices
- Prepare publications on nanomedicine and give input to the media
- Build relationships with national and international Working Groups and expert groups
- Collaboration in national and international Task Forces on nanotechnology (focusing on medicinal products and medical devices)
- Periodical information within Swissmedic regarding new advances and the developments in the field of nanomedicine
- Establishment of priorities and key areas of activities for Swissmedic within the scope of our mandate.

2. Swissmedic is part of the national Action plan for synthetic nanomaterials, adopted by the Federal council in 2008 and planned to continue until 2015. The action plan is not limited to nanomedicines but also includes them. The objectives of the action plan include:
- development of regulatory framework conditions for the responsible handling of synthetic nanomaterials;
- creation of scientific and methodical conditions aimed at identifying and preventing potential harmful effects of synthetic nanomaterials on health and the environment;
- promotion of the public dialogue about opportunities and risks of nanotechnology;
- better utilisation of existing tools for the development and rollout of sustainable nanotechnology applications.

The creation of regulatory framework conditions is divided into two phases:
- Phase 1 (short and medium term): Strengthening of corporate responsibility through different tools (precautionary matrix, guide to self-regulation, support of private-sector codes of conduct, guidelines for nano-specific safety data sheets, improved consumer information, disposal guide)
- Phase 2 (medium and long term): Development of legal framework conditions for the safe handling of synthetic nanomaterials (review of measures exceeding existing provisions and coordination with developments abroad)

3. As part of the International collaboration, Swissmedic has contact with other regulatory agencies to discuss topics on nanomedicines. We also attend and participate regularly at national and international conferences on Nanomedicine. And in Switzerland, we participate at round tables organised to inform the general public about nanomedicine.
In addition, since the agency approves both clinical trials and therapeutic products, this allows us to follow products from the clinical trial development phase up to the marketing application process, considering that the trials are carried out in Switzerland. It is also important to stress the fact that the review of dossiers for Clinical Trial Applications (CTA) and of Marketing Approval Applications (MAA), is done on a case by case basis, and not standardised according to the type of product. For the CTA we evaluate the risk to the patient based on safety and quality aspects of the product, and during the MAA we consider the risk-benefit ratio of the therapeutic product in the context of the intended use and intended population. Swissmedic has created a flagging system for both application processes, in which applicants have to identify if their products are nanomedicines (according to our definition) already in the CTA form. This is not intended as a red flag alert system for the products, but rather as an additional information for the reviewers.
The agency also offers the opportunity to do pre-submission scientific meetings, where applicants can discuss the main issues and questions regarding their products. Manufacturers of nanomedicines are also welcome to request such meetings!
As an agency we endeavour to ensure that authorised nanomedicines are of high quality, effective and safe. By doing so, we make a considerable contribution towards protecting the health of humans, and we also participate in safeguarding Switzerland as a location for industry.

PERSONALIZED VACCINATION WITH LANGERHANS CELLS-TARGETING DNA NANOMEDICINE

JULIANNA LISZIEWICZ, Orsolya Lőrincz, Zsolt Csiszovszki, Eszter Somogyi, Kata Pantya, Jozsef Toth, Levente Molnár and Enikő R. Tőke
eMMUNITY Inc., Budapest, Hungary and Bethesda MD USA

There is no vaccine that exploits Langerhans cells (LCs), the epidermal precursors of dendritic cells that are the natural agent of antigen delivery. We developed a DNA formulation with a polymer and obtained synthetic 'pathogen-like' nanoparticles that preferentially targeted LCs in epidermal cultures. These nanoparticles applied topically under a patch elicited robust T-cell immunity in animals and human subjects*. We showed that the combination of nanoparticle administration and skin treatment was essential both for efficient loading the nanomedicine into the epidermis and for potent activation of the LCs to migrate into the lymph nodes. LCs in the epidermis picked up nanoparticles and accumulated them in the nuclear region demonstrating an effective nuclear DNA delivery in vivo*. Tissue distribution studies revealed that the majority of the DNA was targeted to the lymph nodes. Toxicity of the LC-targeting DNA nanomedicine was limited to mild and transient local erythema. This novel, clinically proven LC-targeting DNA nanomedicine broadens the options to induce T cell immunity against cancer.
Most cancer vaccine trial failed to demonstrate efficacy because low rate of patients had anti-tumor T cell immunity and tumor regression. To improve the success rate, we developed a novel molecular diagnostic test capable of determining whether a person could respond to immunotherapy. We post hoc analyzed several clinical trials and demonstrated the correlation between the patient's complete HLA genotype and T cell immunity. This diagnostic test is suitable to personalize cancer vaccines and to increase their clinical success.

* Lisziewicz & Toke NNBM 2013; Toke et al. Gene Therapy 2014

HUMAN RIGHT FOR HEALTH AS LONG-TERM PERSPECTIVE FOR NANOMEDICINE

BEAT LÖFFLER
MA CEO of the European Foundation for Clinical Nanomedicine

Worldwide there are many initiatives that claim that health should be a human right.
In his time as Secretary General of the United Nations the Nobel Laureate Kofi Annan wrote: …"The aspiration that health finally will be seen not as a blessing to be wished for, but as a human right to be fought for". Today we are far away from this goal and the ethical debate ended in most situations in a quarrel between Ethics and Economics and brought up no solutions. The questions debated were of the wrong ones. CLINAM brings together many scientists of excellence who are pioneers in the field. The CLINAM Summit is Science driven, addressing other scientists, industrials, engineers, policy makers, regulatory authorities and further groups including also political institutions. These drivers of the development to novel therapy and diagnostics to the benefit of the patients and mankind are not specialists to organize and handle the realization of sustainable, less expensive medical care since this is a pure political issue. The intervention elucidates the contexts and shows the configuration between "affordable health for mankind" "human right for health", "Science" "Industry" and "Politics".

MICROWAVE SOLVOTHERMAL SYNTHESIS OF ACTIVE NANO-HAP FOR MEDICAL APPLICATIONS

W. LOJKOWSKI[1], S. Kuśnieruk[1], J. Wojnarowicz[1], T. Chudoba[1], A. Majcher[2], A Mazurkiewicz[2]
[1]Institute of High Pressure Physics, Polish Academy of Sciences, Sokolowska 29/37, 01¬142 Warsaw, Poland, wl@unipress.waw.pl
[2]Institute of Sustainable Technologies, National Research Institute, Radom, Poland

Hydroxyapatite (HAP), chemical formula Ca5(PO4)3(OH), in the form of paste or granulates used to fill small bone gaps, in stomatology, or as a coating to increase biocompatibility of metal or polymer implants, various polymer nano-composites for medical applications. It is an artificial form of the bio-apatite, which is the ceramic component in human bones.
In regerative medicine there is great interest in resorbable scaffold for tissue regrowth, including bone regrow. Such scaffolds support growth of the natural tissue to fill any occurring due to illness or trauma gap. Nano-materials and nanostructures are of particular interest in that respect. Their use may enhance regrowth of natural tissues, as they will replace gradually the implant.
Hydroxyapatite is usually not considered as resorbalbe, or its resorbtion rate is quite slow. However, in its nano-form it becomes resorbable. We developed a microwave technology to produce resorbable nanoparticles of hydroxyapatite1,2,3. We can control the mean grain size from 15 to 25 nm, and the specific surface ranges from 150 m2/g to 300 m2/g. We can also control the Ca/P ratio, which for an equilibrium structure is 1.67, while in calcium deficient materials it may be as low as 1.55. We could show that nano sized and/or calcium deficient nanoparticles are soluble in a standard ISO 10993-14 resorbtion test for biomaterials, and the resorbtion rate depends on particle size and stoichiometry. The nanopowder of our production was registered in the Polish Patent Office under trade name GoHAPTM.

Controlling precisely the nano-structure of GoHAP is possible due to use of microwave energy in the process of hydrothermal synthesis. Microwaves heat quickly and uniformly water with the reaction substrates, and as short reaction times as 90 seconds are possible. Figure 1 show part of the MSS-2 reactor used by us. The microwave power possible is 3 kW, the ceramic chamber volume is 470 ml, and the reagents are never in contact with any metal. Our present GoHAP production capacity is 100 gr/day.

Figure 1. The MSS-2 reactor for solvothermal synthesis of nanoparticles. MW power 3 KW, volume 470 ml, maximum pressure 6 MPa, temperature 260oC.

1. W. Lojkowski et al, Patent application PL396906 "Method for of producing nano-plates of synthetic hydroxyapatite and nanopowder comprising a synthetic hydroxyapatite nano-plates"
2. D. Smolen et al, Hydroxyapatite Nanopowder Synthesis with a Programmed Resorption Rate, Journal of Nanomaterials, Volume 2012, Article ID 841971, doi:10.1155/2012/841971

PEPTIDE-BASED NANOPARTICLES FOR TARGETED DELIVERY OF siRNA

PATRICK LU
Sirnaomics, Inc. Gaithersburg, Maryland 20879, USA and Nanotides Pharmaceuticals, Ltd. Guangzhou, China.

To address unmet needs for siRNA therapeutic delivery, we took advantage of nanoparticle technology for efficient and safe delivery of siRNA drugs to disease tissues with three generations of nanoparticle systems based on their functions and structures. Our first generation of delivery agents uses clinically viable and approved nanoparticle technologies for siRNA delivery in vivo. The 2nd generation siRNA delivery systems use the scaffolds from the first generation nanoparticles along with targeting agents for tissue and cell type specific siRNA delivery. The 3rd generation system involves in a silica-coated upconversion nanoparticle (SC-UCNP) which is equipped with ligand-directed targeting for siRNA systemic delivery. The nanoparticle can release siRNA in response to an external trigger (near infrared). Based on these nanoparticle delivery platforms, Sirnaomics has developed an enriched product pipeline including siRNA therapeutic STP705 (CotsiranibÒ) for Hypertrophic Scar prevention and reduction, STP702 for "Resistent-Proof" influenza therapeutics, and STP909 for HPV and cervical cancer treatment. Currently, Sirnaomics is pushing two siRNA therapeutic programs into clinical study in China with its Chinese pharmaceutical company partners. By entering technology market and building partnerships, Sirnaomics successfully gained manufacturing capability, technical strength and financial support for developing siRNA therapeutics in China. These progresses have provided solid foundation for Sirnaomics global business effort to start clinical study in US and other regions.

NONINVASIVE IN VIVO MONITORING OF NANOCARRIERS IN PRECLINICAL MODELS

KARSTEN MÄDER
Martin-Luther-University Halle-Wittenberg, Institute of Pharmacy, Halle (Saale), (DE)

Only a very low percentage of the drug candidates reach the clinical phase or the market. Promising in vitro data are often followed by disappointing results obtained in vivo. Problems which are commonly faced in drug development and clinical use include poor solubility, poor distribution into the desired tissue, high toxicity and rapid elimination or metabolism. Nanosized Drug Delivery Systems (Nano-DDS) have the potential to solve one or several of these issues. Many attempts have been made to increase the concentra-

tion in the target tissue by Nano-DDS with an optimized distribution. Examples include higher tumor accumulation values by taking advantage of the EPR effect (passive targeting). In addition, several strategies to obtain a specific, stimulus-sensitive drug release (e.g. pH- or other stimuli) have been proposed and investigated. Nano-DDS include a broad range of different materials and structures. Nanoemulsions and liposomes are commonly used clinically. Alternative materials and structures (e.g. polymeric nanoparticles, polymer conjugates, nanocapsules, dendrimers) are in the focus of industrial and academic research. The biofate of a nanocarrier is a result of a complex interplay between the carrier and the biological environment. It is highly desirable to monitor the in vivo fate of the Nano-DDS in the preclinical phase. However, characterization of Nano-DDS is already challenging in vitro due to the submicron size of the structures and even more complex in vivo. If at all possible, noninvasive techniques with high specificity and sensitivity are desired which permit a continuous measurement of the fate of the carrier, the drug and its therapeutic efficiency. The most commonly used methods include MRI, Optical Imaging, Bioluminescence, CT and PET. Unfortunately, in many cases tracers have to be introduced to make Nano-DDS or their load visible. Every method has is strengths and limitations. Our group uses mainly MultiSpectral Optical Imaging (MSOI) in addition to MRI, benchtop-MRI and Electron Spin Resonance to follow Nano-DDS in vivo.

MSOI is capable to record local encoded emission spectra from 550-950 nm. Therefore, in contrast to many other systems, it is possible to use the spectral sensitivity of the fluorophores for the in vivo measurement of important parameters, e.g. the micro-pH in biodegradable implants1. Fluorescent tumor models offer the potential to follow the therapeutic effect of anticancer Nano-DDS noninvasively in vivo. However, quantitative optical imaging is very challenging. Tumors which express GFP are only of partial use due to the limited light penetration depth due to the relatively short wavelength of GFP emission at 509 nm. Our data show that fluorescent proteins with longer emission wavelengths (e.g. TurboFP635, mPlum) are better suitable for quantitative in vivo measurements of tumor growth and tumor regression2dsRed2, TurboFP635, and mPlum. Applying subcutaneous tumor models in different experimental designs, specific correlations between measured total fluorescence intensity (FI.

Many different materials (e.g. lipids, polymers) and structures (micelles, nanoparticles, nanocapsules) have been proposed as carriers for tumor treatment. It is well known, that the biodistribution is influenced by the size, the surface charge, the shape and the flexibility of the carrier. A reproducible performance of Nano-DDS requires a reproducible quality of the nanocarier. Therefore we did characterize our Nano-DDS by flow-field-flow-fractionation (A4F), which permits – in contrast to PCS - a detailed quantitative characterization of molecular weights and particle sizes. Our results indicate that PEG-PLA nanoparticles accumulate in HT29 and A2780 tumors. The outcomes indicate, that rather small changes of the particle size might cause large changes of the nanoparticle tissue distribution[3,4]. The results of these studies did also show that (1) tumor accumulation of PEG-PLA nanoparticles was highest after 1-2 days, (2) liver uptake was still quite high despite pegylation and (3) accumulation in the tumor was dependent on the tumor model and the nanoparticles[4].

Further studies have been performed on carbohydrates[5] and HPMA polymer conjugates[6,7]. Hydroxyethylstarch (HES) and HPMA polymers did show very long circulation times in blood and - in contrast to PEG-PLA nanoparticles – no accumulation in the liver. In the case of the HPMA polymers, a pH-sensitive delivery approach was followed. The Optical Images indicate that the pH-stimulus sensitive delivery from polymer conjugates can be highly efficient to achieve a high tumor accumulation (Figure 1).

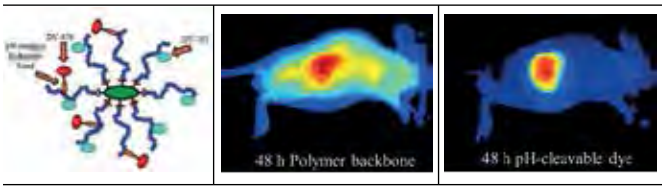

Figure 1: Left: Principle structure of HPMA-star polymers with pH-sensitive drug delivery. In vivo Optical Images of DY-782 labelled HPMA backbone (centre) the pH-dependent cleavable DY-676 (right) in a DLD1 tumor mouse.

The efficiency of the pH-sensitive release was determined in vitro and in vivo as a function of different molecular weights, the polymer architectures (star vs. linear) and the linker[6,7]. For quantification purposes, a tumor accumulation factor (TAV) was introduced[6]. During our investigations of the distribution of Nano-DDS in healthy and tumor-bearing animals, we observed unexpectedly for different DIR-loaded carriers (nanocaparticles, nanocapsules, nanoemulsions) an accumulation in the ovaries[8] (Fig.2). This finding was confirmed in different mice strains and also in rats.

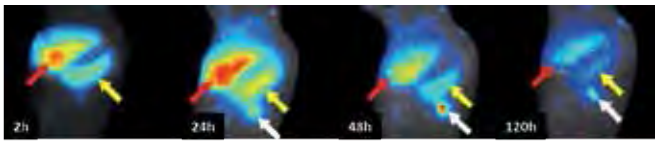

Figure 2: In vivo Optical Images of DiR-loaded PEG-PLA nanoparticles after i.v. injection into mice. The red arrow marks the liver, the yellow one the spleen and the white one the ovaries[8].

A similar a recent publication describes the ovarian accumulation of lipid nanodispersions, which were labelled with the NIR dye DiD or a 99mT c-SSS complex9. This totally independent study supports our findings. One could argue that DiD is very similar to DiR and both might accumulate dye specific in the ovaries. However, the structure of the 99mT c-SSS complex is totally different. We conducted a further study to confirm our findings. Chol-HPMA stabilized F APGA nanoparticles were labeled non covalently by DiR (similar to previous studies). In addition, the stabilizing polymer Chol-HPMA was covalently linked with Dy676. Fig. 3 shows that both the covalently bound dye DY-676 and the highly lipophilic, non-covalently dye DIR accumulate in the ovaries. In conclusion, multispectral Optical Imaging is a powerful tool to follow the distribution and efficiency of Nano-DDS in vivo. High tumor accumulation values can be obtained with pH-sensitive polymer conjugates. The accumulation of Nano-DDS seems to be a rather common phenomenon, which requires further investigations.

Figure 3: Principle structure of double labeled Chol-HPMA FA-PGA nanoparticles. Dy-676 is covalently bound to the amphiphilic polymeric surfactant Chol-HPMA. The highly lipophilic dye DIR is dissolved in the FA-PGA core of the nanoparticles. NIR ex vivo jet color images of the excised uteri with ovaries after i.v. injection. DiR accumulates in the ovaries (middle). The covalently bound dye Dy-676 shows a similar distribution (right).

ACKNOWLEDGEMENT

I would like to thank all members of my group and my cooperation partners in Halle (J. Kressler, T. Müller), in Regensburg (A. Göpferich) and Prague (T. Etrych and K. Ulbrich). The support from the DFG (MA 1648/7 and MA1648/8) is gratefully acknowledged.

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NANOSIZED PROBES FOR MEDICAL IMAGING: CHALLENGES AND OPPORTUNITIES

ALESSANDRO MAIOCCHI
Bracco Imaging S.p.A., Via Ribes 5, Colleretto Giacosa (TO), 10010, Italy

Medical imaging is the technique and process used to create images of the human body (or parts and function thereof) with the goal of enabling early diagnosis or reveal and examine diseases. Consequently, nanotechnology in medical imaging reefers to the use of nanoparticles (NPs) as contrast agents for in-vivo diagnosis in combination with different medical imaging modalities such as CT, MRI PET, SPECT or more recently emerging techniques like Photo Acustic Imaging, (PAI) aimed to achieve a more accurate diagnosis and ensuing improved patient compliance.
The increasing attention in the development of multifunctional nanomaterials is principally due to NPs versatilities offered over the conventional agents. In fact, the availabilities of several surface chemistries, unique magnetic properties and tunable energy absorption and emission properties make the nanoparticles an exciting opportunities on the whole imaging techniques. Besides, the engineered multifunctional nanosystems may facilitate the realization of individual therapy taking advantage from the combination of both therapeutic components and imaging agents. This concept, recently coined as theranostic, is expected to contribute to the development of the imaging-guided therapies and/or drug delivery, aimed to follow the therapeutic response and improve the scheduling of the administration dose. However the theranostic concept applied to nano-sized systems is still far to be really employed in clinical setting and the advantages and challenges the in bringing these fields together need to be discussed.
Nowadays the main results concerning the use of NPs in clinical trials largely deals with therapeutic applications (taking advantages from nanoparticles ability to solubilise hydrophopic and labile drug, to improve drug efficacy varying its pharmacokinetics and minimizing systemic toxicity). The translation to nano-sized system as carrier of imaging agents is still rather complicated and the scenery is even more exiguous. The current clinical contrast enhanced imaging applications are based on relatively fast procedures in which, after intravenous administration of the contrast agent, the patient is imaged in a quite short time. On the contrary, the use of nano-sized agents increases the time required to complete a diagnostic procedure, particularly when the analysis of a wash-in/wash-out curve in a pathological tissue is needed. Moreover the uptake of a nano-sized contrast agent could be the result of several mechanisms, where both active and passive interactions and different localizations in the tissue, are mixed together contributing to the whole signal. The deconvolution of multiple sources of information is not straightforward and requires suitable image analysis tools for signal quantification and clinical validation. A clear evidence of the additional hurdles that should be overcome in the medical imaging applications is provided by the iron oxide-containing nanoparticles. In spite of their promising results in humans and an impressive amount of preclinical studies, the marketing and the clinical applications of iron oxide-containing contrast agents are still highly limited. Although SPIO and USPIO have been approved for clinical use in the past, currently they are no longer available on the market as intravenous imaging agents. The last product on the market with a clinical imaging indication is the oral iron oxide contrast agent, ferumoxsil (Lumirem/Gastromark). Although iron oxide nanoparticles had been reported to have favorable safety profiles, the delayed toxicity effects due to an increased inflammation and oxidative stress cannot be excluded. In addition the negative contrast generated in the images (black spots) offer a more complex information pattern with respect to the traditional positive contrast, requiring a specific training for radiologists. It is important to stress the relevance of toxicity or adverse reactions occurrence in the field of contrast agents because, differently from therapeutics, the level of practical acceptance of toxicological events is very low for a diagnostic procedure.
Several years ago a new disease was observed, referred to as Nephrogenic Systemic Fibrosis (NSF) which was assumed to be associated with the use of the gadolinium- based contrast agents. It was supposed that the free gadolinium, released in body fluids through transmetallation reactions with the endogenous metals, triggered the development of NSF. The NSF is a very rare, highly debilitating, life-threatening disease, which was observed only in patients with severe or end-stage renal disease. With the use of nanoparticles containing gadolinium, the exposure to the free ion could be increased over a larger population due to gadolinium retention in liver, spleen, kidneys and bladder which are typically occurring accumulation sites for the nanoparticle.
Taking all the above into consideration, the aim of this talk is to analyze which are the main challenges in the engineering of nanoparticles for medical imaging application in order to address the development of nano-sized system for clinical purpose. To do this, the main advances and drawbacks of preclinical and clinical nanoparticles are evaluated with particular attention to their physicochemical features aimed to control pharmacokinetics improving in vivo targeting properties and safety.

VULNERABLE ATHEROSCLEROTIC PLAQUES – THE SIGNIFICANCE OF IMMUNE-MEDIATED INFLAMMATION / NANOATHERO

HARALD MANGGE
Prof. Dr. med., Head of the Research Unit on Lifestyle and Inflammation-associated RiskBiomarkers, Coordinator BioTechMed-Graz, Vicespeaker of the Cardiovascular RF Clinical Institute for Medical and Chemical Laboratory Diagnosis, Medical University of Graz (A)

Vulnerable atherosclerotic plaques are the main cause for serious clinical endpoints like myocardial infarction and stroke. Hence, an improved diagnosis and treatment of these dangerous vascular lesions is absolutely essential. Atherosclerosis (AS), the underlying pathologic process, is a chronic immune-mediated inflammation around central lipid deposits involving monocytes, macrophages, T-lymphocytes, and arterial wall cells. The innate and the adaptive immune response, as well as adipokines, chemokines, cytokines, and their receptors are involved in the initiation and perpetuation of AS, and play an important role for the generation of an increased vulnerability.
Hence, an immense number of pro-inflammatory mediators have been investigated in the context of nanomedicine and atherosclerosis but, interestingly, only few anti-inflammatory biomarkers. Nevertheless, the anti-inflammatory axis is always present as a negative feedback if a critical inflammatory perpetuation destabilizes atherosclerotic lesions.
Recently we could show that the immune-modulating, anti-inflammatory molecules, adiponectin and interleukin-10, are useful for molecular imaging of AS plaques. Thus, based on recent publications in animal models of atherosclerosis, we strongly assume that the inflammatory “brake” mechanisms may represent an interesting new tool to specifically target the scenario of vulnerable AS-lesions. Hence, this presentation addresses the potential of adiponectin, interleukin-10 and other anti-inflammatory active molecules towards an improved diagnosis of vulnerable AS lesions. Moreover, promis-

ing nanoparticle biomarker constructs which have been developed within the cooperational interface between Laboratory Medicine (Biomarkers), Nano-Medicine (Nanoparticle Development), and Radiology (Molecular Imaging) will be discussed.

CLINICAL PERSONALIZED MEDICINE PRACTICE BIG DATA VERSUS SMALL DATA ANALYSIS

MIRA MARCUS-KALISH
Tel Aviv University

The new sophisticated and advanced technologies enabling profound insights on the human body and it's functioning, in various levels and degrees of sensitivity, is ushering a new era in medical research and development. Massive amounts of data - "Big Data"- are provided including molecular, physiological, genetic, imaging, environmental data, etc. initiated from various sources while utilizing different techniques and tools.
"In 10 years each individual will be surrounded by a virtual cloud of billions data points" said Prof Leroy Hood (P4 Medicine, 2012). The goal is to translate these "Big Data" outcomes and know how to responsible personalized, preventive and predictive medicine applied in the clinics.
Thus the need is to define the micro and macro factors of the human body functioning in its physical, mental and community environment including the interplay of all these factors. That is to provide the broadest comprehensive insight translated to better healthcare for all.
As the "Big Data" is initiated in various sources utilizing different technologies, it acquires the need to harvest and analyse combined data sets originated in various sources, levels and shapes, such as: age, genomic, single image, time course of images, biomarkers etc. creating an inevitable need to apply and develop sophisticated analysis tools and modelling. Furthermore, beyond the need to analyze simultaneously different types of data, the "Big Data" provides many parameters, such as genetic, imaging and other measurements, sometimes related to few number of people (many parameters, few records) – defined as "Small Data". That acquires the need to tackle the over fitting and to provide reliable and replicable analysis to "Big Data" as well as "Small Data" while creating a comprehensive and well utilized bridge among the two.
The latest publication, such as "Erroneous analyses" in Nature Neuroscience (2011) present massive barriers that are caused by:
1. Leakage in data bases - (Eloyan et al (2012), Brown et al (2012).
2. Replicability – (Baggerly & Coombes-2011, Rosenblatt, Vink & YB-2013)
3. Reproducibility 4. Confidentiality.
For example, hospital data bases suffer from leakage and replicability barriers, as being constructed for different purposes with limited availability of healthy records and different diagnostic tests that the healthy and sick people passed.

Thus, the need is to utilize, converge, modify and adapt current analysis tools as well as to develop new philosophical approaches and innovative tools specially targeted to meet these barriers, based on the coupling of computer sciences, complexity theory, nonlinear dynamics, logic theory, etc.
That is in compliance and in parallel to the statistical models such as generalized linear models or classification trees that are further endowed by new regularization methods, as well as recent developments in FDR (False Discovery Rate) testing and emphasizing the hierarchical structure.
New technologies and targeted tools are developed in the industry, research centres and academia to meet these analyse challenges based on growing sensitivity and specificity needs in representing the medical data variance and its relevance and contribution to personalized medicine.
A targeted data mining rule discovery tool, enabling easier clinical translation and interpretation, will be presented as an example.
This patented rule discovery and prediction tool, enables the simultaneous analysis of multilevel, multisource data (imaging, signals, categorical, numerical and descriptive data). The goal is to relate to the whole data set, as is, with no data manipulation, such as normalization, data neglecting and while handling missing data. The algorithm reveals the underlying rules with the confidence level attached to each rule, identifies the unexpected rules and detects the unexpected cases. In summary the Data Mining and prediction tool is proven to reveal All "If Than" rules, all "If Than Not" rules and a subset of strong rules "If and Only If" (necessary and sufficient conditions), with no prior assumptions.
Few examples utilizing this tool, will be presented, such as: the evaluation of the Epoetin adverse effects to assess long term risks and advancing towards better Epoetin driven treatment modalities, based on the survival analysis of dialysis patients and cardiovascular patients treated with EPO.
The data mining tool was already successfully applied to various applications such as: Neuronal data, autoimmune diseases, Erythropoietin administration on dialysis and cardiovascular patients, drug development, personalized skin treatment, skin genetic data and biomarkers analysis, etc.

NANOPARTICLES FOR THERANOSTICS OF ALZHEIMER DISEASE

MASSIMO MASSERINI, Simona Mancini, Francesca Re
Department of Health Sciences, University of Milano-Bicocca, Via Cadore, 48-20152 Monza (I)

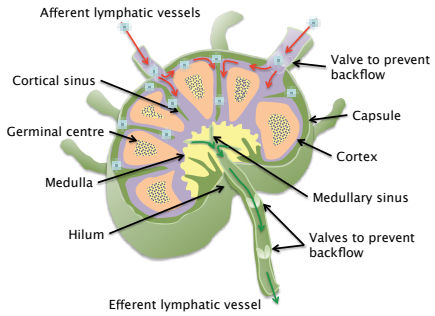
Alzheimer's disease is characterized by a progressive loss of cognitive functions and affects more than 35 million people in the world. A pivotal role in the pathogenesis of the disease is played by beta-amyloid (Aβ), a peptide fragment of the membrane-associated amyloid precursor protein. Accumulation and aggregation of Aβ in the brain, eventually leading to the deposition of extracellular plaques, are histological hallmarks of the disease. Currently, there is no drug to cure the disease, thus a considerable effort has been directed towards the identification of new strategies. Nanoparticles represent a promising tool, relying on the possibility of their multi-functionalization, allowing both the blood-brain barrier (BBB) crossing and the targeting of Aβ.
For this purpose, we rationally designed liposomes, that were bi-functionalized with a modified peptide recognizing the receptor-binding domain of apolipoprotein-E targeting the BBB, and with phosphatidic acid for β-amyloid binding.
Bi-functionalized liposomes (LIP), tested in vitro, showed a strong binding to Aβ (kD = 0.6 μM assessed by Surface plasmon resonance) and the ability to trigger the disaggregation of Aβ aggregates, as assessed by electron microscopy. Experiments on an in vitro BBB model showed that bi-functionalization enhances the barrier passage of LIP in intact form, as assessed by Electron microscopy.
Subsequent in vivo experiments were carried out on animal models of AD (single APPswe and double APPswe/PS1 Tg mice). Intra-peritoneal administration of LIP (3 injections/week, 24 days) decreased total brain β-amyloid1-42 (-33%, p=0.00005), β-amyloid oligomers (-57.5%, p=0.033) and the number of plaques (-29.8%, p=0.008). Plaque reduction was confirmed by PET imaging with [11C]-PIB. Moreover, magnetization transfer contrast imaging (MTC) and resting state fMRI (RSfMRI), also suggested that the treatment lowered amyloid content and restored connectivity in the thalamus. Novel object recognition (NOR) test showed an improvement of mouse impaired memory. Taken all together, our findings promote mApoE-PA-LIP as a well-tolerated valuable new nanotechnological device potentially suitable for AD therapy.

MAGNETIC NANOPARTICLES AND SYSTEMS FOR ONCOLOGY CARE

ERIC MAYES
CEO, Endomagnetics Ltd, Cambridge Science Park, Cambridge (UK)

Nanoparticles hold significant promise in the treatment of cancer, particularly due to size interactions with tumour and related tissue structures. For instance the Enhanced Permeability and Retention (EPR) effect, in which nanoparticles can preferentially transit the leaky vasculature into a developing tumour, holds promise for selective delivery of nano-forumulated drugs and therapeutic nan-

odevices. Also tissues down-stream from tumours, such as lymph nodes, filter at the nanoscale and offer routes to identifying the lymph nodes most likely to drain from the primary tumour. This talk will focus on the role of magnetic nanoparticles and their ability to support the staging diagnosis and adjuvant treatment of tumours, alongside device systems that broadly support the oncology care pathway.



The standard of care for lymphatic staging in breast cancer has been established for over two decades, but the availability of the Sentinel Lymph Node Biopsy (SLNB) technique has been limited due to its reliance on radioisotopes. Endomagnetics was founded to resolve availability through the application of magnetic nanoparticles and novel sensing technology. SentiMag is an extremely sensitive hand-held magnetic susceptometer that can accurately detect minute concentrations of the nanoparticle tracer Sienna+, and the system's efficacy has been demonstrated in 10 clinical studies and trials with over 1,200 patients across 12 European countries since 2012. The system's initial application was as an intra-operative medical device for sentinel node localisation in breast cancer, but it has shown promise in other indications such as melanoma, prostate and colorectal cancer.

By using magnetic nanoparticles, the technology overcomes the disadvantages of the current radioisotope-based technique – such as limited availability, poor workflow, short lifetime, and issues of specialist handling and exposure to radiation – to provide a lower cost, more patient-friendly alternative for clinicians. Magnetic nanoparticles are well established in healthcare, particularly superparamagnetic iron oxide nanoparticles (SPIONs) used as MRI contrast agents. As opposed to the DC magnetic fields of MRI, Endomagnetics has developed a range of AC field devices for the sensing, locating and heating of SPIONs. Dr Mayes will discuss Endomagnetics' development of this device platform, the clinical performance in lymphatic staging for breast cancer, the regulatory challenges leading to marketing authorization and the future of magnetic nanoparticles in the oncology care pathway.

THE POTENTIAL OF RNA-INTERFERING NANOPARTICLES AS A NOVEL THERAPEUTIC FOR THE TREATMENT OF CANCER

JOSHUA MCCARROLL

Tumour Biology and Targeting Program, Children's Cancer Institute, Lowy Cancer Research Centre, Sydney, University of New South Wales (UNSW Australia).

Australian Centre for NanoMedicine, UNSW Australia, Sydney, Australia

Lung and pancreatic cancer are highly aggressive tumours which contribute heavily to cancer mortality. Both cancers are characterised by chemotherapy resistance and aggressive tumour growth. Altered expression of proteins involved in regulating the cell cytoskeleton and mitosis are common in many types of cancer. Indeed, the tubulin / microtubule protein network and proteins involved in modulating mitosis are important drug targets in cancer. For instance, our group has previously demonstrated that the microtubule protein, βIII-tubulin is upregulated in lung and pancreatic cancer cells and that silencing its expression using siRNA/shRNA increased sensitivity to broad classes of chemotherapy and reduced tumour growth and metastases1,2. However, there are no known chemical inhibitors against βIII-tubulin. More recently we have also shown that polo-like kinase 1 (PLK1) a protein kinase involved in regulating mitosis is also upregulated in lung and pancreatic cancer cells, and that silencing its expression using siRNA significantly induced apoptosis. Despite the potential of small molecule inhibitors currently available for PLK1 a number of limiting factors need to be considered such as lack of target specificity and poor tumour bioavailability. Therefore, our goal has been to develop and employ nanotechnology and RNAi to silence the expression of key genes involved in regulating chemotherapy sensitivity and tumour growth in lung and pancreatic cancer cells using in vitro and clinically relevant in vivo models. This presentation will discuss the design and physicochemical properties of 2 novel non-viral nanoparticles (iNOPs and star polymers) as delivery vehicles for siRNA to lung and pancreatic cancer cells and the potential of nanotechnology and RNAi as a therapeutic strategy for the treatment of lung and pancreatic cancer.

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NANOMEDICINE CHARACTERIZATION AT NCI'S NANOTECHNOLOGY CHARACTERIZATION LAB (NCL): RELATING PROPERTIES TO PERFORMANCE

SCOTT E. MCNEILL

The Nanotechnology Characterization Laboratory (NCL) at the U.S. Frederick National Lab for Cancer Research (FNL) conducts preclinical efficacy and toxicity testing of nanoparticles intended for cancer therapeutics and diagnostics. The NCL is a partnership among the National Cancer Institute (NCI), the U.S. Food and Drug Administration (FDA) and the National Institute of Standards and Technology (NIST). As part of its assay cascade, NCL characterizes nanoparticles' physical attributes, their in vitro biological properties, and their in vivo compatibility using animal models. The NCL also looks at trends across nanoparticle platforms, parameters that are critical to nanoparticle biocompatibility, and develops assays for preclinical characterization of nanoparticles. The NCL has developed more than 40 protocols that rigorously characterize nanoparticle physicochemical properties, as well as in vitro immunological and cytotoxic characteristics and ADME/Tox profiles in nonhuman animal models. These assays have undergone extensive in-house validation and are subjected to regular revision to ensure applicability to a variety of nanomaterials. The NCL has assessed more than 300 nanoparticles, including liposomes, dendrimers and other polymers, quantum dots, gold colloids, metal oxides, and fullerenes. This presentation will provide an overview of the NCL, discuss observed trends, and illustrate how physical parameters influence nanoparticle biocompatibility and toxicity and determine nanomedicine performance. This presentation will highlight NCL's efforts to define nanomaterial

Funded by NCI Contract # HHSN261200800001E.

BIOAVAILABILITY ENHANCEMENT OF TAFENOQUINE ANTIMALARIAL USING NANOPARTICLE DRUG DELIVERY SYSTEMS

PAULA MELARIRI, Lonji Kalombo, Patric Nkuna, Rose Hayeshi, Admire Dube, Benhards Ogutu, Liezl Gibhard, Carmen deKock, Lubbe Wiesner, Peter Smith, Hulda Swai,

BACKGROUND

Malaria is one of the world's deadliest infectious diseases. Tafenoquine (TQ), an 8-aminoquinoline drug undergoing clinical trials has a long half-life (2–3 weeks) and could be used for the radical cure of malaria. TQ has relatively poor oral bioavailability and as a result, a high dose required for effective treatment leads to toxicity

especially in individuals with G6PD deficiency. Nanomedicine drug delivery systems present the ability to enhance the therapeutic properties of antimalarials using tafenoquine as a case study.

METHOD

A thermodynamically stable microemulsion drug delivery system, was designed using our novel techniques to enhance drug pharmacokinetic and therapeutic properties.

RESULTS

Our results show that the whole blood maximum concentration (Cmax) of TQ is greatly increased when incorporated into the nano-sized particles (Fig 1) with sizes less than 50 nm. The same is true for the area under the curve (AUC0-inf.). The apparent elimination half-life (t1/2) was 38.3 ± 4.1 hours for the TQ reference formulation and 44.7 ± 1.3 hours for the microemulsion of tafenoquine (MTQ). A major improvement in the bioavailability of TQ when incorporated into the nanoparticle formulations was observed. The bioavailability of TQ reference solution was 55%, and was boosted to 99% from the MTQ (Table 1). In-vitro assays showed that the efficacy of TQ microformulation was more active than free TQ when screened against chloroquine sensitive and resistant strains of P.falciparum.

Conclusion: MTQ has the ability to enhance the oral bioavailability and therapeutic properties of tafenoquine and is a promising delivery system for tafenoquine.

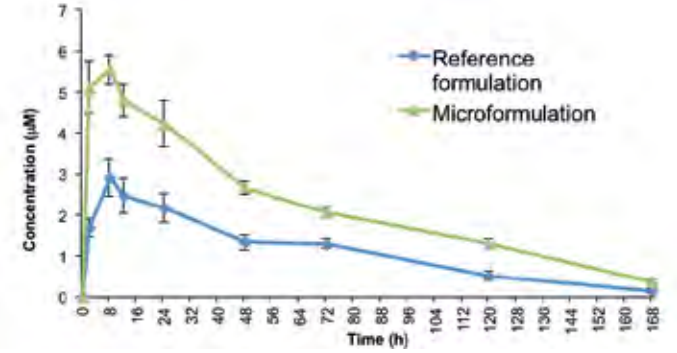

Figure 1: Mean whole blood concentration versus time graphs following oral administration of RTQ and MTQ.

PHARMACOKINETIC PARAMETERS FOR THE TQ REFERENCE FORMULATION AND MICROEMULSION COMPARED (IV N = 5, ORAL N = 4)

Pharmacokinetic parameter	IV administration (2 mg/kg)		FTQ and MTQ (20 mg/kg)	
	Average	SEM	Average	SEM
C_{max} (μM)	n/a	n/a	3,06 5,82	0,37 0,28
T_{max} (h)	n/a	n/a	9,0 6,5	1,0 1,5
Apparent half life (h)	43,7	1,5	38,3 44,7	4,1 1,3
AUC_{0-inf} (min.μmol/L)	2363	146	11368 23842	1232 672
BA (%)	n/a	n/a	55 99	2 1,5

Table 1. A comparison of the pharmacokinetic parameters of the tafenoquine reference formulation and the microemulsion

MULTI-PATHOGEN IDENTIFICATION ON A CENTRIFUGAL MICROFLUIDIC PLATFORM

K. MITSAKAKIS[1,2], S. Hin[1], V. Klein[1], O. Strohmeier[1,2], D. Mark[1,2], F. von Stetten[1,2], R. Zengerle[1,2,3]

[1]Laboratory for MEMS Applications, IMTEK - Department of Microsystems Engineering, University of Freiburg, Georges-Koehler-Allee 103, 79110 Freiburg, Germany
[2]HSG-IMIT - Institut fŸr Mikro- und Informationstechnik, Georges-Koehler-Allee 103, 79110 Freiburg, Germany
[3]BIOSS – Centre for Biological Signalling Studies, University of Freiburg, 79110 Freiburg, Germany

Malaria and infectious diseases of similar clinical symptoms are one of the primary causes of death in endemic areas of the world, where huge numbers denote the extent of the problem: 220 million malaria cases, 80 % of which in Africa, leading to 600 000 deaths (from WHO, World Malaria Report, 2010). Patients often suffer from different disease than malaria but exhibit similar clinical symptoms, resulting in wrong diagnosis and subsequent maltreatment.

The current “gold standard” diagnostic methods are microscopy smear tests, applicable only to malaria, and Rapid Diagnostic Tests (RDTs), available for other infectious diseases too. The latter are often preferred due to their low cost-per-test but have also been disputed for their reliability and accuracy. Furthermore, each test detects only one disease; therefore, if the patient is found negative to malaria, more than one (disease-specific) RDTs need to be employed, which significantly increases the cost-per-patient.

DiscoGnosis aims at developing a fully-automated diagnostic platform for point-of-care use, keeping the cost-per-patient close to RDTs’ level. The platform is based on a disc-shaped microfluidic cartridge (LabDisk, Fig.1a), wherein the fluids are transported by centrifugal forces using a dedicated LabDisk reader (Fig.1b), the latter performing the detection too. Some of the key technological innovations are:

FULLY AUTOMATED SAMPLE-TO-ANSWER ANALYSIS

Minimum external intervention is achieved via: (i) universal adaptor for patient-to-disc interface and sample (whole blood) loading; (ii) utilization of microfluidic unit operations in order to transfer assay protocols from tube to disc: fluid pumping, mixing, metering, aliquoting, reagent storage, and bead handling are integrable on disc in the form of individual, interfaceable modules; (iii) prestorage of dry and liquid reagents, the latter in dedicated pouches encapsulated in the disc, capable of precisely releasing the stored volume at defined rotating frequency.

MULTIPLEXING

(i) On pathogen level, a variety of pathogens will be probed on the same disposable cartridge: parasites (malaria); bacteria (typho and pneumonia); viruses (dengue); (ii) on assay level: dual approach with highly specific immunoassays and isothermal nucleic acid amplification (LAMP) in order to achieve a broad diagnostic window; (iii) on detection level, using quantum dots (QDs) as detection agents.

PRODUCTION TECHNOLOGY

The discs are fabricated via microthermoforming of polymer foils, a technology adapted from the macroscale (blister package production) to the demands of microscale features. The typical steps for fabricating the final kits are: thermoforming; filling with dry/liquid reagents; sealing; cutting and packaging. The polymer-foil nature of the microfluidic cartridge allows adaptability to low-cost scalable production in large batches.

The developed system will be validated in clinical settings at the areas of need, in local hospitals in Senegal and Congo, via partners’ established contacts. The development steps take into account local particularities such as high temperature and humidity, frequent electricity cuts etc, but consider also the general applicability of the system in developed countries. The results will be compared with the “gold standard” methods used for the examined diseases in order to assess the reliability of the system.

(a) (b)

Fig.1. (a) Foil-based disc-shaped microfluidic cartridge (b) The LabDisk reader, integrating user-defined rotating frequency and temperature protocols as well as an optical detection unit (fluorescence)

ACKNOWLEDGEMENT

DiscoGnosis is supported by the European Commission through the objective FP7 ICT-2011.3.2 and under Grant Agreement No. 318408.

COMPARING COMPLEMENT RESPONSES BETWEEN DOXILÂ AND BIOSIMILIARS

S.M. MOGHIMI, P.P. Wibroe, D. Ahmadvand

Centre for Pharmaceutical Nanotechnology and Nanotoxicology, Department of Pharmacy, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen Ø, Denmark

Doxilâ is a sophisticated multi-component liposomal formulation of doxorubicin, and its biological performance is controlled by a complex array of interrelated physicochemical properties including liposome composition, vesicular size (curvature), morphology and surface characteristics, and the internal environment (e.g., volume, pH, sulfate and ammonium ion concentration). Since mid-2011, Doxilâ is in short supply, arising from voluntary shutdown of a third-party manufacturer, and, unfortunately, decision was made to permanently cease production by the end of 2013. However, in February 2013, the US Food and Drug Administration (FDA) approved a 'nano-similar' version of Doxilâ (Lipodox) made by Sun Pharma Global FZE. There are other Doxilâ biosimilars, but manufactured and marketed only in certain countries (e.g., Iran). It is well known that the biophysical characteristics of liposomes can modulate their biological performance, which include vesicular stability and circulation times, enhanced permeability and retention at solid tumors, drug-release rates (at the target site) and toxicity. Indeed, the biophysical properties of the lipid bilayer can affect complement activation. Inadvertent complement activation is a causal factor for infusion-related reactions in humans. The complement system is the first line of defense against intruders, recognizing danger primarily through pattern recognition. Therefore, minor differences in liposome surface curvature, defects and characteristics can incite complement differently and through the binding of antibodies as well as different complement-sensing molecules to include C1q, mannose binding lectin, ficolins and properdin. Further complexity may emerge from the presence of complement activating aggregated contaminants in clinical formulations as well as vesicular structural transformation (resulting from vesicular heterogeneity) in contact with the blood that could elicit immunological reactions. We have now demonstrated subtle difference in complement activation between Doxilâ and some biosimilars in sera of the same individuals, and related these to difference in certain aspects of vesicular morphology and other characteristics. The clinical, manufacturing and regulatory implications of these observations will be discussed.

TEMPORAL CONTROL OF DRUG RELEASE FROM SELF-HEATING HYDROGELS

NASSAJIAN MOGHADAM MOHAMADREZA, Vitaliy Kolesov, Harm-Anton Klok, Dominique P. Pioletti[1]

[1]Ecole Polytechnique Fédérale de Lausanne, Lausanne, Vaud, 1015, CH

mohamadreza.nassajian@epfl.ch

INTRODUCTION

Due to our active life style, knee cartilage is especially at risk and it is very common when a knee injury arises, focal defects are created in the tibial or femoral cartilage. Unfortunately, in this tissue, the native healing aspect is very limited. It has been proposed that drug delivery may enhance the healing process of defected tissues in cartilage defects 1. Several growth factors have been demonstrated to have an impact on articular cartilage repair, but it is how these growth factors are delivered that holds the key for tissue regeneration 1. Mechanical loading has been identified as a key player to improve the potency of these growth factors for the treatment of these tissues 2,3. However, it is important to note that cell receptors are not immediately activated following a mechanical loading but a delay of 5 to 20 minutes has been observed between the initiation of the mechanical loading and the activation of the cell receptor 4. To induce a maximum potency between the drug and the cell reaction, the release of a drug following a mechanical loading should then also be delayed by several minutes.

As hydrogels have viscoelastic and dissipative properties, their temperature may be altered internally by viscous dissipation during cyclic loading over time. In this study we developed a composite hydrogel network, which use the dissipative properties of hydrogels to control mechanically the release of the drug, providing a delay between the initiation of mechanical load and the drug release. The system consists of two components: i) poly(2-hydroxyethyl methacrylate) (PHEMA)-based highly dissipative hydrogel matrix and ii) poly(N-isopropyl acrylamide) (PNIPAM)-based thermosensitive nanoparticles, which are incorporated in hydrogels during hydrogel polymerization. The nanoparticles shrink when temperature passes their lower critical solution temperature (LCST) due to temperature increase induced by viscous dissipation following mechanical loading. The corresponding permeability of the hydrogel increases and ease the release of its payload (Figure 1).

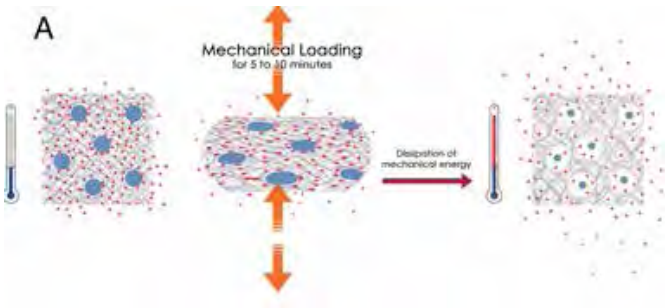

Figure 1. Cartoon of the proposed drug delivery system based on hydrogel dissipative properties, Blue circles: thermosensitive nanoparticles, red dots: payload drug.

EXPERIMENTAL METHODS

The NIPAM based nanoparticles were prepared by room temperature inverse miniemulsion polymerization of NIPAM crosslinked with polyethylene glycol dimethacrylate (PEGDMA550) according to a procedure adapted from Bulmus et al 5. The final formulation of the composite hydrogel based consists of a PHEMA hydrogel cross-linked with 6% mol ethylene glycol dimethacrylate (EGDMA) as cross linker and 40% aqueous phase with dispersed nanoparticles (15 mg/ml) and Xylene Cyanole FF (1 mg/ml) as a model drug. The cylindrical hydrogels were polymerized under UV for 15 min. Cyclic compression load was applied at 1.5 Hz and 15% deformation amplitude in a heat isolated set up, with temperature monitor Sample was immersed in 600 µl water during the test. The amount of Xylene release in water was measured via spectrophotometry.

RESULTS AND DISCUSSION

Size of nanoparticles: Dynamic light scatterting (DLS) results showed that the nanoparticles had a diameter of 340 nm at room temperature and showed a lower critical solution temperature of 37°C. At 37°C, the PNIPAM nanoparticles collapsed and had an average diameter of 255 nm (about 50% decrease in particle volume).
Temperature response of hydrogels: Figure 2 shows the temperature response of HEMA hydrogels. After 5 min of compression, the temperature of hydrogels increased by 1°C due to viscous dissipation.

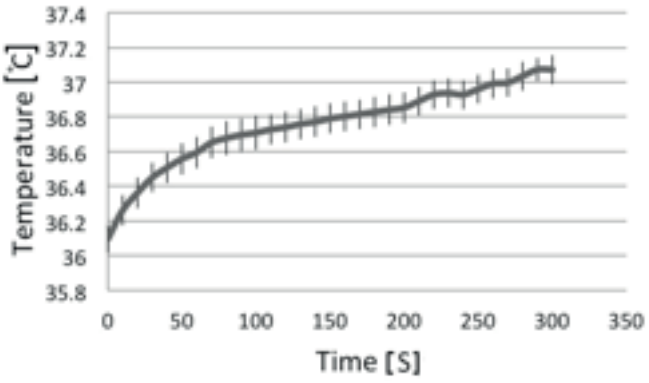

Figure 2. Self-heating of the hydrogel following cyclic compressive load.

Drug release due to dissipation: Under mechanical loading, when environmental temperature was fixed to 36°C, a statistically significant increase (133%) in Xylene Cyanole FF release was observed between the 5 and 8 min loading demonstrating the delayed release of the dye following a mechanical loading (Figure 3). Repeating the experiment at the initial temperature fixed to 34°C (where the temperature of hydrogels stays below LCST when mechanical loading is applied) showed no significant difference in Xylene release between a 5 or a 8 min loading.

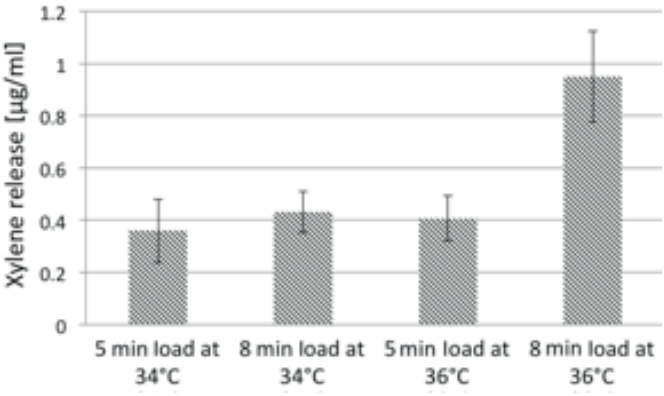

Figure 3. Temporal control of Xylene Cyanole FF release triggered by dissipation properties of the hydrogel.

The most probable effect, which can explain the results presented in Figure 3 is to consider that the temperature-induced shrinkage of nanoparticles due to the dissipation properties of the hydrogel changed the permeability of the hydrogel. To confirm that the release of drug was specifically due to the effect of nanoparticles and not to the hydrogel expansion due to temperature increase, we repeated the measurements with the hydrogels containing no nanoparticles. In this test we observed no significant release. As the change in permeability is obtained following the temperature increase, which in turn is due to the mechanical loading, a time delay is obtained between the initiation of the mechanical loading and the release of the Xylene Cyanole FF.
The developed composite hydrogel could be a good drug delivery system for tissues like cartilage at joints where they are subjected to cyclic mechanical load.

INDIAN 3 KEY OPTIONS OF REGULATORY SYSTEM TO SUPPORT THE SUCCESS OF NANO MEDICINE

S.MOKKAPATI
President, Indian Nanomedicine Society,
Director, Kamineni Health Group, Hyderabad, India
Ex- Deputy Director General, Indian council of Medical Research, New Delhi, India

REGULATORY CHALLENGES

The nanomedicines either approved for marketing or under clinical trials are less than 50. currently under FDA, 591 clinical trials are going on for various liposomal preparations, which is just one type of a nanomedicine; while in India, the total number of nanomedicines under clinical trials are 21. In India, nanomedicines are slowly appearing in the market, for example, Ranbaxyï¿½s ï¿½Voliniï¿½ nanogel. There are few scientific and analytical concerns in the context of regulatory guideline for nanomedicines in Indian context are discussed here.
Firstly, the definition of nanoproducts is not universally accepted and as a result, there is no homogenisation of the acceptable limit, for example, the US National Nanotechnology Initiative (NNI) launched in 2000 considers the dimension from 1-100nm. The definition of size of nanoparticles is important as their size has proportionate increase in surface to volume ratio, admissible changes in physical, pharmacokinetic & pharmacodynamic properties, toxicity and biosafety level, direct or indirect environmental and ecosystem impact.
Secondly, the issues related to the production of nanoparticles such as the infrastructure, training of human resource for handling the nano raw and manufactured materials, occupational hazards and associated health risks, quality control and their assessment in the absence of adequate technology for analysis.
Thirdly, the aspects related to the clinical usage of nanomedicines as they are supposed to deliver the drugs locally in high doses at a particular cellular site. The localised high drug dose may lead to toxicity of a particular cell/organ type (lethal particularly in patients like diabetes and chronic kidney disease). Furthermore, due to the nano size of these medicines, they possess exceptional mobility quality; as a result, they may cross Blood Brain Barrier that may affect brain either severely or for a long term. Also, as a result of their altered biochemical activity, they may bind either with key cellular protein(s) or unwanted molecules such as toxins, and resultantly, interfere with the protein action.
India's engagement with nanotechnology and its potential response to risks emerges in this arduous milieu and is compounded by challenges posed by the nature of the technology itself as it is by the uncertainty in institutional frameworks and capacities for risk regulation and risk governance. The sustainable development of nanotechnologies in India would certainly depend on it as could the shaping of a more sustainable and responsible direction for technology and industrial development which sees a better integration of environment and health issues in its discourse.The department of Biotechnology (DBT), Council of scientific and indutrail research(CSIR),Indian council of medical research (ICMR) are coordinating the regulatory issues of Nanomedicine in India.

FROM GENOMIC PROFILES TO PERSONALIZED CANCER NANOMEDICINE

JAN MOLLENHAUER[1,2]
[1]Lundbeckfonden Center of Excellence NanoCAN
[2]Molecular Oncology, University of Southern Denmark, Odense, DK

It is likely that nanomedicine and targeted medicine will converge in the future to create favorable novel strategies for personalized cancer treatment. Such novel strategies will profit from integration of the most actual knowledge and technologies, including, for example, cancer stem cells and their niches, circulating tumor cell-based diagnostics, and data from genomics-based technologies. Within the latter area, comprehensive international efforts are close to finalization, which will provide mutational catalogues for

most of the prevalent cancer types in humans via sequencing of all 20,000 human genes in several hundreds of cancer cases. Conceptually, this knowledge can be converted into systematic screens for nanodrug components that would selectively eliminate cancer cells with specific molecular fingerprints with prospectively broad therapeutic windows.
The presentation will provide an overview of our strategies in this field. We start from molecular fingerprints from cancer cells in order to first filter out the relevant changes via systematic functional genomics approaches. To this end, we identified several novel modulators of breast cancer growth, metastatic potential and of the breast cancer stem cell phenotype. We use this information to consecutively design automated high-throughput screens for active nanodrug components (siRNAs and miRNA inhibitors) with selective activity against breast cancer and breast cancer stem cells. Data from a first pilot screen indicate that this represents a feasible approach.

SOUNDPHARMA: IMAGE-GUIDED LOCAL DRUG DELIVERY FROM NANOCARRIERS USING FOCUSED ULTRASOUND

CHRIT MOONEN
UMC Utrecht, the Netherlands

OBJECTIVES
The primary goal is to increase the therapeutic index of potent, often toxic treatments through personalized image-guided treatment, ultimately decreasing adverse effects of drugs by better controlling the pharmacokinetics (PK) and pharmacodynamics (PD) of therapy. This is achieved by locally triggering the deposition or activation of drugs via image guided ultrasound triggers.

INTRODUCTION
Ultrasound can be focused within a region with a diameter of about 1 mm. The bio-effects of ultrasound can lead to local tissue heating, cavitation, and radiation force, which can be used for 1) local drug release from nanocarriers circulating in the blood, 2) increased extravasation of drugs and/or carriers, and 3) enhanced diffusivity of drugs. When using nanocarriers sensitive to mechanical forces or to temperature, their content can be released locally. Thermosensitive liposomes have been suggested for local drug release in combination with local hyperthermia more than 30 years ago. Microbubbles may be designed specifically to enhance cavitation effects. Real-time imaging methods, such as magnetic resonance, optical and ultrasound imaging have led to novel insights and methods for ultrasound triggered drug delivery. Image guidance of ultrasound can be used for: 1) target identification and characterization; 2) spatio-temporal guidance of actions to release or activate the drugs and/or permeabilize membranes; 3) evaluation of biodistribution, pharmacokinetics and pharmacodynamics; 4) Physiological read-outs to evaluate the therapeutic efficacy.

METHODS
Thermosensitive liposomes have been suggested for local drug release in combination with local hyperthermia more than 30 years ago. Liposomes may carry both hydrophilic and hydrophobic drugs in their aqueous interior and lipid bilayer membrane, respectively. Nanoparticles may be designed specifically to enhance cavitation effects. Most microbubbles consist of air- or perfluorocarbon-filled microsphere stabilized by an albumin or lipid shell with a size in the range of 1-10 µm.

RESULTS
Several recent publications have shown that ultrasound triggered delivery is feasible (reviewed by 1,2). Real-time imaging methods, such as Magnetic Resonance, optical and ultrasound imaging have lead to novel insights and methods for ultrasound triggered drug delivery. Image guidance of ultrasound has been used to locally release or activate the drugs and/or permeabilize membranes and to evaluate the therapeutic efficacy.

CONCLUSION
The bio-effects of (Focused) Ultrasound can be used for various aspects of local drug delivery and cellular uptake from circulating nanocarriers. MRI guided FUS is particularly useful in case of thermo-sensitive drug nanocarriers. Real-time ultrasound and optical imaging are leading to new insights to increase the therapeutic window with ultrasound.

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2) Frenkel et al. Adv Drug Del Rev 2008

PHARMACOKINETICS COMPARISON OF A NON-BIOLOGIC MICELLAR PACLITAXEL FORMULATION AND NAB-PACLITAXEL– A CASE FOR BIOEQUIVALENCE

KOUROS MOTAMED, Jeff Hsu, Heather Yang and Vuong Trieu
Sorrento Therapeutics Inc., Irvine, CA

STUDY PURPOSE
nab-paclitaxel (nab-Pac/Abraxane®)—a Cremophor-free, albumin-bound, nanoparticle form of paclitaxel—though a breakthrough in paclitaxel formulation, has inherent problems associated with any biologics. IG-001 (Cynviloq/Genexol-PM), a polymeric albumin-free micellar formulation utilizes biodegradable di-block copolymers composed of methoxy poly (ethylene glycol)-poly (lactide) to form nanoparticles with paclitaxel containing a hydrophobic core and a hydrophilic shell and is being developed as the next generation nanoparticle paclitaxel. Its target indications are solid tumors such as Breast, NSCLC, Ovarian, Bladder and Pancreatic cancers, taking advantage of its ability to rapidly deliver paclitaxel to the targeted tissue via albumin-mediated transport, as previously described for nab-paclitaxel. Herein, we report pharmacokinetics comparison between IG-001 and nab-Pac.

METHODS
PK parameters (T1/2, Tmax, Cmax, AUCinf, Vz and CL) of the two formulations were compared following IV injections in mice, dogs, monkeys and human. In the mice and monkeys, the head-to-head comparison of nab-Pac to IG-001 was performed. Blood paclitaxel concentration was quantitated using LC/MS/MS method and PK parameters were obtained using the Phoenix PK program. Dissolution studies of nab-Pac and IG-001 were performed using the drug products reconstituted in saline and diluted in PBS or plasma. Particle stability was monitored by Malvern Nanosizer.

RESULTS
IG-001 and nab-Pac exhibit similar plasma instability such that nanoparticles are not expected in vivo because the Cmax is below the dissolution concentration of both drugs. The data were consistent across 10 different experiments with serum and human plasma (Fig. 1).

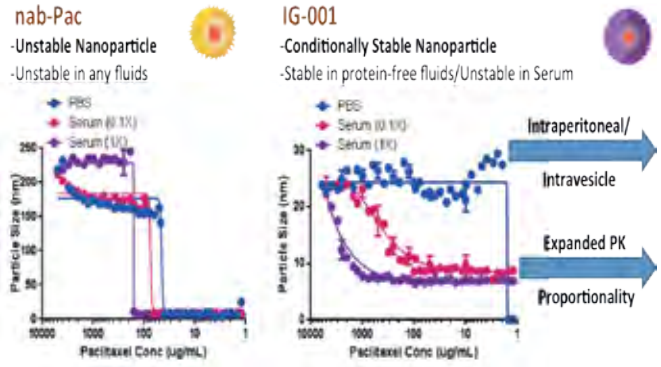
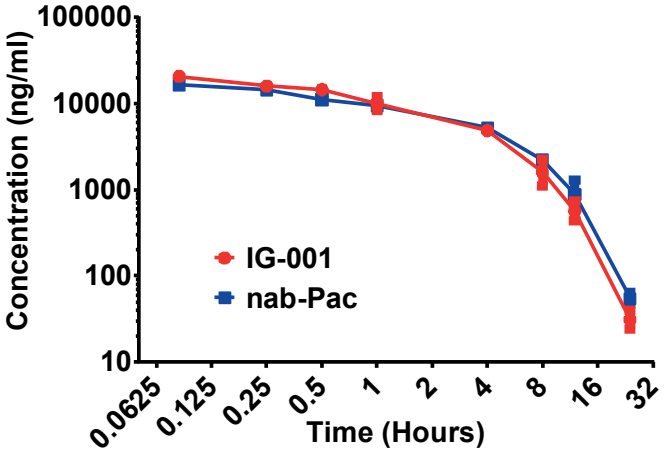


Fig. 1. Comparison of in vitro dissolution profiles of IG-001 and Abraxane®:

- Like Abraxane®, IG-001 nanoparticles are only stable in the vial but not in the blood/serum after administration.
- Like Abraxane®, the nanoparticles dissociated upon administration resulting in the release of paclitaxel.
- Unlike Abraxane®, IG-001 nanoparticles displayed about 8-10-fold enhanced instability in plasma and serum, respectively. 1

• Unlike Abraxane®, IG-001 is a conditionally stable nanoparticle since it displayed a 10-fold enhanced stability in serum-free media (PBS), making it better suited for IP & intravesicle administrations.

Comparison of PK profiles of IG-001 and nab-Pac in mouse revealed a remarkable similarity (Fig. 2).



Drug	$T_{1/2}$ (hr)	T_{max} (hr)	AUC_{inf} (hr*ng/ml)	Vz (ml/kg)	CL (ml/hr/kg)
nab-Pac	2.99	0.08	61561	2104	487
IG-001	2.83	0.08	58151	2104	516

Fig. 2. Comparison of PK parameters of IG-001 and nab-Pac in Mouse

• nab-Pac and IG-001 were administered as an IV Bolus at 30 mg/kg. Blood was collected at 5, 15, 30 min and 1, 4, 8, 12, 24 hr post dosing using 3 mice per timepoint and paclitaxel levels were quantified by LC/MS-MS.

• PK parameters were determined using the Phoenix PK program (Pharsight, CA) and were found to be similar for both IG-001 and Abraxane®.

In monkey, similar PK profiles were observed for both formulations (Fig. 3). More importantly, no unexpected adverse events were observed in the monkey study using the same reconstitution and administration protocol as on the package insert for nab-Pac.

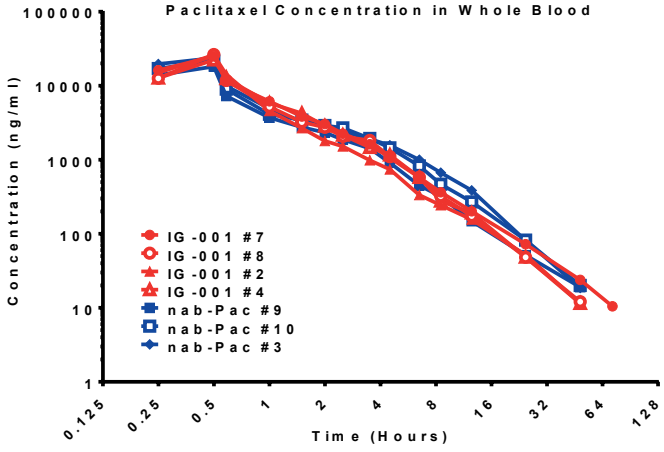


Fig. 3. Comparison of PK parameters of IG-001 and nab-Pac in Monkey:

• Pharmacokinetics of IG-001 (N=4) and nab-Pac (N=3) were examined in Cynomolgus monkeys by a single 30-minute intravenous infusion. Dose levels were 21.7 mg/kg.

• The objective of these studies was to evaluate and compare the pharmacokinetics and infusion reactions of the test articles, IG-001 and nab-Pac. All test articles were directly reconstituted to 5 mg/ml prior to infusion.

• During the course of infusions, no infusion reactions were observed for either IG-001 or nab-Pac.

• The calculated Cmax and AUCinf of IG-001 met bioequivalence criteria; 80%-125% of nab-Pac.

Drug	Reconstitution	$T_{1/2}$ (hr)	T_{max} (hr)	C_{max} (ng/ml)	AUC_{inf} (hr*ng/ml)	Vz (ml/kg)	CL (ml/hr/kg)
nab-Pac #9	Saline	12.65	0.5	18200	21764.57	18190.41	997.03
nab-Pac #10	Saline	9.05	0.5	21600	29083.27	9745.81	746.13
nab-Pac #3	Saline	7.42	0.5	23800	32374.47	7179.92	670.28
Mean		**9.71**	**0.5**	**21200**	**27740.77**	**11705.38**	**804.48**
IG-001 #7	Dextrose	17.23	0.5	25000	29088.93	18545.09	745.99
IG-001 #8	Dextrose	8.73	0.5	26400	26295.02	10395.53	825.25
IG-001 #2	Saline	9.23	0.5	26000	23851.22	12113.52	909.81
IG-001 #4	Lactated Ringer's	8.73	0.5	22300	26493.20	10313.13	819.08
Mean		**10.98**	**0.5**	**24925**	**26432.09**	**12841.82**	**825.03**
BE Criteria	80% - 125%			16960 26500	22192.62 34675.96		

Two previously conducted clinical trials that had used the same dosing regimen for nab-Pac or IG-001 were reanalyzed retrospectively 1, 2 (Fig. 4). Patients in both trials had received 3-hour intravenous infusions for the test articles. Blood samples were collected before infusion and up to 48 hours post-infusion. The paclitaxel concentrations in plasma were quantified by reverse-phase high-performance liquid chromatography for IG-001 and by liquid chromatography atmospheric pressure ionization tandem mass spectrometry for nab-Pac. The results demonstrated that nab-Pac and IG-001 PK parameters reached bioequivalence under the same dosing regimen.

	Nab-Pac 135 mg/m² (n=3)	IG-001 135 mg/m²(n=3)
C_{max} (ng/ml)	1392	1357
AUC_{inf}(ng*h/ml)	5654	5473
$T_{1/2}$ (h)	12.9	12.7
CL (L/h/m²)	27.4	25.5

Fig. 4. Comparison of PK parameters of IG-001 and nab-Pac in Human

CONCLUSIONS

Herein, a clear evidence of bioequivalence between nab-Pac and IG-001 is presented—from in vitro dissolution data to pharmacokinetic data across three different species including human. The ongoing pivotal bioequivalence trial of IG-001 versus nab-Paclitaxel will provide conclusive bioequivalence data. This data will significantly increase our understanding of paclitaxel delivery in clinical setting.

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DRUG DELIVERY AND TISSUE TARGETING WITH ULTRA-SMALL GOLD NANOPARTICLES

JAN MOUS[1], Justin, Barry[2] and Tom Rademacher[3]
[1]PharMida AG, Basel (CH); [2]Midatech-Biogune, Bilbao (ES);
[3]Midatech Ltd., Abingdon (UK)

Midatech's core technology relates to glycan-coated gold nanoparticles (GNPs), a class of self-forming nanoparticles comprised of a noble metal atom core to which an organic layer of carbohydrates or glycans are attached via gold-sulphur bonds. The carbohydrate residues stabilize the metallic core (passivation) and make the particle both water-soluble and biocompatible. During the self-formation process other ligands can be attached to the gold core and these will be interspaced between the glycans. This process results in the single-step synthesis of multi component particles that can deliver multiple copies of a drug with targeting efficiency.

The physical and chemical properties of GNPs are important. The effective hydrodynamic diameter of a GNP is ~3.5 nm (gold core is about 100 atoms of gold and 1.4nm in diameter), which conceivably is smaller than any other delivery vehicle used in medical treatment today and is about the size of a small globular protein. This small size creates several critical qualities for Midatech's GNP-based drugs:

• They are able to pass through the normal pore sizes of blood vessels, and circulate via interstitial flow to normal and diseased organs, to allow delivery to a large number of disease sites

• The GNP's are quickly cleared from the body by excretion in the urine without the need for metabolism thereby increasing the elimination of the product

• Midatech is using gold core GNPs in all its development programmes due to the superior biological properties of gold

Midatech is currently developing its platform technology to target cancer cells with nanoparticle chemotherapeutic agents. The multivalent nature of the gold nanoparticles allows to couple both the anti-tumor agents and various targeting molecules specific for cancer cells on the same nanoparticle.

The targeting agent seeks out specific tumours or diseased organs, using "velcro type" adhesion, and due to the unique uptake properties of small nanoparticles, the nanoparticle drug combination is able to enter cancer cells to deliver its chemotherapeutic payload exactly where it is required.

At present, three oncology projects (ovarian, liver and brain) are being pursued based on similar principles and using similar chemotherapeutic agents with different targeting agents.

First results of in vivo targeting and efficacy studies in human tumour xeno-transplanted mouse models will be presented.

ARTIFICIAL MUSCLES BASED ON NANOTECHNOLOGY

BERT MÜLLER
Prof. Dr., Thomas Straumann-Chair for Materials Science in Medicine, University of Basel (CH)

One of the largest markets, still under-developed by medical device companies, is the treatment of urinary and fecal incontinence. The demographic changes in western countries will lead to a significant increase of incontinent people. For instance, fecal incontinence affects nearly 10 % of people over 60 years of age, and about 2 million people in Europe have daily severe fecal incontinence, which is one of the most devastating of all physical disabilities, since it affects self-confidence and personal image, and usually leads to social isolation. The success of current treatments is disappointing because of numerous complications including infections that often require device removal and the extended use of diapers.

It is the aim of the smart sphincter team to realize prototype devices acting as artificial muscle, termed smart sphincter, to finally treat patients with severe fecal incontinence. The device should replace the destroyed natural muscle function using low-voltage electrically activated polymers controlled by implemented pressure sensors and the patient/medical doctor. The actuator will consist of thousands of nanometer-thin elastomer and conducting layers.

The unique artificial fecal nanotechnology-based sphincter system is driven by an integrated microprocessor, powered by an energy harvesting device and an implantable battery controlling the fluid flow intentionally by the patient and automatically with pressure gauges. The remote control will allow the physician to perform patient-specific adjustments.

The ring-like sphincters is optimized with respect to its macroscopic shape concerning function and comfort applying statistical shape models, with respect to its surface architecture and chemistry to prevent infections and achieve implantation procedures as simple as possible.

The expected benefits for the patient and their physicians are (i) recovery to continence, (ii) short hospitalization periods because of the relatively simple treatment and post-op individual adjustments, (iii) guaranteed reliability (minimal failure rates), (iv) electronically controlled by integrated sensors and managed by the patient.

The initiative «Smart muscles for incontinence treatment» with the duration of four years and a budget of 6.3 million Swiss francs is supported by nano-tera.ch.

THERAPIES IN THE DEVELOPING WORLD WITH REGARD TO ACCESS AND NEW TECHNOLOGIES FOR DELIVERY

MATILU MWAU[1,2] and Doris Night[1]
[1]Kenya Medical Research Institute, Nairobi Kenya
[2]Jomo Kenyatta University of Agriculture and Technology, Juja, Kenya
Email: mmwau@kemri.org

In rich nations, the state of the art in therapy delivery strategies includes lipids, polymers, chemical conjugates, nucleic acid aptamers, and proteins. There are certain hurdles the pharmaceutical industry faces while developing such technologies, including stability, general toxicity, immune-toxicity, pharmacokinetics, and the efficacy and validation of new drug targets in vivo. These hurdles can be overcome.

In fact, it is expected that the controlled delivery of drugs using nanoparticles will enter common usage in the treatment of certain diseases, in the delivery of DNA based and other vaccines, and in personalized medicine in the near future.

In contrast, most therapies in the developing world are delivered in the traditional manner. Nano medicine is unknown in the developing world. Lipids and polymers only exist in the imagination of third world scientists. This is in spite of the fact that non-communicable diseases such as cancer, for which pharmaceuticals have made significant advances, are becoming more and more common in the third world. Paradoxically, the middle class, which is expected to consume a lot of these therapies, is expanding rapidly.

There are several reasons for the gap. Scientific research in the third world is still rudimentary. Pharmaceutical companies may believe that the new technologies may prove unaffordable in the third world. Finally, regulatory authorities will view new technologies for drug delivery with suspicion.

WHAT ARE THE THREE KEY OPTIONS OF YOUR REGULATORY SYSTEM TO BE PUT ON THE TABLE TO SUPPORT THE SUCCESS OF NANO MEDICINE?

MATILU MWAU[1,2] and Doris Night[1]
[1]Kenya Medical Research Institute, Nairobi Kenya
[2]Jomo Kenyatta University of Agriculture and Technology, Juja, Kenya
Email: mmwau@kemri.org

For the success of Nano medicines to be supported by local regulatory authorities in Kenya, there are several considerations. The first one is awareness is very low even amongst medical and pharmaceutical professionals. There needs to be a phased dissemination of information, whichever mode this may take. The second issue is that they could be viewed with suspicion, not unlike genetically modified organisms. To forestall this, local scientists should be incorporated into collaborations for the development and clinical trials of these therapies. Finally, a framework for licensure needs to be thought out, as the mechanisms do not exist.

ULTRA-SMALL, MUCUS-PENETRATING SOLID LIPID NANOPARTICLES ENHANCE THE PULMONARY DELIVERY AND ANTIVIRULENCE OF NOVEL ANTI-INFECTIVES

NOHA NAFEE[a],*, Ayman Husari[a], Christine Maurer[b], Cenbin Lu[b], Anke Steinbach[b], Rolf W. Hartmann[b], Claus-Michael Lehr[c], Marc Schneider[a]
[a]Pharmaceutical Technology and Biopharmacy, Philipps-University Marburg, Marburg, Germany
[b]Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS), Department of Drug Design and Optimization (DDOP), Saarland University, Saarbrücken, Germany
[c]Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS), Department of Drug Delivery (DDEL), Saarland University, Saarbrücken, Germany * Department of Pharmaceutics, Faculty of Pharmacy, Alexandria University, Alexandria, Egypt

INTRODUCTION

Cystic fibrosis (CF) is a life threatening, genetic disorder attacking mainly the respiratory tract due to inherent defect in the CF Transmembrane Conductance Regulator (CFTR) gene. As a result, increased production of thick viscous sputum and failure of mucociliary clearance take place. This represents a perfect environment for opportunistic bacteria namely Pseudomonas aeruginosa (Pa) that grow well protected in 3D- networks known as biofilms.[1] Two main treatment strategies for CF were reported; 1) gene therapy by either gene repair (corrected CFTR gene expression) or

antisense/RNAi-mediated gene silencing, and 2) antibiotic therapy that necessitates multiple courses of antibiotics or antibiotic combinations to eradicate bacteria.[1] Nevertheless, the huge number of possible mutations (>1500) affecting CFTR as well as development of resistant bacterial strains limit efficient CF therapy. Meanwhile, two dominant biological barriers; the thick sputum layer lining the bronchi and the bacterial biofilms prevent the genetic material/antibiotic from reaching their target.[2]

A rather novel therapeutic strategy focuses on developing anti-infectives with novel modes of action known as quorum sensing inhibitors (QSIs) with potential antivirulence activity. Within the biofilms, Pa produce an intercellular signal referred to as "quorum sensing" (QS) that controls of the production of virulence factors. PqsR is a key DNA-binding receptor, specific to Pa and a critical regulator for a set of genes encoding for virulence factors; pyocyanin, elastase B and hydrogen cyanide, thus, represents an attractive target for attenuating bacterial pathogenicity without eliciting resistance. Potent QSI were recently identified reducing pyocyanin in Pa.[3] However, these QSI are lipophilic and thus have limited biomedical application.

In an attempt to improve their delivery, QSI can be encapsulated in solid lipid nanoparticles (SLNs). Ultra-small SLNs (us-SLNs, < 100 nm) are expected to improve drug loading, mucus penetration as well as internalization by bacterial targets. Indeed, the production of ultra-small SLNs remains challenging and was rarely reported.[4] On this basis, the objective of our study implies the preparation of us-SLNs to improve the pulmonary delivery of novel QSI. The delivery system is to be optimized in terms of size, surface hydrophilicity, highest loading, prolonged QSI release and efficient nebulization. In addition, several biological aspects are to be fulfilled including safety on epithelial bronchial tissue, efficient mucus penetration as well as maintained antivirulence activity.

EXPERIMENTAL METHODS

Three lipids (Precirol, glyceryl behenate, and tristearin) were used to prepare SLNs by hot melt homogenization in presence of PVA, tween 80 or poloxamer 407 as emulsifiers. Selected formulations were loaded with QSI or labeled with Nile red. The encapsulation efficiency was directly determined from purified loaded SLNs after extracting QSI with methanol/ dichloromethane mixture. The release was carried out in 50 ml phosphate buffer saline (PBS) or simulated lung fluid (SLF), pH 7.4 at 37 °C. The QSI released was determined in supernatant at predetermined time intervals by LC-MS. Possible cytotoxic aspects were studied using Calu-3 cells incubated with different plain and loaded SLNs at increasing concentrations for 4 h. Cell viability was determined
by MTT assay. The ability of labeled SLNs to penetrate artificial sputum medium (ASM) was investigated by 3D-time laps imaging using confocal laser scanning microscopy (CLSM). Diffused particles were assessed by quantifying fluorescence area using 'Image J' image analysis software. For pulmonary application, SLNs were nebulized using an ultrasonic nebulizer. Particle stability after nebulization as well as nebulization efficiency were verified. The deposition pattern and aerodynamic parameters (MMAD, FPF, GSD) were determined using next generation impactor (NGI) at flow rate of 15 L/ min. The antivirulence activity of free and nanoencapsulated QSI was determined by incubating them with Pa at different concentrations. Pyocyanin produced was measured relative to control. The effect of SLN composition was additionally investigated.

RESULTS & DISCUSSION

1. Characterization of SLNs:

Variation in the formulation parameters lead to the formation of SLNs with tailored colloidal characteristics (diameter 50 - 450 nm, PdI 0.1 - 0.35, zeta-potential -15 to -35 mV). Ultra-small SLNs (< 100 nm in diameter) were selected for drug loading. Encapsulation of QSI did not affect the size of SLNs but occasionally increased polydispersity. DSC thermographs revealed certain reduction in melting temperature and enthalpy of the lipids in SLNs indicating the transformation to less stable polymorphs, whereas, loaded QSI appeared to be in its amorphous form. SEM images showed the spherical shape, smooth surface and homogeneous distribution of SLNs.

2. EE and in vitro drug release:

High EE (60 - 95 %) was recorded for QSI-loaded SLNs according to the lipid-emulsifier combination. The release in PBS showed relatively high burst (~ 40 %) followed by sustained release (60 - 95 %) over 8 h period. In comparison, more prolonged release was observed in SLF with < 20 % burst within the first 2 h and 10 - 70 % released after 8 h, Figure 1. The release kinetics for most formulations followed Higuchi model. It is to be noted that SLNs were stable upon storage at 4 °C for up to 2 months with no drug leakage. Furthermore, particles maintained their colloidal behavior upon incubation in the different buffers and biological media used in the experiments.

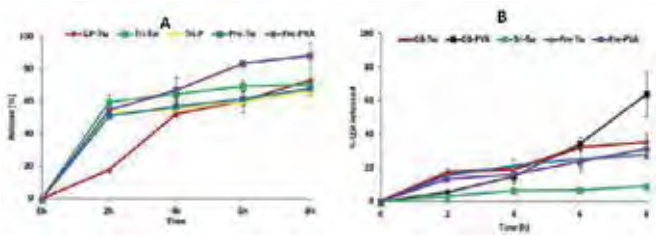

Figure 1: in vitro release of QSI in; (A) PBS, (B) SLF

3. Cytotoxicity assay:

MTT assay revealed that 80-100 % of Calu-3 cells remained viable after incubation with different types of plain and QSI-loaded SLNs at the concentration range tested, except for tween-stabilized glyceryl behenate SLNs, which showed lower viability ranges.

4. Mucus penetration studies:

The diffusion of NR-labeled SLNs in stained artificial sputum (green) was tracked at different time intervals. As indicated in the z-stacks, fluorescent particles can be easily recognized within the ASM 15 - 60 min after deposition according to the emulsifier used. Using image analysis software 'Image J', the total fluorescent signal of combined 3D-laps was determined; for tween-stabilized SLNs, fluorescence was doubled from 15 to 30 min and became 6 times higher at 60 min.

5. Nebulization and lung deposition experiments:

Following nebulization with e-flow, SLNs retained their colloidal nature with certain increase in PdI. The output rate was 0.3 ml/ min. According to the deposition pattern of SLNs in different stages of the NGI; ~ 68 % were deposited in S3-S6 corresponding to the bronchial region, which is our target, Figure 2. Nebulized droplets had an MMAD of 2.2 µm and an FPF of 85 %

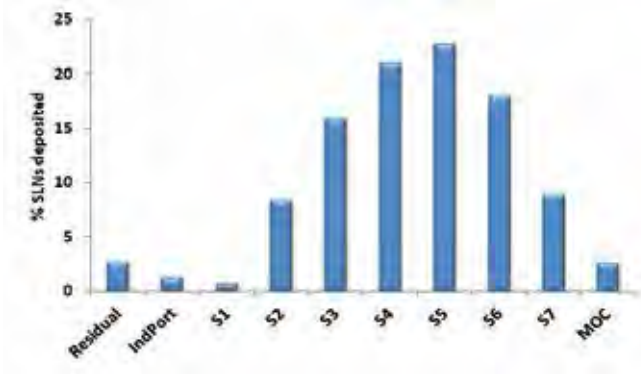

Figure 2: Deposition pattern of nebulized SLNs using NGI (flow rate 15 L/min)

6. Antivirulence activity by pyocyanin assay:

Different types of SLNs loaded with QSI as well as free QSI in solution were incubated with Pa, the concentration of virulence factor pyocyanin was determined. Interestingly, the antivirulence activity represented by inhibition of pyocyanin formation was 5 - 10 folds higher in case of QSI-SLNs compared to free QSI, Figure 3. The mechanism by which SLNs would improve the antivirulence activity is still under investigation. Possible postulations would be improved solubility of the lipophilic compound due to the presence of lipids and/or enhanced uptake by the bacteria.

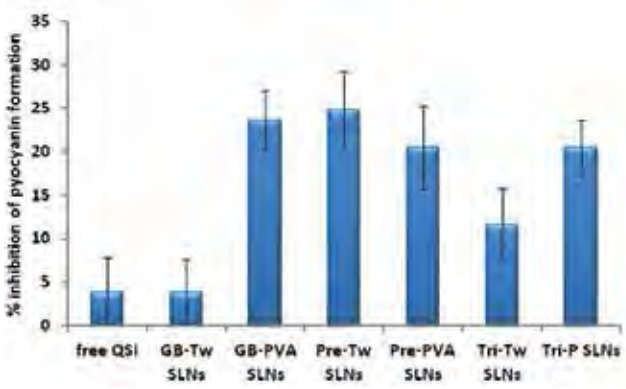

Figure 3: Antivirulence activity of free and nanoencapsulated SLNs by pyocyanin assay Our SLNs represent, thus, a promising carrier for QSI allowing high encapsulation, sustained release, mucus penetration, safety and last but not least improved antivirulence activity.

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BIOLOGICAL FUNCTIONALITIES OF POLYMERIC MICELLE SYSTEMS FOR TARGETING CANCER

NOBUHIRO NISHIYAMA
Professor, Polymer Chemistry Division, Chemical Resources Laboratory, Tokyo Institute of Technology

Polymeric micelles are promising nanotechnology-based carrier systems, since the critical features, including particle size, stability, and drug loading and release properties, can be modulated by engineering the constituent block copolymers. Several micellar formulations of antitumor drugs have been intensively studied in preclinical and clinical trials, and their utility has been demonstrated.
Recently, we have focused our research interest on the treatment malignancies intractable by current therapeutic methodologies including drug nanocarriers. It is known that pancreatic cancer is characterized by hypovascularity and thick fibrosis, leading to limited drug penetration and accumulation in the tumor tissue. We have developed a series of size-different DACHPt (the oxaliplatin active complex)-loaded micelles in the range from 20 to 100 nm, and compared their accumulation, penetration and effectiveness in a pancreatic cancer model. As a result, we found polymeric micelles smaller than 50 nm penetrated the tumor tissue, thereby achieving enhanced antitumor activity [1]. DACHPt-loaded micelles also showed remarkable antitumor activity against spontaneous pancreatic tumors, resulting in prolonged survival of mice [2]. On the other hand, glioblastoma (GBM) is known to be intractable due to the existence of blood-brain tumor barrier (BBTB). Recently, we have demonstrated overcoming BBTB by cyclic RGD peptide (cRGD)-installed DACHPt-loaded micelles [3]. cRGD-installed DACHPt-loaded micelles effectively accumulate in GBM tumors though the active transport pathway, thereby demonstrating significant antitumor activity against orthotopic GBM models.
In my presentation, I will talk about biological functionalities of polymeric micelle systems by introducing the above-mentioned our recent results.

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DRUG TARGETING USING COMPUTER-DESIGNED ANTIBODY AND ANTIBODY FRAGMENTS

YANAY OFRAN

While antibody-based therapies are very promising, the development of new therapeutic antibodies suffers from major hurdles. In particular, it has been proven extremely difficult to raise antibodies against many proteins, including some of the most promising targets, such as GPCRs, ion channels and other membrane proteins. Moreover, existing technologies for the development of antibodies do not allow for the design of antibodies against pre-selected epitopes within the target. To a large extent, existing technologies rely on stochastic process that may or may not yield a binder against some epitope on the target, but could not be directed to yield antibodies that bind a functionally relevant determinant that will result in a biologically active drug. Furthermore, as large multi-chain proteins, antibodies often have limited access to targets, which are hard to manufacture and deliver.
We introduce a novel platform for rational design of antibodies and antibody-fragments that can overcome many of these hurdles. Given an epitope of choice on virtually any target, this platform allows for the design of specific binders that bind that epitope with high affinity and specificity. This allows for the design of both antibodies and CDR-derived peptides that could bind a pre-selected epitope on a target and serve as the basis for a drug with a predefined mechanism of action. Moreover, this platform enables the design of antibodies that are cross-reactive for orthologous targets in different species. It also allows for the design of bi-specific antibodies.
I will discuss the basis of the computational approach we use for the design of antibodies and antibody-fragments. I will show several examples of antibodies that were developed using this method against difficult targets and epitopes.
Finally, I will discuss how this novel approach can be harnessed to develop potent antibody-drug-conjugates.

THE APPLICATION OF NANOTECHNOLOGY FOR OPTIMISATION OF ANTIRETROVIRAL DRUG DELIVERY

ANDREW OWEN

Solid drug nanoparticles (SDNs) have been produced commercially using technologies such as nanomilling where large fragments of crystalised drug are ground into particles with diameters measurable in the nanometer scale (<1mm). This approach has been shown to confer a number of beneficial pharmacological behaviours to active pharmaceutical agents (APIs) with certain physiochemical properties. Such behaviours include overcoming bioavailability issues, maintaining therapeutic exposure with lower doses, long acting / extended release formulations or overcoming food effects for orally administered APIs. Nanomilling has been applied to poorly absorbed and insoluble drugs but does have some limitations because of incompatibility with APIs that have certain physiochemical properties (e.g. low melting point APIs). Using a novel technology that produces SDNs through freeze drying or spray dry-

ing emulsions may be more broadly applicable across drugs. The application of this new approach to the formulation of poorly soluble antiretroviral drugs will be discussed, including benefits for oral bioavailability in pre-clinical species. The SDN formulations have shown the potential for reduced doses and benefits for paediatric administration formats for antiretroviral drugs. These formulations have undergone GMP manufacture and are in stability testing to support regulatory documentation for clinical evaluation in 2014 and the underpinning technology is being commercialised through a recent start-up company from the University of Liverpool called Tandem Nano Ltd.

IN SEARCH OF THE MAGIC BULLET: THE FUTURE OF DRUG TARGETING AND NANOTECHNOLOGY

RICCARDO PANICUCCI
Global Head of Chemical & Pharmaceutical Profiling (CPP)
Novartis Institutes for BioMedical Research, Inc.
500 Technology Square, Cambridge, MA 02139, USA

Despite all the advances in nanotechnology, there is still a long way to go when it comes to treating diseases like cancer. The growth in tumor targeting is in part due to the discovery of the enhanced permeability and retention (EPR) effect. The promise of nanoparticles carrying drugs directly to the tumors without harming normal tissue has exploded the field of drug targeting. At Novartis we have studied many types of nanoparticles both with and without targeting ligands. Diverse nanoparticles chemistries has yielded, more or less, similar observations: Long circulation times, similar bio-distribution and slightly better than free drug efficacy in tumor bearing rats. On the surface, it appears that the chemical and structural diversity in the nanoparticles makes no difference in the results. This is surprising and indicates that there may be some other mechanisms that determine the final fate of the nanoparticles. Ultimately, if nanoparticles are the answer to tumor targeting, there will have to be a major change in the bio-distribution of nanoparticles. Primarily, there will have to be distribution away from organs like the liver and spleen and much higher concentrations in the tumor. It appears that there is still long way to go and it will likely require a different way of thinking about the problem.

THE POWER OF NEXT GENERATION SEQUENCING

RENATO PARO
D-BSSE, ETH Zürich, Mattenstrasse 26, 4058 Basel, Switzerland

The sequence of the human genome as well as the genomes of many model organisms is now complete. However, the primary DNA sequence forms only a foundation for understanding how the genetic program is read and how genome sequence variations determine phenotypes. Genome regulation is very complex and involves the activities of sequence-specific DNA binding proteins as well as layers of ''epigenetic'' information that we only just begin to translate and understand. Heritable epigenetic information is for example stored as methylated cytosine bases, histone modifications, non-coding RNAs and chromatin associated proteins that package the DNA. By regulating chromatin structure and DNA accessibility this layer can influence transcription factor access and creates a context-dependent "epigenome", reflected in a diverse array of developmental stages, tissue types, and diseases. Understanding the regulatory circuitry of chromatin-based events such as transcription, replication and repair requires comprehensive maps of transcriptional start sites, DNA binding proteins and the epigenetic state of the genome for a given cell type and physiological condition.
In parallel to the human genome initiative several new technologies have emerged that allow sequencing at unprecedented throughput and low cost. These approaches are generally referred to as "Next Generation Sequencing (NGS)". They enable researchers to not only resequence genomes and thus to identify genome variations but also to quantify the abundance of experimentally enriched fractions of the genome. One example is the ChIP-Seq method in which a Chromatin-IP is combined with NGS to identify preferential sites of protein binding or nucleosomal modifications. NGS has already been utilized to identify transcript start sites at basepair resolution and to measure quantitatively populations of microRNAs. In addition the new field of metagenomics emerged due to the ability to sequence DNA from diverse biological communities in ecosystems or in infectious diseases. Sequencing of hundreds of cancer genomes is yielding an unprecedented wealth of information of how this deadly disease restructures DNA. It is thus evident that NGS technologies will revolutionize many areas of biology and medicine.
The past decade has witnessed an incredible increase of DNA sequencing capabilities. New technologies have emerged, which parallelize the sequencing procedure in miniaturized reaction vessels and thereby allow the generation of sequences from millions of distinct DNA fragments in single processes. These NGS technologies have substantially facilitated the acquisition of genetic information, boosting the already highly successful discoveries achieved in the past 50 years through the classical tools of molecular biology to a much more integrative level. All tools and equipment have reached a high maturity and reliability, already offering their services as workhorses in many large high throughput sequencing projects (International Cancer Genome Consortium, International Human Epigenome Consortium, ENCODE and modENCODE, etc.). Additionally, new technologies (single-molecule sequencing, etc.) increasing sensitivity and throughput are steadily being developed.
The capability to gather large amounts of sequence information allows customizing the data acquisition from many defined tissue samples or single patients. While the past technologies permitted the assembly of many reference genomes from different model organisms, including human, the current NGS technologies allow to expand and assess the variability of the individual genomes and of the (epi)genetic information defining different cell types of a body.
Sequence genome variation: an important goal of human genetics is to correlate genetic variants like SNPs with distinct phenotypes and diseases. The knowledge of disease-associated SNPs will allow much more refined diagnostic tools and more personalized treatments for specific syndromes. The arrival of NGS technologies has stimulated efforts of large consortia like "1000 Genomes" to map genetic variability and assess its role defining human phenotypes and predisposition to disease. Furthermore, re-sequencing of the whole genomes (or exomes) of cancer cells allows defining the somatic mutations that occur during cancer evolution and progression.
Transcriptomics: RNA sequencing on a high throughput base (RNA-seq) can uncover a much more detailed view of transcript species in the cell. Alternative splice variants as well as transcript fusions often arising in cancer cells can now be comprehensively analyzed in a cell population. RNA-seq led to the discovery of novel classes of non-coding RNAs. For example, new classes of short RNAs have been identified that originate from promoters and gene termini, and many more large, intergenic non-coding (lincRNAs) have been found. The highly quantitative aspect of RNA-seq furthermore enables transcriptional rates in cells to be monitored with high accuracy.
Mapping epigenomes: DNA methylation as well as histone modifications contribute to the epigenetic control of the genome of a cell. NGS technologies as in ChIP-seq allow mapping epigenetic marks on genomes of specific cell types at high precision and resolution. Additional techniques like DHS-seq define regions of open chromatin structure pointing to regulatory sequences. International consortia have recently been established to map epigenomes of specific human cell types and correlate aberrant patterns with disease states.
Genome interactome: both physical and functional interactions of genome sequences are an important part of gene control and the NGS technologies provide means to construct high confidence and high-resolution 3-dimensional maps of interacting elements towards a clearer picture of this still little understood regulatory level. Technologies based on chromosome confirmation capture (3C, Hi-C, etc.) provide snapshots of long-range interactions among regions of DNA, which can be mediated through protein-protein interactions.
Why do we need integrative genomics analyses to better understand fundamental mechanisms of genome function and disease? How might particular risk-associated SNPs affect cellular functions

and lead to specific diseases? What functional sequences exist in the human genome and how are key developmental pathways regulated by epigenetic marks? Many of these questions could not be tackled without a comprehensive view of the genome, as it is the sum of interactions in the nucleus governing the physiology of a cell and ultimately of the organism. The NGS technologies permit to localize genetic as well as epigenetic marks genome-wide at a high resolution and correlate them with specific cellular states. Statistical analyses of datasets allow discriminating causative agents from by-standing or consequential effects. Dedicated algorithms filter noise out of the highly quantitative datasets from NGS and translate the sample information into highly validated genome profiles.
NGS technologies have so far been primarily used in basic science to tackle variability on a genome-wide and organismal level and to detect novel regulatory elements. An impressive range of applications, from the characterization of the evolutionary relationships of ancient genomes to the elucidation of the role of non-coding RNAs in health and disease, have contributed to a much deeper understanding of biology. It is clear, however, that the technology has also a great potential at the applied level for diagnostic and clinical purposes. The ongoing efforts uncover the variability associated to the human haplotypes demarcating novel routes of how phenotypes are created. Consequently, these differences will be useful for diagnostic and predictive purposes. Human genomic variations will in the future be used to devise personalized regimens for drug treatment. Epigenetic profiling will be used as additional diagnostic tool to predict the onset of cancer or neurodegenerative disorders.

PROBING AND MANIPULATING THE IMMUNE SYSTEM WITH NANOMATERIALS

DAN PEER[1,2,3]
[1]Laboratory of NanoMedicine, Dept. of Cell Research & Immunology, George S. Wise Faculty of life Sciences, and [2]The Center for Nanoscience & Nanotechnology, [3]Dept. of Materials Sciences and Engineering, The Iby and Aladar Fleischman Faculty of Engineering, Tel Aviv University, Tel Aviv 69978, Israel; E-mail: peer@tauex.tau.ac.il

The enhanced adjuvant properties of nanomaterials have recently allowed the development of new therapeutic approaches to harness the capabilities of the immune system. This development has been possible principally due to the multivalent presentation of immunogenic chemical groups at the nanoparticle (NP) surface. However, in the presence of biofluids, adsorbed proteins over the NP surface restrict the exposure of the immunogenic groups, which limits control over the type of immunity that can be achieved, and reduces efficiency of immunotherapeutic probes. In my presentation, I will detail examples from the state-of-the-art in material sciences and surface chemistry that can probe and manipulate leukocytes function. I will also discuss the use of RNAi payloads that can modulate the immune response by silencing specific genes and together with the NPs may act as robust adjuvants or reprogram the function of activated leukocytes. Special emphasis will be made to novel approaches in personalized medicine using Omics technologies and their implications in inflammatory bowel disease (IBD) therapeutics and diagnostics standpoints with some current examples from B cell malignancies.

NANOTECHNOLOGIES IN PEDIATRIC CARDIOLOGY

GIACOMO PONGIGLIONE

The application of nanotechnology to medicine includes the utilization of nanoscale structured materials and devices (biosensors, drug delivery technology); advanced proteomics and genomics with gene correction techniques; and machine systems (instantaneous diagnosis and terapy of disease, cellular surgery). The cell is a perfect natural nano-factory. A goal of nanotechnology and nonomedicine is the diagnosis and treatment of disease at the cellular level, developing nanomachines that can work within a cell. Pediatric Nanomedicine include Gene Correction Techniques, particularly in the treatment of single gene defects (Sickle Cell Disease, Cystic Fibrosis, etc.) or in the cure of HIV infections by removal of viral DNA from the body.
Due to their electrical, chemical, mechanical and thermal properties, cardiac nanotubes are promising materials for the electronics, computer and aerospace industries. Our aim is to develop a novel non-toxic CNT-based vector for the nucleic acid delivery in human primary cells (i.e. endothelial cells). These novel systems are able to modulate the expression of genes (up- or downmodulation), thus representing a potentially novel therapeutic tool.

THE FUTURE OF DECISION SUPPORT

GIACOMO PONGIGLIONE

Medical data have unique features that make difficult to create a decision support system. With the increasing amount of genetic data, there are difficulties in discriminating what the real features of a disease are with the consequence of an unprecedented complexity of data sets that should be analyzed jointly.
It is necessary therefore to create large digital repositories where groups of homogeneous patients can be identified and where it is possible to go beyond the classic pair comparison, utilyzing artificial intelligence tools for data analysis.
Furthermore, an individualized prediction can be obtained from disease modelling where tailoring a generic heart model according to the patient's specific features a patients' specific heart model can be obtained on which therapeutic procedures can be simulated.

NANOTECHNOLOGY APPROACHES FOR IMPROVING DRUGABILITY OF THE NEUROPEPTIDE VIP: TARGETED DRUG DELIVERY TO PROSTATE CANCER CELLS AND ENHANCED STABILITY BY ENGINEERED NANOPARTICLES

DAVID POZO* and Rebecca Klippstein
CABIMER; Center for Molecular Biology and Regenerative Medicine.
Spanish National Research Council (CSIC)-University of Seville, Seville, Spain
Av. Americo Vespucio, Seville, E41092, Seville, Spain
*corresponding author: david.pozo@cabimer.es

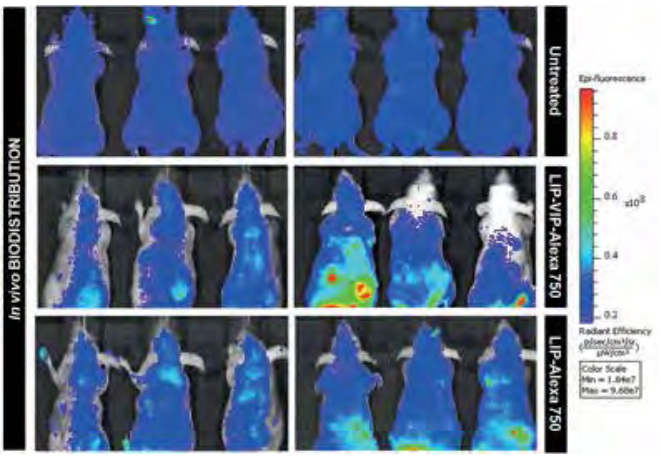
Among the molecules in need of lead optimization, ligands for G protein-coupled receptors (GPCRs, also known as seven-transmembrane domain receptors, 7-TM receptors) are of particular interest, as GPCRs are involved in virtually all physiological processes, with at least 40% of drugs currently in the market thought to modulate GPCRs. A relevant number of endogenous ligands for GPCRs are neuropeptides. Neuropeptides and their receptors provide a mechanistic basis for the mutual biochemical language between the endocrine, the immune, and the nervous systems. Potential advantages of treatments targeting neuropeptide systems in comparison to classical neurotransmitter systems and ion channels revolve around the subject of efficacy as well as the reduced likelihood of side effects, thus making them attractive candidates for the development of new clinical applications for various disorders.
In the present communication, we disclose the advantages of two nanotechnology approaches for VIP improved based-drug delivery systems as a proof of concept, one based on VIP-functionalized gold NPs and the other exploiting VIP-functionalized liposomes as a targeting agent to transport drugs to human prostate cancer cell lines.
Even though there have been several important improvements in the development of neuropeptide therapeutics, they have shown limited success, mainly due to poor bioavailability after protease degradation. Thus, the development of small, non-peptide mimic ligands have resulted in molecules that often have reduced affinity and selectivity compared to their endogenous ligand counterparts. One the other hand, the development of inhibitors of specific proteases increases the chances of adverse effects. In this sense, despite its potential, the neuropeptide VIP, as a paradigm of other peptide-based therapeutics, is still not available for treating clinical

problems. For these reasons, we looked for an alternative strategy that simultaneously: a) targets the protease substrate (neuropeptide) instead of the protease, and b) makes use of the entire neuropeptide molecule to retain its full biological activity. Remarkably, although it has been hypothesized that surface functionalization of proteins and bioactive peptides on noble metallic nanoclusters might protect from protease degradation, so far there are no formal proofs in this sense. Our aim is to prove that coating gold NPs with the neuropeptide VIP impairs the hydrolytic activity of extracellular proteases, leading to VIP-mediated functional responses after harsh conditions resembling the extracellular circulating proteases milieu. By combining physical and chemical characterization to determine size, dispersion and homogeneity of VIP AuNPs, by AFM and TEM analysis and quantifying the amount of peptide, we carry out VIP-receptor-effector functional studies in several settings. Indeed, this is the first study to address the potential protection from protease degradation upon AuNPs functionalization of a given peptide.

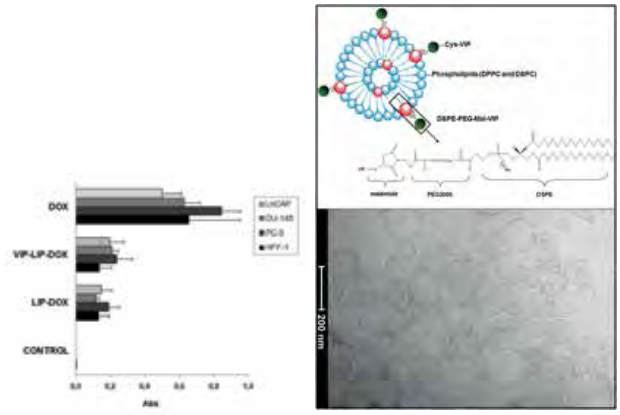
Beside the potential of noble metal NPs, the use of liposomes is recognized as a promising strategy for improving the delivery of anticancer drugs to tumours, leading to a reduction in drug toxicity and improving the therapeutic outcomes. Conventional chemotherapy is the most applied treatment for many cancers but has low specificity and limited effectiveness due to its severe side effects. Doxorubicin (Adriamycin) is a broad-spectrum antitumor antibiotic that has been widely used for treatment of several cancers, including breast, ovarian, and prostate cancers. The effectiveness of doxorubicin is limited due to its high toxicity and side effects, including myelosuppression, alopecia, acute nausea, vomiting, stomatitis, cumulative cardiotoxicity, and strong multidrug resistance response in tumour cells after repeated administration. Biomarkers that differentiate cancerous tissues from normal tissues can be used as targets for this purpose and one of these attractive molecular targets is VIP receptors which are overexpressed in human PCa compared to normal prostate tissue. In particular, in human and rat PCa, VIP receptors are mainly VPAC1 receptors, and the same occurs in the prostate cancer cells lines LnCAP and PC-3. The use of liposomes is recognized as a promising strategy for improving the delivery of anticancer drugs to tumours, leading to a reduction in drug toxicity and improving the therapeutic outcomes. Furthermore, VIP phospholipid liposomes were used to encapsulate doxorubicin in order to deliver it to PCa cells. The aim of this study was to assess the potential of VIP as a ligand for PCa targeting by liposomal nanocarriers. Moreover, we wanted to evaluate the effect of a peptide coupling method on the cellular uptake, cytotoxicity and apoptosis of doxorubicin liposomal formulations. We also addressed in vivo experiments in a preclinical setting in order to evaluate the VIP active driven targeting of the prostate cancer cells by liposomes.

Besides the implications in the field of neuropeptides, our study places the concept of surface functionalization in the broader perspective of novel proteins escaping from extracellular proteases, which could represent a major driven force and an added value to steer the research in the field of engineering NPs and peptide-derived treatments. Moreover, we have developed a reliable method to synthesize homogenous, stable and properly characterized VIP functionalized liposomes.VIP-liposomes demonstrated significant cellular binding and uptake by VIP receptor expressing cells (PC-3, DU-145 and LnCAP) in contrast to unconjugated liposomes. VIP-liposomes showed higher cell-death efficacy in VIP receptor expressing cells than unconjugated liposomes, being confirmed by cytotoxicity and apoptosis studies. In vivo biodistribution studies showed VIP-liposome accumulation in liver, kidneys and spleen, whereas unconjugated liposomes showed preference for liver and kidneys after 12 hours of intravenous injection.

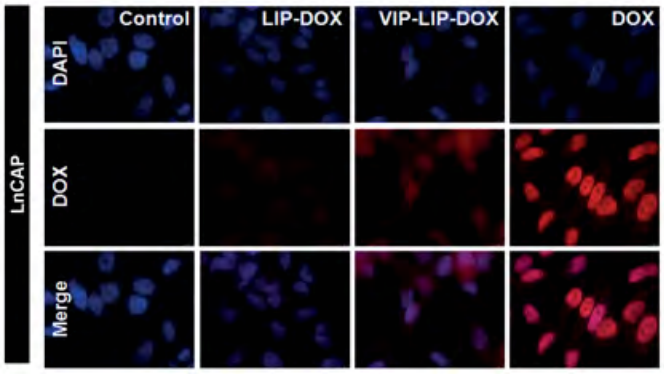
REPRESENTATIVE FIGURES



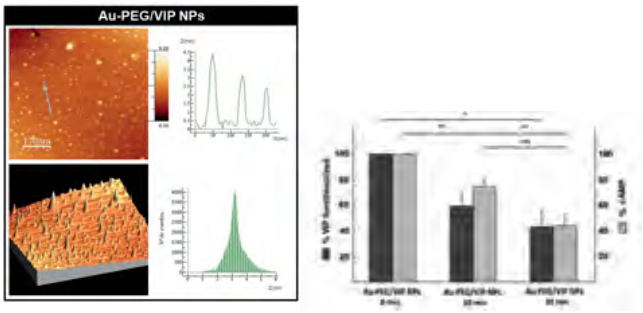
Biodistribution of VIP functionalized liposomes determined by near infrared fluorescence imaging 12 hours post treatment in human hormone-independent prostate tumour xenograft (DU-145) in SCID mice. From left to right, data from two independent experiments of untreated, LIP-Alexa 750 and VIP-LIP-Alexa 750.



Short-term differential cellular uptake of VIP-functionalized liposomes in human cancer cell lines determined by spectrofluorometry.
HFF-1, LnCAP, PC-3 and DU-145 cells were incubated for 2 hours with different DOX formulations (DOX, VIP-LIP-DOX and LIP-DOX). The red line indicates the difference in the average between VIP-LIP-DOX and LIP-DOX treatments. All values are presented as mean ± SD of at least three independent experiments.



Differential cellular uptake of VIP-functionalized liposomes after 2 hours incubation by fluorescence microscopy in human LnCAP PCa cells. LnCAP cells were incubated with media, LIP-DOX, VIP-LIP-DOX and DOX respectively. The blue colour indicates DAPI staining whereas the red colour indicates the uptake of DOX. Purple colour is seen due to the combination of blue and red colour. These are representative images of three independent experiments.



*Characterization of Au-PEG/VIP NPs by AFM. Top-left: Tapping mode topography; Top-Right: measurement of NPs height (indicated in the topographic image); Bottom-left: 3D AFM image; Bottom-right: statistical analysis of colloids height evaluated from AFM image. These are representative images of 2 independent experiments performed. Au-PEG/VIP NPs peptide quantification and cAMP intracellular levels after exposure to proteases. Remaining peptide quantification after 10 and 30 minutes of exposure to proteases (black bars) compared to non-treated Au-PEG/VIP NPs (0 minutes). Intracellular cAMP levels in human PC-3 cells treated for 30 minutes with non-treated and treated Au-PEG/VIP NPs (hatched bars). Values are mean ± S.E.M. * P<0.05, ** P<0.01, *** P<0.005.*

CELL-SPECIFIC TARGETING TO MYOFIBROBLASTS: IMPLICATIONS IN CANCER AND FIBROTIC DISEASES

JAI PRAKASH
Department of Targeted Therapeutics, MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, Enschede, The Netherlands

Myofibroblasts, also known as activated fibroblasts, are elongated spindle shaped cells that are either derived from local fibroblasts, myeloid cells or differentiated from other cell types. These cells play a crucial role in the pathogenesis of several diseases including cancer and fibrotic diseases such as liver fibrosis and renal fibrosis. In cancer, myofibroblasts are referred as cancer-associated fibroblasts that are located in tumor stroma. Cancer-associated fibroblasts (CAFs) provide oncogenic signals to the tumor cells and also induce angiogenesis. CAFs are also involved in induction of metastasis. In liver fibrosis, myofibroblasts are mainly derived from hepatic stellate cells while in renal fibrosis they are reported to be originated from local fibroblasts, bone marrow derived myeloid cells and epithelial cells via epithelial-mesenchymal transition. In fibrotic tissues, myofibroblasts produce an excessive amount of extracellular matrix leading to scar tissue formation and eventually organ failure. Therefore, it is highly essential to develop novel targeted therapies against these cells. Myofibroblasts strongly express Platelet-derived growth factor beta receptor (PDGFbR) as found in human tissues from liver fibrosis, kidney fibrosis and certain tumor types. We designed a cyclic peptide (PPB) against PDGFbR which we conjugated to an antifibrotic cytokine interferon gamma (IFNγ) chemically using polyethylene glycol linker. The PPB-IFNγ was shown to be effective in vitro in mouse and human myofibroblasts to inhibit their activation. We examined the anti-fibrotic effects of PPB-IFNγ in CCl4-induced liver fibrosis mice model and in unilateral ureteral obstruction model for renal fibrosis in mice. Treatment with PPB-IFNγ significantly inhibited the progression of both liver and renal fibrosis. In addition, there were no side effects found with this therapy. In addition, we found that a similar conjugate (PPB-albumin-IFNγ) significantly inhibited the tumor progression of B16 subcutaneous mouse model. The latter effects were attributed to the inhibition of pericytes that also express PDGFbR. Although PDGFbR is commonly present in myofibroblasts, the inter-individual variation and variation in different tumor types for the expression of PDGFbR has been shown in literature. Currently, we are designing new peptides against receptors that are highly expressed on the surface of cancer-associated fibroblasts. Also, we are investigating novel highly potent therapeutic agents such as microRNA and biologicals to block the activities of cancer-associated fibroblasts. To deliver these therapeutic agents, we are applying different nanoparticles as drug carrier for the delivery of these therapeutic agents. In conclusion, cell-specific targeting to myofibroblasts is in potential an attractive strategy for the development of novel therapies against cancer and fibrotic diseases.

MUCOADHESIVE THIOCHITOSAN COATED LIPOSOMES FOR ORAL ADMINISTRATION OF DRUGS

R. PRASSL[5], K. Gradauer[1], S. Dünnhaupt[2], J. Barthelmes[2], E. Roblegg[3], E. Fröhlich[4], G. Leitinger[4] and A. Bernkop-Schnürch[2]
[1]Faculty of Pharmaceutical Sciences, Setsunan University, Osaka, Japan and Novo Nordisk A/S, Måløv, Denmark,
email: kerstin.gradauer@gmx.at
[2]Department of Pharmaceutical Technology, Institute of Pharmacy, University of Innsbruck, Innrain 80/82, 6020 Innsbruck, Austria.
emails: sarah.duennhaupt@uibk.ac.at; jan.barthelmes@uibk.ac.at
andreas.bernkop@uibk.ac.at
[3]Institute of Pharmaceutical Sciences/Pharmaceutical Technology, Karl-Franzens University, Universitätsplatz 4, 8010 Graz, Austria.
email: eva.roblegg@uni-graz.at
[4]Center for Medical Research, Medical University of Graz, Stiftingtalstraße 24, 8010 Graz, Austria. emails:eleonore.froehlich@klinikum-graz.at ; gerd.leitinger@medunigraz.at
[5]Institute of Biophysics, Medical University of Graz, Schmiedlstraße 6, 8042 Graz, Austria. email: ruth.prass@medunigraz.at

The development of polymer coated liposomes as an oral delivery system is projected to improve the systemic delivery of degradation sensitive and poorly absorbed drugs, such as proteins or peptides. A particular challenge in this context is the efficient transport of high amounts of drugs through the intestinal membranein the gastrointestinal tract. To achieve this objective, we have designed new nanocarriers based on the coating of liposomes with thiolated polymers, named thiomers. Thiomers appear very suitable as they show improved mucoadhesion, as well as permeation enhancing and efflux-pump inhibitory properties [1]. Thiomers, more precisely chitosan-thioglycolic acid (chitosan-TGA) of two different molecular weights (77 and 150 kDa) and an S-protected version thereof were covalently bound to the surface of preformed maleimide functionalized liposomes (Figure 1).

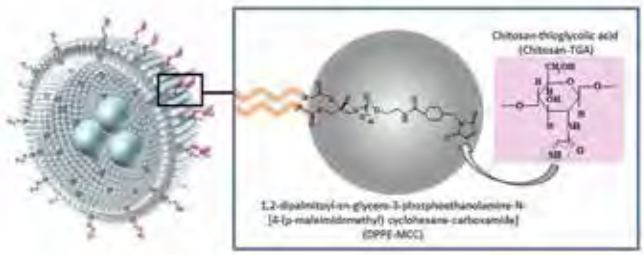


Figure 1: Chitosan-thioglycolic acids (chitosan-TGA) of different molecular weight or an S-protected modfication thereof were covalently linked via maleimide functionalized lipids to preformed drug loaded liposomes.

Efficient coupling of chitosan-TGA to the liposomal surface was observed as an increase in the particle size of approximately 150 nm and a positive zeta potential. The elevated polydispersity index could be explained by crosslinking of single liposomes via polymer chains as visualized in freeze fracture electron microscopy images (Figure 2).

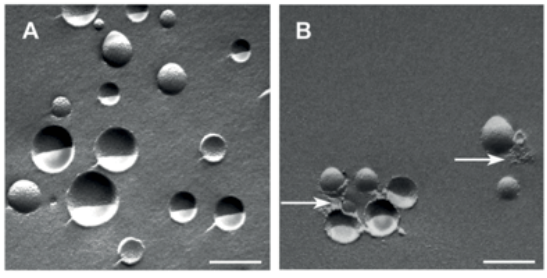


Figure 2: Freeze fracture electron micrographs of (A) uncoated liposomes and (B) chitosan-TGA coated liposomes. Arrows indicate the polymer coat. Magnification: 30.000x. Scale bar: 200 nm.

To test the stability and release behavior in simulated gastric- (SGF) and simulated intestinal fluid (SIF) ex vivo, the fluorophore/quencher-couple ANTS/DPX (8-aminonaphthalene-1,3,6-trisulfonic acid/p-xylene-bis-pyridinium bromide) was entrapped within liposomes. A slow and continuous release of encapsulated ANTS/DPX was observed in SIF over 24 hours, while in the same period less than 10% ANTS/DPX was released in SGF. To assess the mucoadhesive properties of thiochitosan coated liposomes we have monitored the adsorption of rhodamine-123 (Rho-123) labelled liposomes to porcine small intestine using a modified falling liquid film technique [2]. We observed an almost two-fold increase in the mucoadhesion of chitosan-TGA coated liposomes in comparison to uncoated ones. With fluorescence microscopy, we saw a tight adherence of coated particles to the intestinal mucus [3].

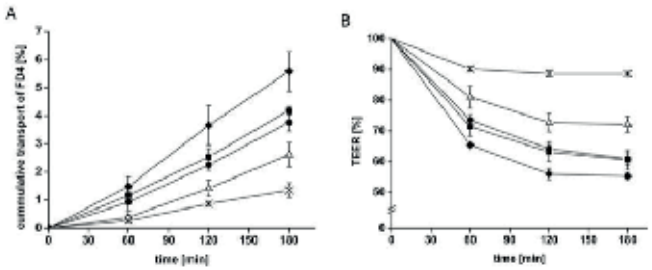


Figure 3: Absorptive permeation of FD4 across rat intestinal mucosa (A). Thiochitosan coated liposomes (●,■,◆) efficiently improved the transport compared to uncoated liposomes (r) or FD4 control (×). (B) A significant decrease of the transepithelial electrical resistance (TEER) was observed after adding uncoated liposomes (r) and thiochitosan coated liposomes** (●,■,◆) in comparison to the FD4 control (×). Indicated values are the means ± SD of at least three experiments (*p<0.05, **p<0.01 compared to the FD4 control solution).*

To evaluate the permeation enhancing- and efflux pump inhibiting properties of thiochitosan coated liposomes the transport of fluoresceinisothiocyanate-dextran (FD4) and Rho-123, respectively, was monitored using rat small intestine. Polymer coated liposomes induced considerably improved permeation enhancing- (Figure 3A) and efflux pump inhibitory effects when compared to uncoated liposomes. The best results were obtained for liposomes coated with S-protected thiochitosan probably due to a higher reactivity of S-protected thiol groups compared to unprotected ones. As shown in Figure 3B drug permeation goes hand in hand with a decrease of the transepithelial electrical resistance (TEER). Finally, to assess the potential of thiomer coated liposomes for oral peptide delivery in vivo, salmon calcitonin (sCT) loaded liposomes were orally administered to rats, and the blood calcium level was monitored over 24 hours. A remarkable reduction of the blood calcium level by about 35%, determined as area above the curve (AAC), was observed for liposomes coated with S-protected thiomers compared to free sCT administered orally in the same amount. Uncoated liposomes as well as liposomes coated with conventional thiomers also led to a considerable, but much smaller reduction [4].
To sum up, liposomes coated with thiochitosan, especially applying an S-protected version thereof, revealed optimized drug release properties, enhanced mucoadhesion and improved permeation of encapsulated compounds. Preliminary in vivo studies in rats demonstrated the high potential of thiochitosan coated liposomes as oral peptide delivery systems.

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GOLD NANOPARTICLES AS CARRIER FOR INTERNALIZATION INTO LUNG ADENOCARCINOMA CELLS: 3 TIERED APPROACH FOR THERAPEUTIC APPLICATIONS

ADRIELE PRINA-MELLO[1,2]
[1]School of Medicine, Trinity College Dublin, College Green, D2, Dublin, Ireland
[2]Centre for Research on Adaptive Nanostructures and Nanodevices (CRANN); Email: prinamea@tcd.ie

Gold nanomaterials have been getting growing interest in clinical diagnosis, therapeutics for chronic pathologies, and other biomedical applications. In particular, engineered gold nanomaterials have been used for developing novel approaches and treatments of cancer. In my talk I will present some potential application of gold nanoboxes (AuNBs) as carriers as perspective pre-clinical nano-enabled systems for personalized medicine approaches against lung cancer. The utilization of a 3 tiered approach integrated in a safe-by-design approach allowed for systematic experimental testing during the selection of uncoated AuNBs and then on coated, drug-loaded nanomaterial. The results presented in the talk showed that uncoated AuNBs could effectively penetrate into human lung adenocarcinoma (A549) cells when in simple (mono-cultures) or complex (co- and three-dimensional-cultures) in vitro environments mimicking the alveolar region of human lungs. Furthermore, uncoated AuNBs were biologically inert in A549 cells and demonstrated signs of biodegradability. Preliminary data also revealed that coated, drug-loaded AuNBs could efficiently deliver a chemotherapeutic agent to A549 cells, supporting the hypothesis that could be used for personalized nano-enabled systems for lung cancer treatment [1].

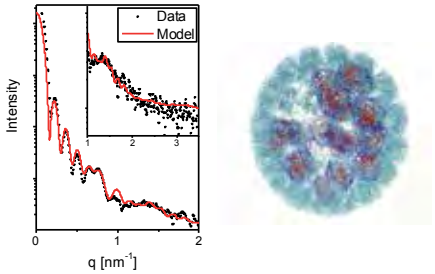
[1] Movia D., Gerard V., Maguire C.M., Jain N., Bell A.P., Nicolosi V., O'Neill T., Scholz D., Gun'ko Y., Volkov Y. and Prina-Mello A. (2014) Biomaterials, (in press, avaialble online) DOI: 10.1016/j.biomaterials.2013.12.057

Supported by the EU FP7 NAMDIATREAM project (NMP-2009-LARGE-3-246479), EU FP7 MULTIFUN project (NMP-2010-LARGE-4-246979), CRANN (CRANN Pathfinder to DM) and Science Foundation Ireland (SFI) under the CRANN CSET.

X-RAY: HI-RESOLUTION, STRUCTURE, DYNAMICS, AND INTERACTIONS OF SUPRAMOLECULAR SELF-ASSEMBLIES

URI RAVIV
The Institute of Chemistry, The Hebrew University of Jerusalem

Using SAXS, in combination with Monte Carlo simulations, and our unique solution x-ray scattering data analysis program, we resolved at nearly atomic resolution, the exact manner by which wtSV40 packages its 5.2kb circular DNA about 20 histone octamers in the virus capsid. This structure, known as a mini-chromosome, is highly dynamic and could not be resolved by any microscopy methods(NAR, 41, 1569, 2013). Using time-resolved solution SAXS, stopped-flow, and flow-through setups the assembly process of VP1, the major caspid protein of the SV40 virus, with RNA or DNA to form virus-like particles (VLPs) was studied in msec temporal resolution. By mixing the nucleotides and the capsid protein, virus-like particles formed within 35 msec, in the case of RNA that formed T=1 particles, and within 15 seconds in the case of DNA that formed T=7 particles, similar to wt SV40. The structural changes leading to the particle formation were followed in detail (J. Am. Chem. Soc. 134, 8823, 2012).



ELECTRON MICROSCOPY IMAGING OF DRUG DELIVERY SYSTEMS IN LIQUID STATE

OREN REGEV

Department of Chemical Engineering, Ben-Gurion university of the Negev, Beer-Sheva, Israel

The structural study of dispersed nanometric-size objects in liquids is challenging. Conventional electron microscopy techniques fail to deliver honest information on aqueous systems due to sample drying or staining. In low-temperature, cryogenic-transmission electron microscopy (cryo-TEM) technique we thermally arrest the motion of the objects, facilitating direct imaging of the liquid state at nanometer resolution.

In my talk, I will present the basics of the cryo-TEM technique, employed in handful of pharmaceutical studies (see Figure 1), and show few examples of applications in drug delivery (Doxil). I will focus on extraction of analytical information from the images.

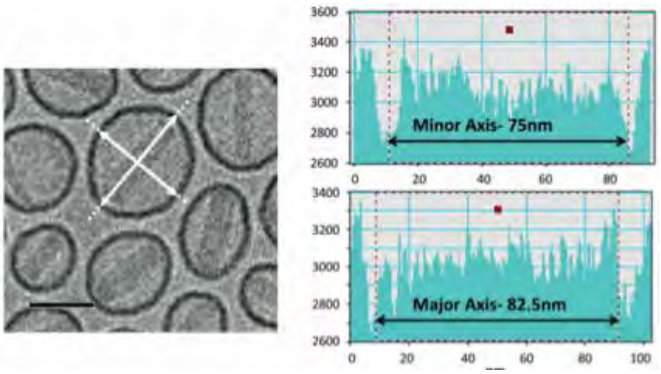


Figure: Cryo-TEM micrograph of drug-encapsulated liposomes (left) and grayscale intensity values along line profiles (right). Note a liposome aspect ratio greater than 1. Bar=50nm

CRIPEC® DOCETAXEL – NANOMEDICINE WITH HIGH AND SUSTAINED EFFICACY IN ONCOLOGY

CRISTIANNE RIJCKEN

PharmD, PhD, Cristal Therapeutics, Oxfordlaan 55, 6229 EV Maastricht, The Netherlands

Cristal Therapeutics is a pharmaceutical company developing innovative nanomedicines based on its proprietary polymeric technologies (CriPec®). Cristal Therapeutics' mission is to improve the therapeutic performance of new and existing drugs in various therapeutic areas. The application of CriPec® results in better efficacy and improved tolerability of drugs by an improved disposition in the body and controlled exposure to the sites of action.

CriPec® docetaxel is the company's lead product, designed for the treatment of various solid tumours, with gastric adenocarcinoma, endometrium and bladder cancer as potential indications of initial focus. In CriPec® docetaxel, the active molecule docetaxel is temporarily covalently bound within stabilised nanoparticles. The 70 nm-sized CriPec® docetaxel nanoparticles are designed to target the tumour tissue efficiently. Concomitant chemical hydrolysis results in release of only parent docetaxel in a well-controlled manner, and ensures a sustained exposure of the tumour to docetaxel.

The nonclinical efficacy profile of CriPec® docetaxel is currently evaluated in vivo in xenograft subcutaneous tumour mouse models, including gastric and breast cancer models. The maximum tolerated dose (MTD) of CriPec® docetaxel in nude mice (q7d3 injections) was shown to be 3-fold higher than of Taxotere, 90 mg/kg vs 30 mg/kg respectively. A single injection of CriPec® docetaxel (90 mg/kg) induced more profound suppression of both small as well as established MDA-MB-231 breast xenograft tumours when compared to Taxotere (30 mg/kg), with complete regression observed in at least 7 out of 10 animals. Recently, another study in small MDA-MB-231 breast tumours demonstrated the superior therapeutic efficacy upon equimolar (60 mg/kg) dosing of CriPec® docetaxel versus Taxotere.

In the NCI-N87 gastric cancer model, weekly i.v. doses of CriPec® docetaxel (75 mg/kg; 3 doses in total) also resulted in a more sustained suppression of tumour growth compared to Taxotere dosed at its MTD, viz. 25 mg/kg.

The efficacy findings in both breast as well as gastric models are consistent with the observed 50-fold higher tumour uptake of total docetaxel with CriPec® docetaxel compared to Taxotere, and with sustained presence of docetaxel for at least 96 hours after dosing.

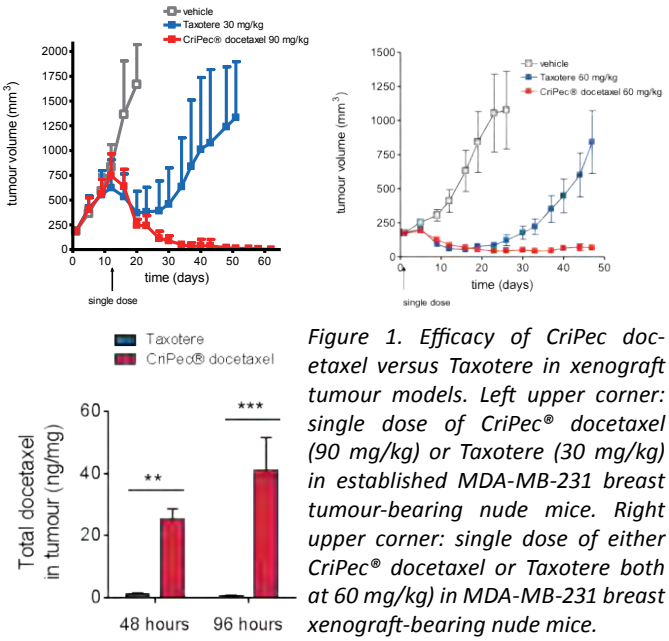


Figure 1. Efficacy of CriPec docetaxel versus Taxotere in xenograft tumour models. Left upper corner: single dose of CriPec® docetaxel (90 mg/kg) or Taxotere (30 mg/kg) in established MDA-MB-231 breast tumour-bearing nude mice. Right upper corner: single dose of either CriPec® docetaxel or Taxotere both at 60 mg/kg) in MDA-MB-231 breast xenograft-bearing nude mice. Left down corner: Total docetaxel levels in tumour following a single injection of 30 mg/kg CriPec® docetaxel or Taxotere. Data represent mean +/- SEM.

The preclinical safety and tolerability studies performed so far indicate that the MTD of CriPec® docetaxel is higher than Taxotere. In both groups, expected patterns of docetaxel toxicity were observed, but the toxicity to key targets was of significantly less intensity in CriPec® docetaxel-treated rats compared with Taxotere, despite an approximately 150% higher dosage. Equally important, the control arm with CriPec® empty showed no signs of any toxicity.

Data to date support the potential of CriPec® docetaxel as novel nanomedicinal product in oncology with improved efficacy/safety balance compared to other taxane-based treatments. Clinical evaluation in a phase I/IIa study in patients with solid tumours is scheduled end 2014.

Next to CriPec® docetaxel, the CriPec® product portfolio comprises various early-stage products in various therapeutic areas. Cristal Therapeutics develops these products independently, as well as in collaboration with other parties.

THE SOCIO-ECONOMIC ASPECTS OF NOVEL NANOMEDICAL TECHNOLOGIES

DOUGLAS K. R. ROBINSON

For over a decade, there have been promises about socio-economic impacts of nanotechnologies. By now they begin to permeate different application domains and in a variety of ways. In parallel, decision makers in various positions call for indicators or measures of socio-economic impact.

Those attending this talk will be provided insights into the types and forms of soico-economic impacts visible today, or predicted for the future, stemming from nanomedicine related technologies and describes the method of Impact Pathway Analysis (IPA). The short presentation, is part of a larger activity conducted by the speaker both as a consultant and in collaboration with academic partners in the Netherlands, UK and France.

ANTITUMOR ACTIVITY OF A BCL2-DIRECTED DNAI DRUG IN A PHASE II STUDY OF PATIENTS WITH RELAPSED OR REFRACTORY NON-HODGKIN'S LYMPHOMA

DR. WENDI V. RODRIGUEZA

PhD. CSO and VP, Product Development, ProNAi Therapeutics, Inc., Plymouth, MI (USA)

BACKGROUND

The derangement of BCL2 regulated control mechanisms is a defining characteristic of a variety of cancers including follicular and diffuse large B-cell lymphoma. PNT2258 is a 24-base chemically unmodified phosphodiester DNA oligonucleotide encapsulated in a specialized liposome (SMARTICLESÒ) that has demonstrated antitumor activity against human cell lines and xenograft models that include NHL, prostate cancer and melanoma. The oligonucleotide sequence is designed to hybridize with genomic sequences that reside within 5'-untranscribed regulatory regions of the BCL2 gene to block transcription of BCL2 through a mechanism called DNA interference (DNAi). In a previously conducted phase I study, PNT2258 was safe and well tolerated at doses through 150 mg/m2 and PNT2258 showed evidence of BCL2-targeted effects on a variety of biomarkers.

METHODS

This study is a multi-center, single-agent, pilot Phase II investigation of PNT2258 administered at a dose of 120 mg/m2 as a 3-hour IV infusion on days 1-5 of a 21-day cycle. Objectives included characterization of anti-tumor activity and safety data in patients (pts) with relapsed or refractory lymphoma undergoing treatment with PNT2258 for six cycles or until progressive disease or unacceptable toxicity. Adult pts with measureable disease that was FDG-PET positive at baseline were eligible. Additional criteria included ECOG PS <2, adequate bone marrow, renal and hepatic function, and prior treatment that included rituximab and cytotoxic chemotherapy. Standard CTCAE and Cheson criteria were used for evaluation. Patients were scanned with CT/PET at baseline, after 2 cycles and at study end.

RESULTS

To date, 13 pts with DLBCL, FL, MCL and CLL have been treated with PNT2258. Antitumor activity was observed in the majority of patients and results will be presented.

CONCLUSIONS

PNT2258 is a first-in-class DNAi where a native chemically unmodified DNA oligonucleotide is delivered to a nuclear genomic target via a protective liposomal nanoparticle, and the first to target the regulatory upstream region of the BCL2 gene. PNT2258 is well tolerated with an acceptable safety profile and shows significant single agent anti-tumor activity in pts with heavily treated relapsed NHL, particularly in pts with FL and DLBCL.

ANTIBODY AND BISPECIFIC ANTIBODY TARGETED NANOPARTICLES FOR SELECTIVE CANCER THERAPY

STEVE R. ROFFLER

Institute of Biomedical Sciences, Academia Sinica, Taipei 11529, Taiwan

Selective delivery and uptake of therapeutic nanocargos into target cells is important to realize the potential of nanomedicine. Here we describe several experimental approaches to study and perform preferential delivery of active anticancer agents to cancer cells via nanoparticle delivery systems.

Polyethylene glycol (PEG) is a water-soluble, non-toxic, non-antigenic, biocompatible polymer that has been approved by the Food and Drug Administration for human intravenous, oral, and dermal applications. PEGylation of nanoparticles (to create "stealth" NPs) can reduce NP uptake by macrophages, prolong circulation time in the blood, and enhance tumor accumulation.

We developed the first anti-PEG monoclonal antibodies that bind to the repeating subunits of the PEG.[1, 2] We recently generated additional monoclonal antibodies to PEG that can detect PEGylated proteins, liposomes and nanoparticles.[3] These anti-PEG antibodies have been widely used in proprietary assays throughout the pharmaceutical and biotechnology industries for analysis and pharmacokinetic measurements of PEGylated compounds.[4, 5]

Here we investigated using these anti-PEG antibodies as the basis of a universal method to target PEGylated stealth NPs to specific cellular addresses. We constructed and produced recombinant bispecific antibodies in which one arm of the antibody can bind to PEG while the other arm can bind to receptors expressed on the target cell surface. We show that tumor targeting capability can be simply conferred to stealth NPs in a one-step method by mixing the NPs with bispecific antibody (Fig. 1). This simple strategy may be used to confer targeting specificity to any PEGylated NP.

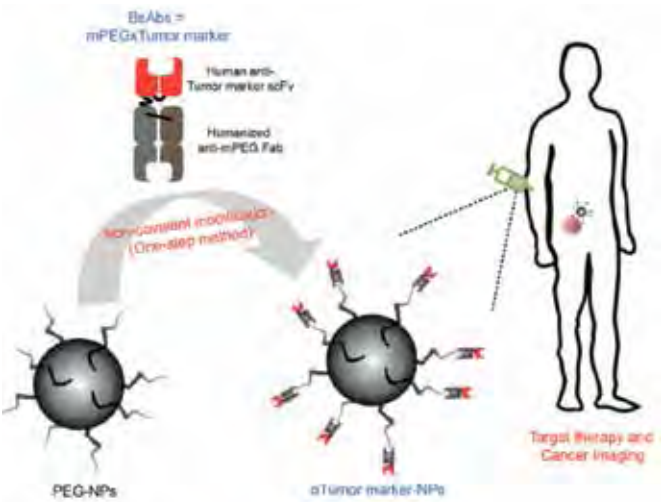


Figure 1. Bispecific antibodies for targeting stealth nanoparticles to cancer cells. A humanized anti-PEG Fab fragment was fused to human anti-EGFR or anti-HER2 single-chain antibodies (scFv) to form anti-PEG bispecific antibodies. The anti-PEG portion can bind to PEG molecules on stealth NPs to endow tumor tropism for selective cancer imaging and therapy.

Another approach to confer target specificity to NPs is to covalently attach peptides or antibodies to their surface. Basic investigations of targeted NPs, however, are hampered by difficulties in making comparisons between various NP formulations, tedious and complicated coupling reactions which may harm NP integrity, and the need to characterize and adjust each targeted NP for similar bioactivity.

Here we describe preliminary work toward establishing a platform to rapidly compare different stealth NP properties and formulations to accelerate the development of targeted NPs. We previously reported that a chimeric receptor composed of an anti-PEG Fab fused to the TM and cytoplasmic tail of the LDL receptor can bind and facilitate receptor-mediated endocytosis of PEGylated NPs into cells.6,7 We are working to extend the utility of this system by fusing anti-PEG antibodies to truncated cancer cell receptors including the epidermal growth factor receptor (EGFR), HER2/Neu and CD19 to act as model receptors for human cancer cell targeting (Fig. 2).

We anticipate that anti-PEG receptors can bind and induce the endocytosis of any NP that has PEG chains exposed on its surface. These artificial receptors may therefore mimic the behavior of cancer-targeting NPs.

Potential advantages of the anti-PEG receptor platform include ability to simultaneously compare multiple stealth NPs, creation of cells expressing defined numbers of receptors and elimination of the need to covalently couple peptides or antibodies to NPs. This system may therefore be useful to systematically investigate the influence of important variable including receptor density, NP size, hardness, or composition on NP binding, selectivity, uptake, routing and efficacy. This system can be extended to additional cancer targets by fusing the anti-PEG antibodies to the appropriate receptor.

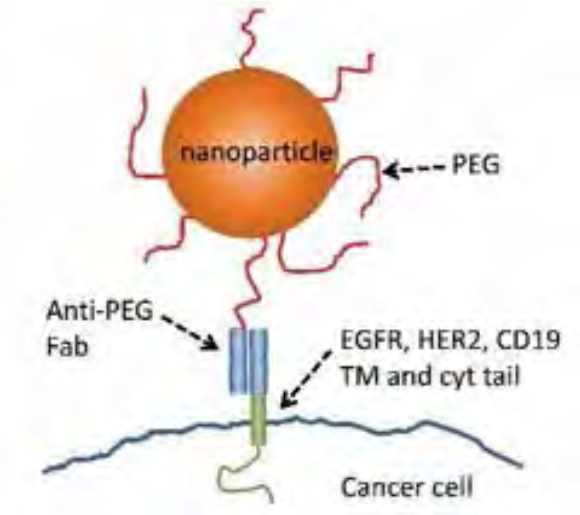


Figure 2. Anti-PEG stealth NP receptors. Transgenes coding an anti-PEG antibody Fab fragment fused to TM and cytoplasmic tails derived from receptors that are often overexpressed on cancer cells (ie, EGFR, HER2 and CD19) allow controlled expression of the receptors on mammalian cell. The receptors can bind to PEGylated NPs and cause endocytosis of the NPs into the cells. These receptors may mimic the behavior of NPs decorated with ligands that bind the receptor of the corresponding TM and cytoplasmic tail. Anti-PEG receptors may help investigate factors that influence NP binding, uptake, routing, metabolism and therapeutic efficacy.

We are also working to develop proactive NPs for more selectively delivery of anticancer agents to cancer cells. The NPs are loaded with proactive anticancer drugs, which are designed to display minimal systemic toxicity but become activated to display cytotoxicity in lysosomes of cancer cells after receptor-mediated uptake of the NPs (Fig. 3). This may facilitate generation of high concentrations of anticancer drugs in cancer cells while normal tissues are largely spared from drug exposure.

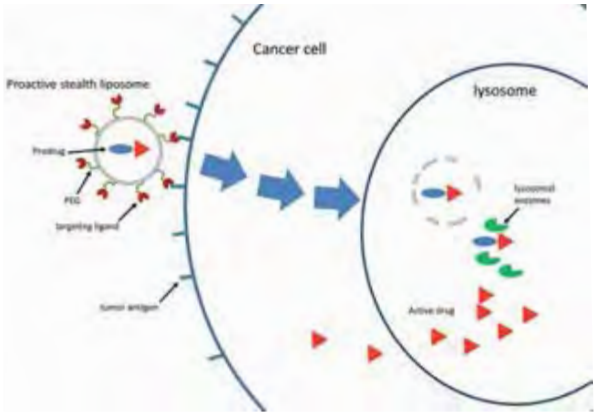


Figure 3. Proactive stealth liposomes. An anticancer prodrug is encapsulated in a PEGylated liposome. Targeting ligands (such as a single-chain antibody) are attached to the PEG chains to facilitate binding and endocytosis of liposomes into cancer cells. Liposomes are routed to lysosomes where prodrug is released after degradation of liposomal membranes. Prodrug is hydrolyzed by lysosomal enzymes to release active cancer drug for specific killing of cancer cells.

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THE GOOD THE BAD AND THE UGLY: CURRENT PRECLINICAL NANOMEDICAL STRATEGIES AGAINST DENGUE AND CHAGAS DISEASE

EDER LILIA ROMERO and Maria Jose Morilla
Nanomedicine Research Program. Quilmes National University, Roque Saenz Peña 352 Bernal, Buenos Aires, Argentina

The Chagas disease or American trypanosomiasis and the mosquito-borne Dengue fever affect 10 and nearly 400 million people worldwide respectively. Both infections are caused by the protozoan Trypanosoma cruzi and by a single positive-stranded RNA flavivirus. Currently, the only accepted drug against Chagas disease is the 2 nitroimidazole benznidazole-an extremely toxic and incapable of eradicating the intracellular amastigotes, responsible for the chronic cardiac cardiomyopathy and potential sudden death- antichagasic agent. There is no specific antiviral medication against Dengue and both diseases lack of preventive vaccines. In this presentation we offer an overview on the recent nanomedical approaches for the treatment and prophylaxis of these tropical neglected diseases. We will focus on the preclinical developments of tetravalent vaccination for Dengue as well as the recent strategies for antichagasic nano-drug delivery.

AN ECONOMICS PERSPECTIVE ON PERSONALIZED MEDICINE

MICHAEL ROSSBACH
Genome Institute of Singapore; rossbachm@gis.a-star.edu.sg

The concept of personalized medicine not only promises to enhance the life of patients and increase the quality of clinical practice and targeted care pathways, but also to lower overall healthcare costs through early-detection, prevention, accurate risk assessments and efficiencies in care delivery. Current inefficiencies are widely regarded as substantial enough to have a significant impact on the economies of major nations like the US and China, and, therefore the world economy.

Personalized medicine aims to use state-of-the-art genomic technologies, rich medical record data, tissue and blood banks and clinical knowledge that will allow clinicians and payors to tailor treatments to individuals, thereby greatly reducing the costs of ineffective therapies incurred through the current trial and error clinical paradigm.

Pivotal to the field are drugs that have been designed to target a specific molecular pathway that has gone wrong and results in a diseased condition and the diagnostic tests that allow clinicians to separate responders from non-responders.

However, the truly personalized approach in medicine faces two major problems: complex biology and complex economics; the pathways involved in diseases are quite often not well understood, and most targeted drugs are very expensive. As a result of all current efforts to translate the concepts of personalized healthcare into the clinic, personalized medicine becomes participatory and this implies patient decisions about their own health. Such a new paradigm requires powerful tools to handle significant amounts of personal information with the approach to be known as “P4 medicine”, that is predictive, preventive, personalized and participatory.

P4 medicine promises to increase the quality of clinical care and treatments and will ultimately save costs. The greatest challenges are economic, not scientific.

UPTAKE OF NANOPARTICLES INTO CELLS AND SKIN

E. RÜHL[1], C. Graf[1], Q. Gao[1], D. Nordmeyer[1],
V. Haucke[2], I. Schütz[2],
J. Lademann[3], A. Vogt[3], S. Ahlberg[3], M.C. Meinke[3], F. Rancan[3],
A. Jordan[4], N. Bohmer[4],
A.D. Gruber[5], L. Mundhenk[5], A. Ostrowski[5], C. Holzhausen[5]
[1]Physical Chemistry, Institute for Chemistry and Biochemistry, Freie Universität Berlin, Takustr. 3, 14195 Berlin, Germany
[2]Biochemistry, Institute for Chemistry and Biochemistry, Freie Universität Berlin, Takustr. 6, 14195 Berlin, Germany
[3]Department of Dermatology and Allergy, Charité-University Medicine Berlin, Berlin, Germany
[4]Project for Biomedical Nanotechnology (PBN), Charité – Universitätsmedizin, Berlin, Germany
[5]Institute of Veterinary Pathology, Freie Universität Berlin, Robert-von-Ostertag-Str. 15, 14163 Berlin, Germany

Recent results on the uptake of intentionally made inorganic nanoparticles into cells and skin are presented. These nanoparticles were prepared in narrow size distributions by colloidal chemistry approaches. They were functionalized by organic ligands so that their electrostatic stabilization and aggregation in biological media can be controlled.[1] Most successful for avoiding aggregation were functionalizations by N-(6-aminohexyl) 3-aminoproplytrimethoxy silane (AHAPS) ligands. It is shown that AHAPS functionalized silica particles (55± 2 nm) were readily and efficiently taken up by HeLa cells, so that their uptake mechanisms could be determined. Further uptake studies were performed on variable size functionalized silica particles ranging between 42±2 nm and 291±9 nm. Detailed uptake studies into HaCat cells as well as freshly isolated skin cells (keratinocytes and Langerhans cells) are reported, indicating particle size and cell type dependent uptake properties of nanoparticles.[2] Functionalized nanoparticles containing a superparamagnetic iron oxide core have been used to study the adsorption kinetics of plasma proteins by gel electrophoresis in greater detail.[3] It is shown that there is no exchange of a hard protein corona that binds rapidly to the particles. Furthermore, results from knockdown experiments are reported which provide detailed information on the cellular uptake process of nanoparticles. In addition, it is shown that the toxicity of nanoparticles depends critically on the phases of the cell cycle.

The uptake of variable size nanoparticles into skin is reported. Various uptake routes and transport through the uppermost horny layer (stratum corneum), epidermis, and dermis can be considered, such as intercellular and transcellular transport. In addition, the role of hair follicles for the uptake of nanoparticles will be discussed.[2]

Results from standard approaches, such as confocal laser scanning microscopy and electron microscopy, as well as X-ray microscopy are presented. X-ray microscopy has the specific advantage that it combines chemical sensitivity with high spatial resolution, so that detailed information on the uptake processes of nanoparticles into cells and skin is derived. [4]

Another advantage of X-ray microscopy is that entire cells without fixation can be investigated, permitting along with plunge freezing detailed tomography studies, so that 3-dimensional information on the location of nanoparticles within cells can be performed. These studies allowed us to identify those nanoparticles which can penetrate even into the cell nucleus. Finally, the importance of the stratum corneum providing a barrier against nanoparticle uptake is discussed in detail. Damage of this barrier has been induced by tape stripping, oxazolone-induced allergic contact dermatitis, and mechanical impact (pricking). The uptake of nanoparticles into damaged skin is reported, indicating that only pricked skin shows evidence for nanoparticle uptake into deeper skin layers. Perspectives for the use of the reported results regarding novel concepts of drug delivery in topical therapy of inflammatory skin diseases will be briefly discussed.

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CURRENT INITIATIVES IN JAPAN FOR NANO-MEDICINES

KUMIKO SAKAI-KATO
Section Head, Division of Drugs, National Institute of Health Sciences (NIHS), Ministry of Health, Labour and Welfare (MHLW)

Nanomaterials often have physical, chemical, or biological properties that are different from those of bulk materials. These properties may have potential impacts on a variety of products, and nanotechnology application to pharmaceuticals or medical devices is a typical such example. In Japan, nanomedicines have been regulated within a general framework of the Pharmaceutical Affairs Law on a product-by-product basis. In January,2014, Ministry of Health labour, and welfare (MHLW) has issued the Joint reflection paper with European Medicines Agency on the development of block copolymer micelle medicinal products which discusses principles for the pharmaceutical development, and non-clinical and early clinical studies of block-copolymer micelles.

Based on the "Science and Technology Basic Law" enacted in 1995, MHLW has been promoting applications of nanotechnology to therapeutics and diagnostics through the allocation of research grants and collaboration with other ministries. A new section was established at the National Institute of Health Sciences (NIHS) for conducting regulatory science researches on development of an evaluation strategy of nanomedicines from the standpoint of quality, efficacy and safety. Because the size-specific interaction with biological systems or biodistribution may have significant impacts on the efficacy and safety of nanomedicines, studies of the physicochemical properties, and nonclinical pharmacokinetics, pharmacodynamics, and toxicology have been conducted. These studies would contribute to identify which quality attributes of nanomedicines are critical to efficacy and safety.

Because research into the application of nanotechnology to pharmaceuticals is very active in Japan, regulators at MHLW, reviewers at Pharmaceuticals Medical Devices Agency, and government researchers at NIHS should keep up with the latest relevant scientific findings. Open discussion would be followed between industry, academia, and regulatory authorities about the appropriate regulation of nanomedicines, for enhancing the medical applications of this technology. International cooperation with other organizations would also be appreciated.

Based on these findings, research outcomes, and dialogue with industry and academia, MHLW is developing points-to-consider documents for the development and manufacturing of nanomedicines and exploring the possibility of future regulation of nanomedicines.

ENTRY OF NANOPARTICLES INTO CELLS: MECHANISMS AND CONSEQUENCES

KIRSTEN SANDVIG

Nanoparticles can be used as tools both in basic cell biology and to deliver drugs or other substances both in vivo and in vitro (1-3). To enter cells the particles exploit the endocytic machinery, and they may even induce changes in cellular uptake and intracellular transport (4,5). To optimize delivery into cells it is important to understand which cellular mechanisms that are involved in their uptake. Such information may help in deciding the type of particle to use, the size of the particle as well as which components to add at the surface of a particle. Today we know that cells have different types of endocytic mechanisms (6), some giving rise to small vesicles (60-200 nm diameter), whereas other endocytic mechanisms such as macropinocytosis can be involved in uptake of larger particles. Depending on the particle, it may even contribute to induce its own transport into the cell. Thus, knowledge about cellular mechanisms and correct interpretation of results obtained from cellular studies are essential. One should be aware of that cells growing in a polarized manner are likely to have different endocytic mechanisms which are under differential influence of signaling substances at the two poles (6), cholesterol is involved in several endocytic processes including macropinocytosis (6), and increased cell density may induce changes in membrane lipids and intracellular transport (7). Robust methods to determine whether a particle is internalized or only at the cell surface are important to provide the investigator with correct data about uptake efficiency. Furthermore, as internalized particles may induce unexpected changes in intracellular transport, detailed cellular studies are warranted.

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THE CLOCKS TICKING WITHIN OUR BODY

GOTTFRIED SCHATZ
Biozentrum, University of Basel; Gottfried.schatz@unibas.ch

All life on earth is controlled by internal biological clocks. This is also true of the human body which houses several distinct biological time-keepers. One of them is our circadian clock; it links our metabolism, our hormone status and our well-being to the rotation of planet earth. This clock is an oscillator measuring periodic events in the order of days. Other clocks control our development, our aging and our life span. These clocks resemble irreversible hour-glasses and are based on several different molecular mechanisms. Controlling these clocks, slowing them down or even stopping them altogether is a hotly debated issue of today's society.

MINDS: MICROVESICLE-INSPIRED DRUG DELIVERY SYSTEMS

RAYMOND M. SCHIFFELERS

Delivery of biological drugs is difficult, especially if they have an intracellular site of activity. Over the past years, extracellular vesicles have emerged as endogenous carriers of biological molecules. These extracellular vesicles are a heterogeneous class of nanosized particles consisting of a lipid bilayer. They are released by cells through various routes, from the cell membrane or intracellular organelles, constitutively or in response to triggers. In addition to the lipid bilayer they contain membrane proteins, cytoskeletal proteins and cytoplasmic proteins. Interestingly, these vesicles also contain a variety of nucleic acids, like mRNA, regulatory RNAs such as miRNAs and lncRNAs, and DNA fragments. Their composition reflects the cell of origin, but certain molecules are selectively enriched while others are depleted. Interestingly, the proteins and nucleic acids in the vesicles have shown to be functionally transferred from the producing cell to acceptor cells.
The purpose of the MINDS grant is to learn how extracellular vesicles achieve functional delivery. We are studying how they bind and interact with cells and by which routes contents are delivered intracellularly.
During execution of the grant a new instrument was introduced by the ERC to promote translation of findings to commercial exploitation: the Proof of Concept grants. In this work we identified market opportunities for our research. It turned out that, in particular, our ample experience with liposomes was valuable to introduce standard materials for analysis of extracellular vesicles that share the main structural element, the lipid bilayer. Up to then, the standards were composed of polystyrene beads which have completely different scattering behavior, biochemical characteristics and density compared to extracellular vesicles. This resulted in the foundation of EXCYTEX a company dedicated to develop tools for extracellular vesicle research.

TOXICITY OF ABRADED PARTICLES FROM AN EPOXY/CARBON NANOTUBE COMPOSITE

LUKAS SCHLAGENHAUF[abc,] J. Buhab[c], T. Buerki-Thurnherr[d], P. Wick[d], F. Nüesch[a] and J. Wang[bc]
[a]Laboratory for Functional Polymers, EMPA, Dübendorf, Switzerland
[b]Laboratory for Analytical Chemistry, EMPA, Dübendorf, Switzerland
[c]Institute of Environmental Engineering, ETH Zurich, Switzerland
[d]Laboratory for Materials-Biology Interactions, EMPA, St. Gallen, Switzerland

Epoxy/carbon nanotube (CNT) nanocomposites exhibit excellent mechanical properties. Compared to the pure epoxy, they have additional properties like electrical conductivity and thermal conductivity. They are used for applications where low weight, high strength, and high conductivity are required. The potential hazard of such materials has become a significant concern to researchers, manufacturing industries and customers. The exact environmental and health impact of nanoparticles released into the ambient air either during manufacturing or in applications is still unknown and a matter of debate. To estimate the hazard potential of epoxy/CNT nanocomposites, a project was started at the Swiss Federal Laboratories for Materials Science and Technology (EMPA) that addresses the release of particles during abrasion and their potential acute cytotoxic effects.
Recently, we could show that free standing CNTs were released from epoxy/CNT nanocomposites during abrasion (Schlagenhauf et al. 2012). The released particles have now been further characterized and in vitro cytotoxicity tests have been carried out. For the first time, the toxicity of abraded particles from a nanocomposite, whereof release of the nanofiller has been observed, will be presented. Tests with human alveolar epithelial cells A549 and human monocytic cells THP-1 were conducted. After treatment of the cell cultures, cell viability, formation of reactive oxygen species, pro-inflammatory response, and DNA damage were investigated.

L. Schlagenhauf et al. Release of Carbon Nanotubes from an Epoxy-Based Nanocomposite during an Abrasion Process, Environ. Sci. Technol. 2012, 46, 7366-7372

PERSONALIZED CANCER NANOMEDICINE

SIMO SCHWARTZ

It has been hypothesized that drug delivery by nanoparticles may well circumvent the resistance machinery of cancer stem cells (CSC). To be able to study efficacy of nanomedicines in population of CSC, we first developed an in vitro model in which CSC are tagged by a fluorescent reporter gene under the control of a CSC specific promoter. Using this system, we demonstrated that while bulk cancer cells die, CSC population augments after paclitaxel (PTX) treatment. We then investigated the prospects of different targeted and non-targeted delivery systems loaded with PTX and functionalized with specific antibodies against cancer stem cell populations in regular breast cancer cell lines, as well as in our CSC models. Our data shows that reducing tumor resistance of cancer stem cells might be related to specific active targeting of DDS and not attributed to a general mechanism of action of nanomedicines.

NANO--FORMULATED ENZYMATIC BASED CHEMO-ADJUVANTS

PABLO SCODELLER, Kazuki Sugahara, Erkki Ruoslahti
Sanford-¬-Burnham Medical Research institute, La Jolla California.
Email: pscodeller@sanfordburnham.org

We describe a new biocomposite material as a targeted, enzymatic-¬-based chemoadjuvant. Fluorescent, 130nm sized silica particles were synthesized following a published modified Stober synthesis. A Silane-¬-Fluorescein (Fitc) conjugate in the core is protected by a shell of Silica matrix1 providing bright and photostable particles. These colloidal surfaces were modified with the enzyme Hyaluronidase (Hyal) using the layer-¬-by-¬-layer technique for derivatizing with enzymes and preserving catalytic activity2. For that, first a monolayer of positively charged Poly(allylamine) (PAH) was self assembled onto the negatively charged silica particles; followed by adsorption of the enzyme Hyal which at water pH is a polyanion. These deposition steps were followed by Zeta potential.
Hyaluronidase is the hydrolase that cleaves Hyaluronic acid (HA). In many tumors, HA is highly overexpressed and because high molecular weight HA binds water so tightly, this HA overexpression poses a resistance to the flow of liquid, impeding the diffusion of drugs to the tumor. Hyalorunidase is used as a spreading factor for oncolytic drugs because it degrades high Mw HA to small molecular weight and solvatable oligosaccharides3.
The Hyal modified particles were characterized using Dynamic Light Scattering, Zeta potential, TEM, UV and Infrared Spectroscopy, Hyal enzymatic activity assay, and fluorescence emission spectra.

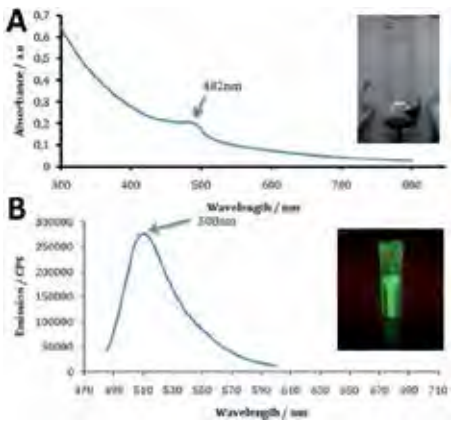
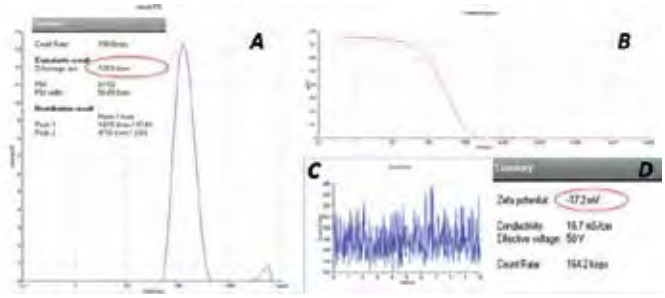


Fig 1. Absorption (A) and emission spectra (B) of Fitc-¬-SiNp in PBS. Emission spectrum was collected with 490nm excitation. Emission peak of Fitc-¬-SiNp shows a 4nm blue shift respect to free Fitc in solution, indicating encapsulation of dye.

Fig 2. Dynamic light scattering and Zeta potential characterization of Hyal/FitcSiNP. Hydrodinamic radius of Hyal/SiNp showing particle diameter of 129nm (A) and the corresponding autocorrelation function for that determination (B). A stable photon count rate (C) with time is indicative of particle stability. (D) Shows a negative Zeta potential for these Hyal/FitcSiNp. All measurements are taken in PBS buffer.



Particles were injected intraperitoneally in a Luciferase modified mouse model of human gastric cancer (MKN45P), a model that develops many metastasic nodules in the peritoneal cavity. Two hours after injection of particles, fluorescence is observed only in the tumors and not the control organs. Besides, cross sections, and also tumor sections imaged with fluorescence microscopy, show green signal deep within the tumor and not the control organs, suggesting deep tumor-¬-selective penetration of these HA degrading nanostructures.

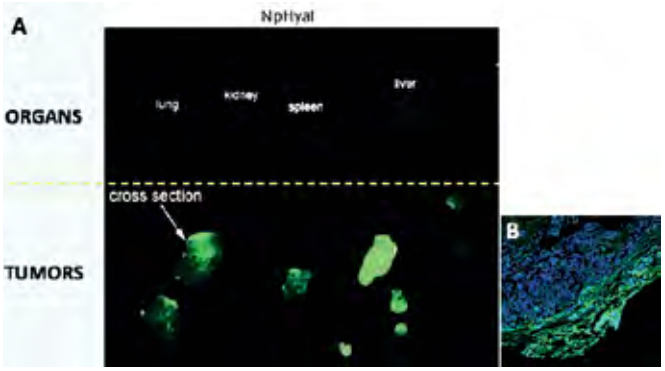


Figure 3. Hyal/FitcSiNp selectively accumulate and penetrate in tumors. (A): fluorescence image in the green channel: all tumors show green signal (bottom panel, A) while none of the control organs show any signal (top panel, A). (B) Fluorescence microscopy on a section of one of the tumors from panel A (green signal is native Hyal/FitcSiNp fluorescence, and blue is DAPI staining). In bottom panel A note the signal coming deep within the tumor in the cross section.

When injected intraperitoneally, controls with bare FitcSiNp particles showed no binding of particles to tumors, and heat inactivated Hyal/FitcSiNp showed less binding and no deep tumor penetration of particles, compared to Hyal/FitcSiNp. Also, when these same Hyal/FitcSiNp were injected intravenously no particle homing was observed in the tumors.
Staining with Alcian Blue (typical stain used for HA detection) of tumors and control organs revealed intense HA overexpression in the rim of the tumors and not so in the organs.

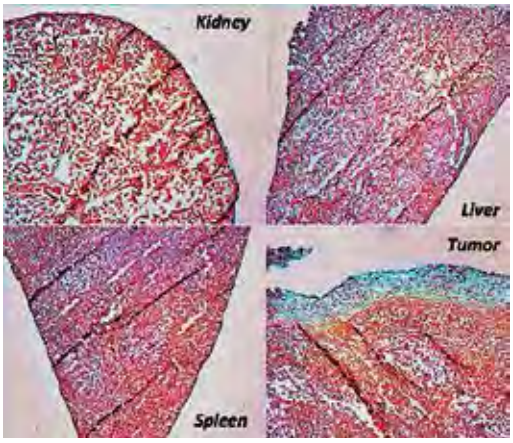


Figure 4. Alcian Blue staining reveals extensive Hyaluronic acid overexpression in the rim of the tumor and absence of HA in the other organs.

Also, iRGD, a known tumor penetrating peptide4, was evaluated as an additional spreading factor.
Substrate-¬-enzyme interactions between Hyal and HA in the rim of the tumor are speculated to be responsible for this tumor homing feature of Hyal/FitcSiNP when these are injected intraperitoneally and experiments are now under way to demonstrate this.
This new, targeted chemoadyuvant could signify a major improvement of current intraperitoneal chemotherapy used in the clinic.

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UNTAPPED NEW PHYSICAL METHODS FOR DIAGNOSTIC APPLICATIONS AND DEPOSITED PATENT ON HOW TO COUNT AND CHARACTERIZE CTCS

GIACINTO SCOLES
Prof. Dr., Nanotechnology for Medicine Laboratory, University Hospital and Department of Medical and Biological Sciences, University of Udine, Udine (I)

I will briefly but understandably review two breakthroughs that were achieved recently in our laboratories. In the first I will show how it is possible at present to detect, using oscilllating pillars, sub picomolar concentrations of PSMA (that in contrast with PSA is thought to be a reliable biomarker for prostate cancer) using the micromechanical analog of a sandwhich Elisa test in which an selective primary antibody is bound to the surface of a pillar while a Secondary Antibody COnjugated to a MAssive Nano-particle (SACOMAN) makes the frequency of the pillar measurably change when ten to hundred SACOMANs recognize the protein layer that has docked into the first Ab. In the seciond I will show how it is possible to detect Circulating Tumor Cells (CTCs) using a mcrofluidic device in which the cells, obtained from the blood of patients, and embedded in a flowing stream of microdroplets are detected via the acidity generated into the droplets by the cells after they are expose to various sugar concentrations measured via laser fluorescence. We have already showed that cells from cell lines behave as expected. But we have also collected expected evidence that WBC from a healthy blood have a minority population that produces relatively high values of acidity (false positives) and we are in the process to evaluate the possibility to treat the false cells to get rid of the acidity while leaving the cancer cell intact.The results of the latter series of tests will be reported at the meeting. (Work in collaboration with F. Del Ben, M. Turetta, D. Cesselli UniUD, W. Huck and A. Piruska, Radboud U.)

WHAT ARE THE THREE KEY OPTIONS OF YOUR REGULATORY SYSTEM TO BE PUT ON THE TABLE TO SUPPORT THE SUCCESS OF NANOMEDICINE?

HRIPSIME SHAHBAZIAN
Health Canada

INTRODUCTION
Nanomaterials (NMs) are increasingly being used in the marketplace in a wide range of products and substances that Health Canada is responsible for regulating. It is recognized that nanomaterials exhibit unique physical and chemical properties which can be exploited for improved therapeutic benefits; however, these unique properties may lead to unanticipated behaviors. Health Canada acknowledges that new approaches may be necessary for risk assessment and risk management of nano-based health products to keep pace with advances in this area as there is inadequate information on risks associated with nanomaterials at this time.

CURRENT REGULATORY APPROACH
Health Canada relies on authorities within existing legislative and regulatory frameworks, which require the assessment of potential risks and benefits of products to the health and safety of Canadians before they can be authorized for sale. All health products, including those that contain nanomaterials are regulated by the Food and Drugs Act, and associated Regulations. Nanomaterial containing products are subject to the same rigorous health and safety regulations that apply to conventional health products. New health products can be sold in Canada once they have successfully passed a review process to assess their safety, efficacy and quality.

IDENTIFICATION OF PRODUCTS THAT CONTAIN NANOMATERIALS
To identify regulated products and substances that may contain nanomaterials and to ensure a consistent approach across diverse regulatory programs Health Canada developed a general working definition which is described in the Policy Statement on Health Canada's Working Definition for Nanomaterial. The working definition is relevant for all products and substances regulated by Health Canada. The Policy Statement on Health Canada's Working Definition of Nanomaterials was adopted on October 6, 2011.1 The "Working Definition" is intended to be used as a tool to help the Department gather safety information about nanomaterials to improve the understanding of nanomaterials in its risk assessment and risk management activities. It enables the Department to establish internal inventories, to ask for additional information, and to integrate new knowledge into regulatory decision making processes.

TRACKING OF PRODUCTS THAT CONTAIN NANOMATERIALS
To facilitate identification and tracking of nanomaterial containing drug submissions Health Canada released a revised Drug Submission Application Form for Human, Veterinary, Disinfectant Drugs and Clinical Trial Application/Attestation (HC/SC 3011).2 The form asks the sponsor to self-identify when their application concerns a nanomaterial or 'nano-product'.

TRANSPARENCY AND VISIBILITY
To add visibility and transparency, the HPFB created a nanotechnology webpage entitled Nanotechnology-Based Health Products and Food.3 The webpage outlines applications of nanotechnology, and provides general guidance to stakeholders regarding health products containing nanomaterial. It advises sponsors and other stakeholders to communicate with responsible regulatory areas early in the development process if their products contain or make use of nanomaterial and provides examples of the type of information that may be required for a nanotechnology-based product's safety assessment.

CHALLENGES
Currently there are no Health Canada guidance documents specific for nano-based health products. Maintaining a flexible approach is important to integrate new knowledge about risks and benefits related to nanomaterials into regulatory decision-making processes.
Health Canada believes that, in general, its current risk assessment methodologies are applicable for nanomaterials as they allow for sufficient flexibility. To address unique physical, chemical and biological properties of nanomaterials each product is assessed on a case-by-case basis.
Health Canada encourages sponsors and other stakeholders to communicate with the responsible regulatory authority early in the development process, especially for combination products that are, contain or make use of nanomaterials. In order to identify and assess potential risks and benefits of nanotechnology based health and food products, the Department encourages manufacturers to request a pre-submission meeting with the responsible regulatory authority to discuss type of information that may be required for their product's safety assessment.

Health Canada is continuing to monitor the emerging scientific studies to ensure appropriate action is taken should any substantial safety concerns be identified. Health Canada reserves the right to request information, material or define conditions in order to allow the Department to adequately assess the safety, efficacy or quality of a therapeutic product.

INTERNATIONAL COOPERATION

There are many regulatory and scientific issues that must be addressed before nano-based health products are authorized for sale in Canada. The state of science around nanomaterials is evolving. Joint efforts are needed to accelerate the achievements promised by Nanotechnology.

Health Canada continues to work closely with domestic and international partners toward consistency with relevant international norms, to ensure application of sound regulatory practices and standards which are consistent, whenever possible, with international norms, strengthen and facilitate existing mutual cooperation with international jurisdictions in scientific and regulatory areas; address the challenges of globalization, new technologies and timely approval of new medicines and reduce risks associated with therapeutic products marketed in Canada. request a pre-submission meeting with the responsible regulatory authority to discuss type of information that may be required for their product's safety assessment.

IMPLANTABLE SIRNA DELIVERY SYSTEM - TREATMENT FOR PANCREATIC CANCER, PHASE 2

AMOTZ SHEMI

RNAi-based medicine presents a huge promise for many therapeutic areas. However, effective delivery of the RNAi-based drugs to the target tissue still remained a major challenge. Local and prolonged delivery of siRNA is a viable solution for many indications, specifically for solid tumors. Pancreatic cancer (PC) is an aggressive disease that leads to a high mortality rate. Over 90% of patients carry a mutation in K-Ras to which the tumor is addicted to. We developed an implantable system for controlled regional drug delivery, by designing a miniature biodegradable polymeric matrix that encompasses an anti-KRASG12D siRNA drug, named siG12D LODER™. The LODER™ prevents siRNA degradation in-vivo along months, and releases the drug regionally within a pancreatic tumor during four months. Treatment of pancreatic cells with siG12D LODER™ resulted in a significant inhibition of KRAS mRNA and reduction in its protein levels. Decrease of KRAS inhibited cell proliferation and reduced EMT inducing protein levels. In vivo, in mice implanted with siG12D LODER™ the growth of human PC cell lines was retarded, the survival was significantly improved, and development of new metastasis was halted. Evidences from histology analysis showed drug distribution throughout the tumor in a typical rate of one mm per day. We completed a phase I clinical study with the siG12D LODER™, which was implanted into patients with locally advanced PC by using a standard endoscopic ultrasound device. The results of the clinical study show a high safety profile. Moreover, patients had an extending overall survival and a retardation of tumor progression. Multinational controlled Phase II clinical trial (NCT01676259) is now in progress. Next generations LODERs in which we have incorporated novel nano-technologies are in development, tailored to the specific requirements of tumors in cancers such as prostate and brain.

PREDICTION OF NANOPARTICLE DISTRIBUTION THROUGH PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELLING

MARCO SICCARDI, Darren Moss, Rajith Rajoli, Marco Giardiello, Tom McDonald, Neill Liptrott, Phil Martin, Steve Rannard, Andrew Owen

Favourable pharmacokinetics of therapeutic agents can be hindered by several factors, such as poor absorption, low penetration into target tissues and high metabolism rate. Nanotechnology is currently being utilised to improve drug delivery. However, the broad spectrum of available nanoformulations means that a large, almost overwhelming, range of delivery strategies are available for research and potential future application. Polymers can be used as carriers for therapeutic agents, encapsulating drugs through the construction of vehicles such as liposomes, micelles and nanoemulsions . The direct conjugation of drugs to polymers has been successfully used to enhance circulatory times and a wide variety of inorganic oxides have been used to create nanoparticles, such as gold, silver, silica and iron. Understanding the interactions between nanoparticles and the human body is of central importance to the engineering of future materials. Numerous molecular and physiological processes mediate the pharmacokinetics of nanoparticles and the absorption, distribution, metabolism and elimination (ADME) properties of nanoparticles often differ greatly from traditional formulations (figure 1). The effects of chemical composition and other nanoparticle properties on the pharmacokinetics are clearly significant, but large and essential knowledge gaps exist. An innovative pharmacological approach for predicting nanoparticles distribution and to inform their design is represented by physiologically based pharmacokinetics (PBPK) modelling, which has already found widespread application for traditional formulations.

PBPK modelling is a bottom-up technique which simulates pharmacokinetics by combining system data describing a population of interest (e.g. demographics, physiology, anatomy and genetics) with experimental in vitro data (e.g. intestinal permeability, formulation release rate, protein binding, macrophage uptake) through a mathematical description of ADME. Such modelling gives a mathematical description of all the physiological and anatomical processes involved in nanoparticle distribution, offering the opportunity to identify important determinants of pharmacokinetics. The PBPK models used for simulating nanoparticle pharmacokinetics include specific nanoformulation characteristics and consequently appropriate algorithms and modelling strategies are required.

A novel PBPK model has been developed, simulating the application of solid drug nanoparticle (SDNs) for sustained release formulations for parenteral administration of anti-HIV drugs. Injectable sustained release nano-formulations of anti-HIV drugs represent a viable pharmacological option to simplify treatments, reduce therapy costs and improve patient adherence. Following intramuscular injection of SDNs into the depot, the active agent is released at a controlled rate into the tissue fluid and then has to traverse the interstitium to reach the blood capillaries. The PBPK model has been applied to simulate this process and assess the suitability of existing anti-HIV drugs for intramuscular administration. Moreover, the theoretical target dose and release rate from the SDN depot for once monthly administration were hypothesised for each drug. The dose of anti-HIV drug and release rate were adjusted to obtain plasma concentrations above therapeutic cut-offs to effectively inhibit viral replication. Additionally, a microdialysis system to experimentally measure the release rate of anti-HIV drugs from the SDNs has been developed. This experimental approach allows the quantification of the release rate in simulated interstitial fluid and therefore complements the PBPK model.

There is an urgent need for a rational approach to nanoparticle design and PBPK models can assist in answering questions that cannot otherwise be examined in pre-clinical development. Additionally, property–distribution relationships can be integrated in PBPK models, giving the opportunity for a rational nanoparticle design, identifying further strategies to maximise the efficiency and safety of novel nanotechnologies. The application of PBPK modelling will benefit our understanding of the mechanisms underpinning nanoformulation ADME and favour a more rapid and accurate determination of their kinetics.

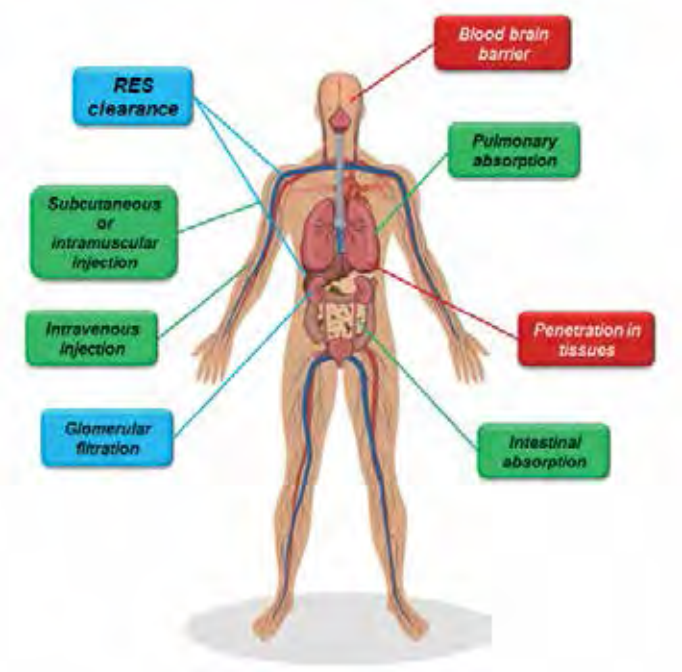

Figure 1. Relevant absorption (green boxes), distribution (red boxes) and elimination (blue boxes) processes regulating the pharmacokinetics of nanoparticles

BIODEGRADABLE VERSUS NON-DEGRADABLE NANOPARTICLES: TOXICOLOGY AND COST OF DEVELOPMENT

TORE SKOTLAND
Centre for Cancer Biomedicine, The Norwegian Radium Hospital, Oslo University Hospital and University of Oslo, Norway

There are huge expectations for the use of nanoparticles (NPs) to deliver therapeutics and for imaging of different diseases, such as cancer. Carefully designed experiments, both in vitro and in vivo, are essential in order to fully explore this technology. Despite many promising NPs being made during recent years, the biological studies performed with such NPs very often do not have the quality needed to support the conclusions drawn (1,2,3). There is a huge need for more interdisciplinary collaboration to improve the quality of such studies.

With a long experience from pharmaceutical R&D, I will discuss improvements that should be made in biological studies with NPs. The design of animal studies, including which time points to take samples and which parameters to analyze, is critical when aiming at developing drugs for clinical use (1). Biodistribution, metabolism and excretion studies are extremely important not only to generate such data (e.g. for an imaging agent), but also to evaluate safety and to predict whether it is likely that the NPs studied ever can receive market approval for clinical use (3).

It is of utmost importance that NPs made of non-endogenous substances are degraded and excreted. The impact of having biodegradable versus non-degradable NPs on toxicity studies, cost of development and the risk/benefit analyses one can expect pharmaceutical companies to perform will be discussed (3).

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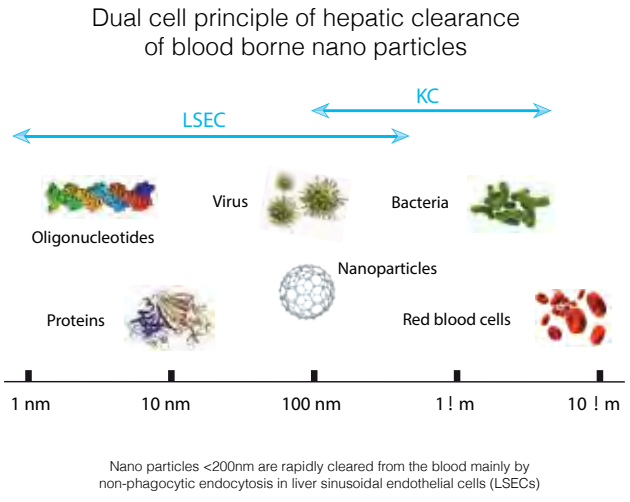
ROLE OF SPECIALIZED HEPATIC SCAVENGER CELLS IN CONTROL OF BIODISTRIBUTION OF BIOLOGICS AND NANOPHARMACEUTICALS

BÅRD SMEDSRØD[1,2], Kjetil Elvevold[2], Britt Fuglesteg[2]
[1]Dep Medical Biology, University of Tromsø, Norway; [2]D'Liver, Tromsø, Norway

In spite of the great therapeutic potential of biologics and nano delivery systems, and the increasing amount of resources that are being spent on their development, the progress in this field has been limited when it comes to biodistribution control. When developing biologics and nano therapeutics a major goal has been to produce compounds that are able to target the cell type where the wanted therapeutic activity is to be delivered. A great part of the preclinical work to establish a functional drug compound of this category has been to test interaction and uptake of the compound in cultured cells. However, subsequent testing in animal models have often resulted in the disappointing observation that the compound disappear by uncontrolled rapid uptake in the liver. Chemical modification may sometimes lower the liver uptake of the compounds. However the same modification may also result in a reduced ability of the compounds to target the tissue in which the therapeutic activity is to be delivered.

To solve the challenge of uncontrolled liver clearance of biologics and nano delivery systems it is necessary to understand the mechanism(s) of uptake. Blood borne compounds, including biologics and nano particles <200 nm, are cleared mainly by non-phagocytic uptake by liver sinusoidal endothelial cells (LSECs), employing specialized endocytosis receptors, whereas larger nano particles (>200 nm) are taken up by phagocytosis in the liver macrophages, or Kupffer cells, KCs (Am.J.Physiol 2012,303:R1217). Hence, to understand the hepatic uptake of nano particles it is important to realize that size matters (Fig 1). Representing only a few percent of the liver mass, these extremely active scavenger cells line the minute liver capillaries (termed liver sinusoids) that make up the vasculature of the liver. Since nano particles of practically any type are thus prevented from getting in direct contact with the liver parenchymal cells, false results will be obtained if including only hepatocytes in ADMET assays monitoring liver uptake and metabolism of nano particles. In conclusion, a detailed knowledge about the scavenger function of hepatic scavenger cells and their receptors are a prerequisite to design strategies to avoid unwanted liver uptake of biologics and nano pharmaceuticals. The same knowledge is required to understand the mechanism of hepatotoxicity caused by administration of nano particles.



Nano particles <200nm are rapidly cleared from the blood mainly by non-phagocytic endocytosis in liver sinusoidal endothelial cells (LSECs)

FINDING THE BALANCE BETWEEN INNOVATION AND BENCH-TO-BEDSIDE TRANSLATION IN HIV THERAPY

ALEJANDRO SOSNIK
Group of Pharmaceutical Nanomaterials Science, Department of Materials Science and Engineering, Technion-Israel Institute of Technology, Technion City, 32000 Haifa, Israel
E-mail: alesosnik@gmail.com, sosnik@tx.technion.ac.il

A recent report on the global situation of the Human Immunodeficiency Virus (HIV) indicates that about 35-40 million infected people live worldwide. The High Activity Antiretroviral Therapy (HAART), a combination of at least three antiretrovirals (ARVs) that tackle the replication cycle of the virus in different stages made the infection manageable. However, low patient compliance remains one of the greatest challenges to control the progress of the infection towards the active phase, the Acquired Immunodeficiency Syndrome (AIDS). Over the last years, we have dedicated efforts to investigate diverse technologies (e.g., polymeric micelles) to improve the therapy of the disease. Protease inhibitors (PIs) are a group of ARVs that inhibit the activity of the viral protease. They usually exhibit low aqueous solubility that leads to a poor oral absorption and low bioavailability. In addition, they display relatively short half-life. Thus, to maintain therapeutic concentrations in plasma, frequent administration schedules are demanded what usually leads to low patient compliance and eventually to treatment cessation. To improve the biopharmaceutical performance of PIs, we have developed a novel pure drug Nanoparticle-in-Microparticle Delivery System (NiMDS) employing indinavir free base (IDV) as model drug. Pure drug nanoparticles were initially produced by nanoprecipitation or a supercritical anti-solvent method and encapsulated within alginate/chitosan microparticles that were finally film-coated with a mucoadhesive and water insoluble poly(methacrylate). After the through characterization of the systems in vitro, the oral pharmacokinetics was assessed in mongrel dogs employing an IDV single dose of 10 mg/kg. A dramatic increase of the oral bioavailability and the half-life for both IDV nanoparticles and IDV-loaded NiMDSs was observed with respect to the unprocessed drug. Furthermore, NiMDSs supressed the burst effect usually associated with drug toxicity. Overall findings support the potential of this platform to reduce the dose and the frequency of administration and the associated medication costs, improving the HIV therapy and potentially patient affordability. First, the most urgent needs faced in the treatment of HIV will be overviewed. Then, this novel technology will be discussed in the context of the challenges faced to transfer innovative pharmaceutical products for poverty-related diseases to the market.

Acknowledgements: The author thanks the European Union's - Seventh Framework Programme under grant agreement #612675-MC-NANOTAR.

EXTRACELLULAR VESICLES AS POTENTIAL BIOMARKERS AND THERAPEUTIC TARGETS IN CARDIOVASCULAR DISEASE

EWA STĘPIEŃ[1,2]
[1]Department Clinical Biochemistry, Faculty of Medicine, Jagiellonian University Medical College Krakow, Poland
[2]Department of Medical Physics, Faculty of Physics, Astronomy and Applied Computer Sciences, Jagiellonian University Medical College Krakow, Poland

MICROVESICLES CHARACTERISTICS

The term "extracellular vesicles" defines a large group of different membrane subcellular structures and extracellular bodies, varied in diameter from 25 nm to 1 µm [1,2]. This heterogenous population of vesicles we can divide into at least 3 divers subclasses: Ex -exosomes (the smallest ones), MPs - microparticles and apoptotic bodies (over 1 µm in size). Whilst Ex are produced in the endocytic-lysosomal system and released from cell by the fusion of multivesicular bodies (MVB) with plasma membrane, MPs are formed in the process of cell membrane shedding (Fig. 1). In vascular system, endothelial dysfunction (an encompassing term for a shift from healthy to stressed/damaged endothelium) is usually accompanied with the release of pro-coagulation and pro-inflammatory MPs.

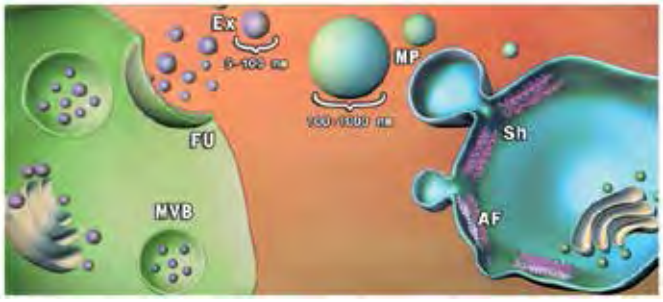


Figure 1. Exosome and microparticle formation by stimulated cells.

Microvesicles are abundantly present and widely distributed in every kind of body fluids: blood, cerebrospinal fluid, saliva, urine, uterine fluid, breast milk, were they can be easily collected and identified. In cardiovascular patients, as well as in healthy subjects, the most abundant peripheral blood microvesicles are platelet derived microparticles - PMPs, representing 70 to 90% of all circulating MPs [3]. As well, vascular endothelial and blood cells (lymphocytes, macrophages) release MPs, in response to cell activation, stress (hypoxia) or as a result of cell apoptosis (Figure 2). By stimulating endothelial cells in vitro, with numerous cytokines and apoptotic stimuli, we can suspect how the mechanism of their formation runs in vivo, whereas, by their isolation from peripheral blood and their characterization we can suspect what is their biological impact on various disorders [4].

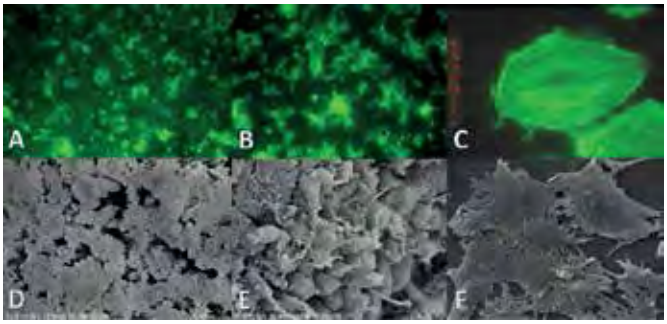


Figure 2. Formation of microparticles (MPs) by platelets and endothelial cells documented by fluorescence and scanning electron microscopy (SEM). F-actin staining of relaxed (A) and stimulated platelets (B) shows cytoskeleton reorganization during microparticle release. SEM images show that activated platelets form protrusions which are the source of MPs. Endothelial cells form MPs during membrane shedding process, where actin remodeling is also observed (C). Finally MPs are formed as cell vesicles, released from a cell surface (F).

VISUALIZATION OF PERIPHERAL BLOOD MICROPARTICLES IN CARDIOVASCULAR PATIENTS

A variety of methods can be applied in microparticles research. The method of choice is flow cytometry. Despite its limitations - many microparticles are below the limit of detection due to their low diameter and refractive index – this method is widely applied in clinical diagnostics of stroke, myocardial infarction or acute coronary syndrome (Figure 3) [3].

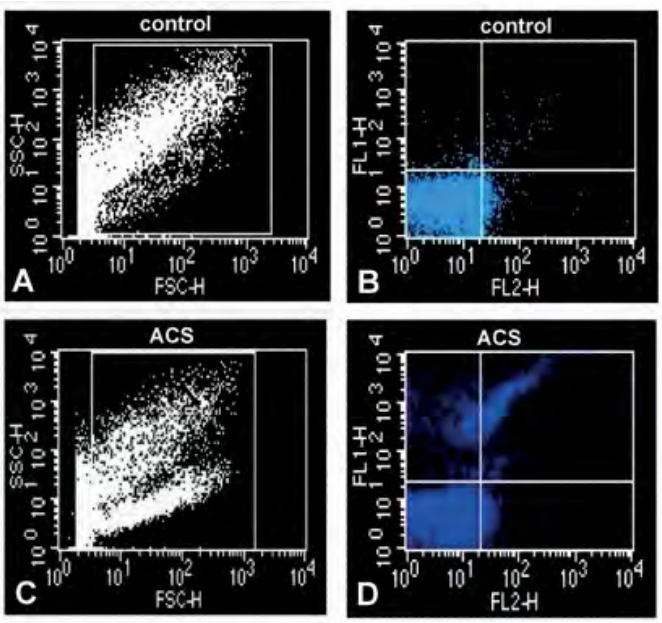


Figure 3. Representative dot plot of circulating microparticles (MPs) in a patient with acute coronary syndrome (ACS) (A) and in a control voluntary (C). MPs were initially gated by forward (FCS-H) and side scatter (SSC-H) in logarithmic scale; fluorescence plots show MPs binding of annexin V-FITC (FL1-H) and anti-CD142-PE (FL2-H) monoclonal antibody (B,D).

The method of choice is Nanoparticle Tracking Analysis (NTA) which provides a new tool for microparticles sizing (Figure 4).

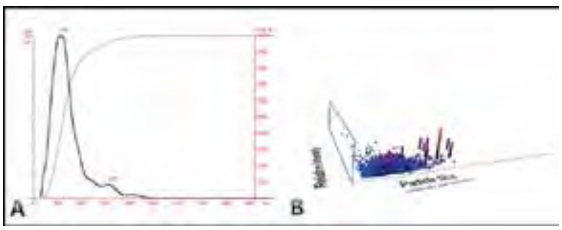


Figure 4. Microparticles sizing in plasma separated from a patient with advanced coronary disease. Two populations of microvesicles are observed: ~ 100 nm and ~ 300 nm in size (A). The relative number of larger MPs is low (B).

CLINICAL SIGNIFICANCE OF MICROPARTICLES RESEARCH

Grooving clinical and scientific interest of MPs is due to their potential application as biomarkers for diagnostic and prognostic purpose as well as therapeutic applications. They participate in the horizontal intercellular communication, and they can transfer a variety of biologically active molecules including proteins, lipids and micro-RNA [5]. Pro-coagulation role of MPs has been proved in myocardial infarction (MI) and their role in modified fibrin clot structure should be considered (Figure 5). [6].

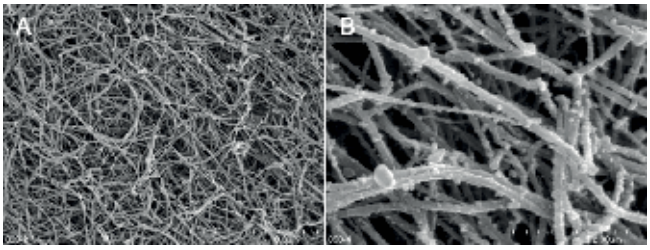


Figure 5. SEM images of clots formed form plasma obtained from a patient with myocardial infarction (MI). Dense structure of clot is visible (A); a higher magnification image shows that fibrin fibers are incrusted with numerous small sized MPs (B).

CONCLUSIONS

The impact of MPs on coagulation, inflammation and vascular function seems to be undeniable and has made them important targets in cardiovasculat disease diagnostic and treatment. Precise knowledge of their release and activity in vivo may help to identify patients with increased cardiovascular risk in the future and perhaps apply appropriate therapies before acute complications occur.

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LIPOSOMAL NANOMEDICINES TO TREAT MULTIPLE SCLEROSIS

G. STORM[1,2], Bart Metselaar[2], Jens Schmidt[3] and Ralf Gold[3]
[1]Dept. Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, PO Box 80082, 3508 TB Utrecht, The Netherlands. Email: G.Storm@uu.nl
[2]Dept. Targeted Therapeutics, MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, Enschede, The Netherlands
[3]Dept. Neurology, Clinical Research Group for Multiple Sclerosis, University of Wutzburg, Wurzburg, Germany

Multiple sclerosis (MS) is one of the most common inflammatory disorders of the CNS. Its pathological hallmarks are demyelination and cellular infiltration of T cells and macrophages. The most favoured pathophysiological hypothesis includes a T cell-dominated autoimmune reaction. Despite long-term immunotherapy, relapses occur, which are commonly treated by repeated i.v. injections of high dose (pulse) glucocorticosteroids. The main goal is to prevent ongoing tissue destruction with loss of oligodendrocytes, axons and neurons leading to permanent functional deficits. In MS, a high dose i.v. pulse therapy with 10 mg/kg methylprednisolone for 3-5 days is the standard regimen in relapse therapy. The aim of this presentation is to show the positive effects of long-circulating prednisolone-phosphate liposomes in animal models of MS. The objective of drug targeting with this formulation is to achieve high tissue levels of drug in the inflamed target area along with a reduction of side unwanted side effects, as compared with an equivalent dose given as free drug. If available, early clinical experience will be shared.

PITFALLS AND PROMISES IN TRANSLATING NANOMEDICAL TREATMENT FROM EXPERIMENTAL TO CLINICAL ATHEROSCLEROSIS

ERIK STROES
Vascular medicine, AMC

BACKGROUND

Atherosclerosis is the predominant pathological process leading to cardiovascular disease. Three major pillars are considered to contribute to atherogenesis, namely dyslipidemia, inflammation and

lipoapoptosis. Non-invasive imaging modalities such as MRI and PET/CT provide valuable atherosclerosis biomarkers for testing novel agents directed against plaque formation in cardiovascular trials. We tested the efficacy of a liposomal nanoparticle encapsulating prednisolone (LN-PLP) in vitro, experimental animal models and patients with atherosclerosis.

METHODS AND RESULTS

In patients, we observed a favourable pharmacokinetic profile of LN-PLP with an increased half-life (t½ 45-65hrs) facilitating delivery in atherosclerotic lesions. In patients undergoing endarterectomy due to symptomatic peripheral artery disease, LN-PLP delivery could be achieved in >75% of the macrophages isolated from excised plaque tissue following LN-PLP infusion. When testing anti-inflammatory efficacy, however, no anti-inflammatory effect was observed following LN-PLP treatment in CV-patients (target-to-background-ratio from 1.78 ± 0.31 to 1.90 ± 0.38 on PET/CT; p = 0.03 and Ktrans from 96±17 to 116±13 10-3min-1 on DCE-MRI; p = 0.20). Subsequent in vitro and animal data show that the lack of a beneficial effect relates to the induction of lipo-apoptosis in the lipid-rich micromilieu of the atherosclerotic plaque.

CONCLUSION

This study shows successful local delivery of a liposomal compound, prednisolone, into plaque macrophages in CV-patients. The absence of an anti-inflammatory effect of LN-PLP in plaques is shown to be caused by the induction of lipo-apoptosis in a lipid-rich environment. These data advocate rigorous assessment of the effect of future anti-inflammatory compounds in a lipid-rich environment prior to its evaluation in CV-disease in the clinical setting.

REENGINEERING-CANCER: RE-ENGINEERING THE TUMOR MICROENVIRONMENT TO ALLEVIATE MECHANICAL STRESSES AND IMPROVE DRUG DELIVERY

TRIANTAFYLLOS STYLIANOPOULOS

Tumor growth in the confined space of the host tissue results in the generation of physical forces. These forces are high enough to compress and eventually collapse fragile intratumoral blood and lymphatic vessels. Blood vessel collapse compromises tumor perfusion and induces an acidic and hypoxic micro-environment. Furthermore, compromised perfusion hinders the delivery of blood-borne therapeutic agents to the tumor site, and thus, reduces the efficacy of drugs. Recently, we introduced a new therapeutic strategy, which we refer to as the "stress-alleviation" treatment, to improve drug delivery and the efficacy of the therapy (1,2). With this strategy we aim to alleviate physical forces in the microenvironment of solid tumors in order to decompress tumor blood vessels and improve perfusion and delivery of chemotherapeutic agents and nanomedicines. In my talk, I will present my research work, which has established the stress alleviation strategy as a promising therapeutic modality to optimize drug delivery to highly desmoplastic and most difficult to treat tumors, including pancreatic and breast cancers and soft tissue sarcomas.

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COMPLEMENT-MEDIATED HYPERSENSITIVITY TO NANOMEDICINES: A NEW PAN-SPECIFIC COMPLEMENT ASSAY FOR ANIMAL MODELS

JANOS SZEBENI[1,2], Tamás Mészáros[1], Rudolf Urbanics[2], László Dézsi[1], Tamás Fülöp[1], Marieluise Wippermann[3]

[1]Nanomedicine Research and Education Center, Semmelweis University, [2]Seroscience Ltd, Budapest, Hungary and [3]TECOmedical AG, Sissach, Switzerland

Complement (C) activation has been proposed to underlie the acute, non-IgE-mediated hypersensitivity (or infusion) reactions (HSRs) called C activation-related pseudoallergy (CARPA), that arise during intravenous therapy with liposomal (e.g., Doxil, AmBisome), micellar (e.g., Taxol, Taxotere) and many other nanoparticle-containing "nanodrugs". These reactions can occasionally be severe, or even lethal, therefore they represent a major immune barrier to the therapeutic use otherwise "state-of-the-art", highly effective pharmaceuticals. However, the evidence for a causal role of C activation in these reactions has been indirect to date, mainly based on the 1) in vitro capability of these agents to activate C in human serum, 2) the known physiological effects of C activation byproducts (i.e., anaphylatoxins), which explain the symptoms, and 3) the demonstrated strong correlation between C activation in vitro and extent of HSRs in vivo in animal (pig, dog and rat) models, including the fact that C inhibitors can inhibit the HSR. Direct evidence for C activation during HSRs in animal models has, however, not been available to date, partly because of the lack of a validated, sensitive assay for C activation in animal blood. This gap in immunological methods has been filled by a universal, species independent C3 ELISA kit, recently launched by Quidel Corporation (MicroVue Pan Specific C3 Complement Reagent Kit, PS-C3). The assay contains a proprietary C matrix and a C3 converter reagent that converts the activity of C3 in the animal specimen to human SC5b-9 that is quantifiable with the human SC5b-9 specific ELISA. The method allows sensitive and quantitative measurement of C3 in animal blood, plasma or serum, which, to the extent C3 is consumed prior to the assay, also provides a measure of C activation. The converter's use to measure C3 levels was demonstrated in 13 species to date (cat, dog, donkey, goat, goose, horse, minipig, mouse, pig, rabbit, rat, sheep and turkey). The goals of the study presented in this lecture were 1) to show C3 consumption by reactogenic nanomedicines, and, hence, provide direct evidence for the casual role of C activation in CARPA, and 2) to validate the PS-C3 kit in the rat and porcine models of CARPA. We have treated these animals with i.v. bolus injection of Doxil and AmBisome, as well as with zymosan and cobra venom factor (CVF) as positive controls, and recorded the symptoms of CARPA in parallel with PS-C3 measurements. In both species our results indicated dose-dependent, highly significant correlation between the symptoms of HSR (i.e., systemic hypo- or hypertension, pulmonary hypertension, leukopenia/leukocytosis, thrombocytopenia, rise of plasma thromboxane-B2, drop of plasma CH50/ml) and C3 consumption in blood. Thus, the PS-C3 kit provided a key evidence for the CARPA theory (i.e., C activation during the reaction), and, at the same time, proved itself as a useful test that enables, among many potential veterinary diagnostic and research applications, preclinical quantitation of the risk of nanomedicines and other i.v. pharmaceuticals to cause CARPA.

TOXICOLOGY AND SAFETY ASSESSMENT OF NANOMEDICINES: THE PIONEER CASE OF COMPLEMENT TESTING FOR THE PREDICTION OF ANAPHYLACTIC REACTIONS TO I.V ADMINISTERED NANOPARTICLES

JANOS SZEBENI[1,2,3], Tamás Mészáros[1], Rudolf Urbanics[2], László Dézsi[1], Tamás Fülöp[1], László Rosivall[3], Marieluise Wippermann[4]
[1]Nanomedicine Research and Education Center, Semmelweis University, [2]Seroscience Ltd, [3]Institute of Pathophysiology, Semmelweis University, Budapest, Hungary, and [4]TECOmedical AG, Sissach, Switzerland

The unique toxicity profiles of some nanomedicines tested in animals or humans to date, which arise as a consequence of the exceptional physicochemical properties of nanoparticulate material, have led to the recognition of an urgent need for novel toxicity tests that can predict unprecedented adverse effects in the body or in the environment. It seems to be a daunting task to live up to this need for all nanomedicines under development, with all possible organ toxicities evaluated, but one example at least highlights the feasibility of a widely applicable immune toxicity test scheme that helps the prediction of nanoparticle-induced hypersensitivity (infusion- or anaphylactic) reactions (HSRs) that can be life threatening, or even deadly in man. The ill-understood and until now unpredictable inflammatory reaction is caused by activation of the complement (C) system, hence it is called C activation-related pseudoallergy (CARPA). It can be triggered by a variety of nanoparticulate medicines that are recognized by the immune system as foreign, including liposomes, micellar anticancer drugs, antibody therapeutics, polymers, PEGylated proteins, radiocontrast media, etc. Research of the phenomenon over the past 20 years provided substantial evidence that measuring the C activating capability of nanoparticles in vitro, in undiluted human or animal serum, or in vivo, in pigs or dogs, provide useful measures of the risk of anaphylactic reactions in man. Although the correlation with C activation and clinical reactions in man is not perfect, C activation clearly plays a causal or contributing role in these reactions, making its measurements the best available predictor of CARPA. The presentation will detail the in vitro C assays that can be applied, as well as the animal models, with particular focus on the differences among the pig, dog and rat models. The most recent development in this field highlighted in the lecture is the application of the whole blood C assay for the prediction of rituximab (Rituxan)-induced HSRs, and the development of a universal, species-independent (pan species) C3 ELISA for the measurement of C activation in animals.

SELF-ASSEMBLING PEPTIDE NANOFIBER CONTAINING LONG MOTIF OF LAMININ INDUCES NEURAL DIFFERENTIATION, TUBULIN POLYMERIZATION AND NEUROGENESIS; IN-VITRO, EX-VIVO AND IN-VIVO STUDIES

SHIMA TAVAKOL[1,2], Ali Gorji[3], Hadi Aligholi[3], Seyed Mahdi Rezayat[1]*, Jafar Ai[4]*
[1]Department of Medical Nanotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Iran
[2]Student's Scientific Research Center, Tehran University of Medical Sciences, Tehran, Iran
[3]Shefa Neuroscience Research Center, Khatam al-Anbia Hospital, Tehran, Iran
[4]Department of Tissue Engineering, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
Sh_tavakol@razi.tums.ac.ir

Spinal cord injury (SCI) in humans remains a devastating and incurable disorder. A very important obstacle in axonal regeneration after spinal cord injury is astroglial scaring. The use of self-assembling peptide nanofiber, a hydrogel mimicking extracellular matrix, has been suggested as a scaffold for spinal cord regeneration and inhibition of astrogliosis. However, neurogenesis potential of laminim has been proved. The purpose of this study was to investigate the role of self-assembling peptide nanofiber containing long motif of laminin (SAPN-LL) in neural differentiation of hEnSCs in-vitro, in polymerization and depolymerization of tubulin isolated from sheep brain via UV-Vis Spectroscopy ex-vivo and assess the supportive effects of this hydrogel in an animal model of SCI at two concentrations. Results showed that although nanaofibers strongly differentiated hEnSC towards neuron and there were not significant differences between their neural differentiations at two concentrations but motor recovery results demonstrated that concentration of laminin influences motor recovery. However, both of in-vitro and in-vivo results showed that SAPN-LL inhibited astrogeneis. Based on our results it might be concluded that, SAPN containing long motif of laminin holds great promise for spinal cord injury recovery and it must be further emphasized the importance of different responses to concentration in in-vitro and in-vivo studies.
Keyword: self-assembling peptide nanofiber, Long motif of laminin, Spinal cord injury, polymerization of tubulin.

INTRODUCTION

Spinal cord injury (SCI) is a serious disabling condition that is associated with paralysis and is defined as a loss of axon integrity that could result in persistent deficit. One of the inhibitors of axonal regeneration is astroglial scarring. Glial fibrillary acidic protein (GFAP), an intermediate filament protein and a marker of astrocyte, is expressed in reactive astrocyte in lesions leading to astroglial scarring. Use of nanofibrous hydrogels as injectable biomaterials holds great promise with minimum tissue damage and invasion in treatment of SCI 1. It is demonstrated that fiber diameter significantly influences neural differentiation and proliferation.
In preformed scaffold implantation procedure, void space, poor graft integration, inflammation and low cell viability in the site of SCI induce further damage and inhibit repair at the injury site. To overcome these obstacles, use of self-assembling peptide nanofiber, a hydrogel mimicking extracellular matrix (ECM), is been suggested. Self-assembling is one of several methods for nanofibers synthesis. These oligopeptides are converted into nanofiber upon contact to ionic environment such as cell culture media and in-vivo environment. Early studies demonstrated that long motif of laminin can better mimic laminin conformation and improve neurogenesis. Based on these data, for the first time in this study, (RADA)4 was used as a backbone and chemically linked to a long motif of laminin (SPAN-LL).
Human endometrial-derived stromal cells (hEnSCs) are abundant and available adult stem cells with low immunological incompatibility, which could be considered for cell replacement therapy. The purpose of this study was to investigate the role of SPAN-LL in polymerization and depolmerization of tubulin via UV-Vis Spectroscopy and neural differentiation of hEnSCs in-vitro and assess the supportive effects of this nanofiber in an animal model of SCI.

MATERIAL AND METHODS

With regards to in vitro approach, hEnSCs were isolated from Human endometrial tissue and after 3 passages, cells were labeled with FITC-conjugated mouse anti-human CD146, CD105, CD90, CD34 and CD133 antibodies at concentrations recommended by the respective manufacturers and analyzed using flow cytometry (Partec, Germany). Then, hEnSCs were encapsulated into SAPN-LL at 0.125 and 0.25 concentrations and cell viability and cell membrane damage were assessed. Cells encapsulated into SAPN-LL were treated with neural differentiation medium for 18 days, and then neural genes and protein markers were analyzed using real time-PCR and immunocytochemistry (ICC). DAPI staining was performed to investigate the effect of concentration of nanofibers on chromosomal structure.
With regards to ex-vivo approach, to investigate the effect of nanofibers on tubulin structure as a key factor for neurite outgrowth, microtubule was extracted from sheep brain and after chromatography, tubulin was achieved. Then, tubulin polymerization and depolymerization were assessed at 3 different concentrations via UV-Vis spectroscopy.
With regards to in- vivo approach, SAPN-LL was implanted into rats with SCI and followed for 42 days using a behavioral test. Then, histological specimens were prepared for H&E, Nissl and IHC staining.

RESULT AND DISCUSSION

DAPI staining results showed no significant differences between damaging potential of nanofibers on chromatin structure (Fig.1b). Cell viability results showed extremely significant difference between cell viability of two nanofibers, however nanofiberes at concentration of 0.125 % induced higher level of cell viability as compared to 2D cell culture and exhibited more suitable microenvironment for cells (Fig.1c).

Real-time PCR showed that TH, GABA and Olig 2 were not expressed in cells encapsulated with nanofibers. Gene expression analysis indicated that there are no significant differences between relative gene expression of Nestin, Tuj-1, NF, MAP2 and Bcl2 in two groups. However, nanofibers containing long motif of laminin induced strongly neural differentiation of hEnSCs. Cell membrane contains ECM receptors that can bind to laminin and induces neural differentiation and neurite outgrowth via α6β1 integrin receptors 2. Nanofiber diameter plays a critical role in ECM production and neural differentiation. When cells attach to microfibers and plate (2D), they do not sense a 3D external ECM owing to the similarity in size of cells.

It is notable that both of nanofibers suppressed GFAP expression at the level of gene and protein which is involved in asteroglial scar formation. Bcl2 through β- catenin signaling promotes neurogenesis. It has been said that Bcl2 overexpression induces apoptosis resistance, neurogenesis and axonal regeneration and the reduction in BMP-4. BMP-4 inhibits astrogenesis, as seen in this study.

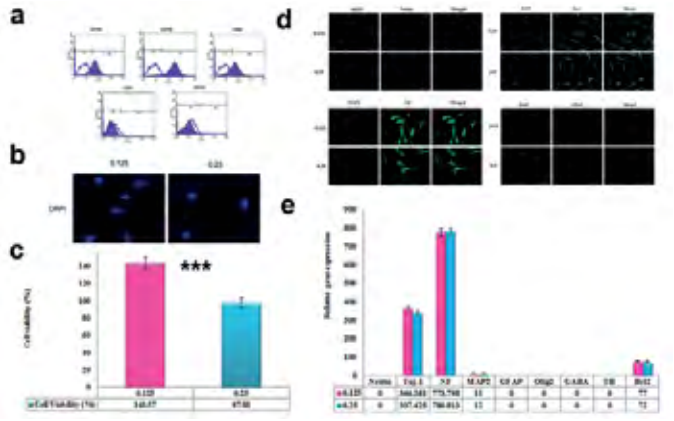

Fig.1. a) Flowcytometry of isolated hEnSC indicated that up-regulation of stem cell markers on the cell surface. b) DAPI staining of encapsulated cell into nanofibers after 18 days of post-incubation. C) Cell viability of cells encapsulated into nanofibers after 48 h d) ICC of cells by using Nestin, Tuj-1, NF and GFAP markers. e) Relative gene expression of cells encapsulated into nanofibers. There was no significant difference in up-regulation of neurogenic genes and proteins after 18 days post-incubation.

Investigation on the effect of tubulin polymerization and depolymerization showed that nanofibers exhibited different manner in polymerization of tubulin and these effects were related to concentration. Substantial recoveries of motor function were observed in animals implanted with SAPN-LL. Motor function recoveries were assigned 15 and 8 at concentrations of 0.125 and 0.25 %, respectively. However, in the control group assigned 2 (Fig.2b). It is notable that although nanofibers at different concentrations over-expressed almost the same neural markers at the levels of genes and proteins in-vitro, but they had different anti-astrogeliosis, neurogenic and motor recovery effects in in-vivo. Nanofiber containing long motif of laminin exhibited significant motor recovery in rats.

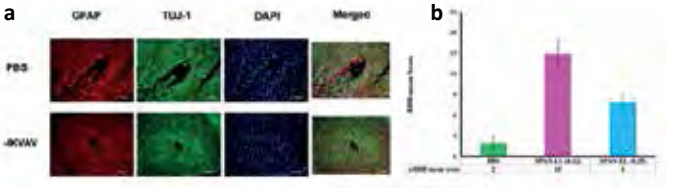

Fig. 2. a) ICC of cells treated by PBS and SAPN-LL via Tuj-1, GFAP and DAPI. Scale bar is 200µm. b) BBB score of rats implanted with PBS and SAPN-LLs at two concentrations

Hisomorphometery results showed higher percentage of neurogenesis in nanofiber group and less amounts of asterogliosis in rats implanted with self-assembling peptide nanofiber than PBS and control groups (Fig.2a). These results demonstrated the neurogenesis and anti-asterogliosis potential of this nanofiber in-vitro and in-vivo. Self-assembling peptide nanofibers containing long laminin motif fills the void spaces and small cyst that form after injury and well integrated to implant site and conform defect shape. Its well-integrative property eliminates cyst formation and glial scar.

CONCLUSION

The results of the current study indicate that treatment with nanofiberous scaffold induces neural differentiation of hEnSCs and tubulin polymerization, produced beneficial effects on functional recovery following SCI in rats, possibly via assimilation to cytoskeleton fiber, high surface/volume ratio, spatial interconnectivity and containing long adhesive motifs that more mimic conformational structure of laminin than short ones, enhancement of anti-astrogliosis, neuronal extension and neuronal regeneration effects.

ACKNOWLEDGMENT

This work was supported by grant from Iran International Science Foundation: INSF, .and Student's Scientific Research Center, Tehran University of Medical Sciences, Iran

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EMERGING CONCEPTS IN DENDRIMER-BASED NANOMEDICINE: PREDICTIVE, CNDP-DIRECTED OPTIMIZATION OF NANOPARTICLE PROTOTYPES FROM DISCOVERY TO CLINICAL TRANSLATION

DONALD A. TOMALIA

NanoSynthons, LLC, Mt. Pleasant, MI, University of Pennsylvania, (Dept. of Chemistry), Philadelphia, PA and Virginia Commonwealth University, (Dept. of Physics), Richmond, VA (USA)

Important predictive nanoperiodic property patterns/relationships observed for all well-defined, Hard/Soft Nanoparticle Categories (i.e., nano-elements/superatoms) are now widely recognized by both chemists[1-3] and physicists[4-5] since they were first introduced in 2008.[1,6] These nanoperiodic patterns/rules are driven by six--Critical Nanoscale Design Parameters (CNDPs) associated with these nanoparticles; namely: 1) size, 2) shape, 3) surface chemistry, 4) flexibility/rigidity, 5) architecture and 6) elemental composition. These same predictive nanoperiodic principles are now being applied to many well-defined, nanoparticle platforms (i.e., dendrimers, metal nanoclusters, proteins, DNA/RNA, etc.) that are playing pivotal roles in nanomedicine (i.e., nano-diagnostics/therapies). Recent progress in the use of these predictive nano-periodic patterns for optimizing important nanoparticle features related to nanotoxicology, biodistribution and pharmacokinetics (Figure 1) will be briefly overviewed.[7]

Figure 1. Soft and Hard Nano-element Categories (center). A graphic overview of six-CNDPs that may significantly affect nanotoxicology, pharmacokinetics and biodistribution parameters for all Soft and Hard Nano-element Categories in various nanomedical applications.

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THE COSMOPHOS-NANO PROJECT

PANAGIOTIS N. TROHOPOULOS

The CosmoPHOS-nano Project is a Large-scale EU FP7 NMP Funded Translational Nanomedicine R&D Project in Cardiovascular Diseases, and more specifically in Atherosclerotic Heart Disease. The Project co-funded by the European Union under the FP7 Programme / NMP Theme (Nanosciences, Nanotechnologies, Materials and New Production Technologies) with 8,5 Million Euros, and additionally co-funded by All Project Participants with 4,5 Million Euros, having a total project budget of 13 Million Euros. The CosmoPHOS-nano is a Five-year R&D Project started on March 1, 2013 and will be concluded on February 28, 2018, and it is a Multidisciplinary R&D Project consisting of 19 World-Class Participants, including 13 Universities and Research Foundations and 6 Companies, from 11 European Countries, Japan, and USA, with a wide variety of complementary and cutting-edge scientific, technological and manufacturing expertise and know-how. The CosmoPHOS-nano Project is the World's Largest R&D Project of Nanomedicine in Cardiology aiming to develop a Radical Innovative Theranostic (Diagnostic and Therapeutic) "Smart" Nanomedicine Product, the CosmoPHOS System, to enable: a) Molecular Imaging by using Near-Infrared Fluorescence (NIRF), b) Targeted Therapy by using Photodynamic Therapy (PDT), and c) Real-time and Follow-up Therapy Monitoring of Atherosclerotic Coronary Artery Disease (CAD) of the Heart which causes the myocardial infarctions (heart attacks) and is the number one cause of human death and morbidity in Europe and worldwide. The CosmoPHOS System is anticipated to significantly reduce the number of deaths and the morbidity caused by CAD. This is forecast to result in a significant decrease of the European and global healthcare costs caused by CAD, increase the income of the European healthcare industry from CAD market which is the global largest, and alleviate the European and global society. The CosmoPHOS-nano Project is the First EU FP7 NMP Funded Large-scale R&D Project planning to apply Nanomedicine for Cardiac Patients. It foresees conducting during the final Project-year, a First-in-man Phase-I Clinical Trial in CAD Patients, to evaluate the safety and feasibility of the novel CosmoPHOS System for human use. The CosmoPHOS Ltd, a European SME for Translational Nanomedicine based in Thessaloniki, Greece (Ellas), is the Founder and the Scientific / Exploitation / Strategic Coordinator of the CosmoPHOS-nano Project.

(E-mail: panagiotis.trohopoulos@cosmophos.com)

ADVANCED PARTICLE ANALYSIS: TUNABLE RESISTIVE PULSE SENSING (TRPS) CLEARS THE PATH FOR NANOMEDICINE DEVELOPMENT AND REGULATORY ACCEPTANCE

HANS VAN DER VOORN

Nanomedicine is a high growth area in medical research but there are still only a few nanoparticle based therapeutics in clinical use. Regulatory approvals for nanomedicine products have been problematic with most of the traditional particle characterisation techniques unable to provide confidence in their accuracy or reproducibility. More detail in the characterisation is required and would avoid the need for the highly detailed prescription on the manufacturing process.

Regulatory oversight by the regulators and gaining regulatory approvals by nanomedicine developers can be considerably simplified if accurate and reproducible hard data on particle properties can be provided in sufficient detail.

TRPS is a high resolution nanopore based technique enabling accurate measurement of the size, charge, and concentration on a particle by particle basis. Accurate calibration is inherent in the technique so absolute values can be provided with confidence and these are independent of the user and the user assumptions on unknown parameters.

TRPS can now measure individual particle charge: electrophoretic mobility which can be converted to zeta potential. Many parties consider particle charge to be as important in predicting particle behaviour as size and therefore accurate charge distribution may be as important as an accurate size distribution.

All of these measurements can be done in physiological conditions and the recommendation is that particle aggregation be checked in higher molarity solutions than their expected use to provide a safety factor.

There is an obvious need for analytical results to be transparent and able to be understood by the user and any subsequent reviewer of the information.

PROBLEMS AND OPPORTUNITIES TO DEVELOP LIPOSOMAL PRODUCTS

PETER VAN HOOGEVEST

Lipoid GmbH, Ludwigshafen (D)

Since the US-FDA claims that the CMC of the lipid components of liposomes should be provided at the same level of detail expected for a drug substance, the development of liposomes for parenteral/intravenous administration requires the use of phospholipids with highly reproducible quality and composition. In general, as derived from the phospholipid composition of the liposome products Ambisome, Doxil, Caelyx and Visudyne, phospholipids originating or derived from natural sources and synthetic phospholipids can be used.

Natural phospholipids are derived from renewable sources and produced with more ecologically friendly processes and are available in larger scale at relatively low costs without possible toxic by-products compared to synthetic phospholipids. They comply with all requirements of the regulatory authorities and are safe in use for any administration route and any dosage form. Synthetic phospholipids contain chemically specific, defined polar head groups and fatty acids but are synthesized with various chemicals and solvents. They may contain intermediates or by-products and unnatural enantiomers may be formed. Synthetic phospholipids are only available in relatively small amounts at high prices.

In the overall phospholipid excipient market, synthetic phospholipids play, compared to natural phospholipid (including hydrogenated and enzyme modified phospholipids), from number of drug products comprising these phospholipids, a very minor role. Their use is restricted to some parenteral liposomal products. If the use of synthetic phospholipids is unavoidable, synthetic phospholipids using the GPC synthesis route should be used. At this way the natural stereochemical configuration is guaranteed and the extra production steps compared to natural phospholipids can be performed with minimal additional usage of solvents and chemicals.

In order to avoid scale up problems during pharmaceutical development and production, natural phospholipid excipients instead of synthetic phospholipids should be selected for liposomal formulations/products whenever possible.

NOVEL NUCLEIC ACIDS FOR TARGETED THERAPY OF SOLID CANCERS

RAKESH N. VEEDU[a,b]*
[a]Nucleic Acid Center, Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Campusvej 55, 5230 Odense M, Denmark; [b]School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia, Brisbane, 4072 Australia.
rnv@sdu.dk, rakesh@uq.edu.au

Cancer has a tremendous impact on society. Despite much advancement towards tackling cancer, the number of victims continues to rise. Current treatment has several limitations like cancer tissue specific drug targeting, severe side-effects and a very high cost. Aadvanced targeted cancer therapy using monoclonal antibodies (MAbs) attracted significant interest, and there are some FDA approved MAbs in the clinic for treating some solid tumours including human epidermal growth factor receptor-2 targeting trastuzumab, epidermal growth factor receptor targeting cetuximab and panitumumab, and the vascular endothelial growth factor targeting bevacizumab [1]. However, there are often issues with the delivery and stability of the antibody conjugate in vivo, poor pharmacokinetics and in some cases, initiation of a deleterious immune-response in patients. Thus, the development of new approaches for targeting cancer cells that is effective and safe in vivo, is a high priority for nanomedicine researchers. Aptamer-therapy is one approach that has recently received significant interest from the scientific community.

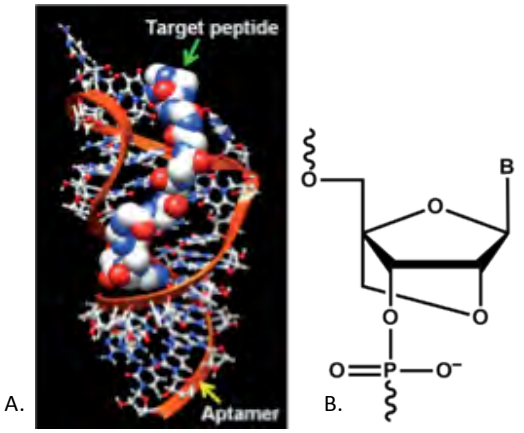

Figure 1. A. Example structure of an aptamer bound to its specific target (adopted from Ref. 8); B. Structure of LNA nucleotide for improving aptamer bio-stability and affinity (letter B on the structure denotes a base).

Aptamers [2] are short single-stranded DNA or RNA oligonucleotides that can bind to their targets (ranging from small molecules to complex proteins) with very high affinity and specificity because of their ability to adopt three-dimensional conformation in solution (Figure 1A). More recently, one aptamer-based drug, Macugen, was approved for the treatment of age-related macular degeneration (AMD). But, aptamers composed of natural DNA and RNA pose serious limitations for therapeutic development as they are rapidly degraded in a biological environment in addition to possess poor binding affinity. To overcome these limitations, chemically-modified nucleotides are used. Locked nucleic acid nucleotide (LNA, Figure 1B) is one of the most prominent and successful nucleotide analogues developed in recent years because of their high target binding affinity and remarkable nuclease resistance [3, 4]. In my laboratory, we explore the development and therapeutic application of LNA-modified aptamers. Our group has developed a novel single step selection methodology [5] for developing nucleic acid aptamers and also developed a method for developing LNA-modified aptamers by conventional selection methods [6-8].
We have recently developed LNA-modified aptamers targeting vascular endothelial growth factor (VEGF) and amyloid beta peptide (Aβ) for targeted nanotherapy against solid tumours and Alzheimer's disease respectively. In this lecture, I will describe our results to inhibit the proliferation of breast cancer cells using LNA aptamers.

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NANOMECHANICS BY WHICH MACROPHAGES CLEAR BACTERIA

VIOLA VOGEL
Laboratory of Applied Mechanobiology, Department of Health Sciences and Technology, ETH Zurich, Switzerland.
E-mail: viola.vogel@hest.ethz.ch

Antibiotics have saved millions of lives, but what comes next? Many strategies have been applied to fight bacterial infections, primarily designed to either kill bacteria via antibiotics or more recently to prevent their adhesion to surfaces and host tissues. Little attention though has been given to ask how these strategies might affect the ability of our immune cells to pick up bacteria from surfaces. To clear pathogens from host tissues or biomaterial surfaces, macrophages have to break a large cluster of adhesive bonds by which bacteria hold on to surfaces or tissue fibers. Novel insights into nanomechanical aspects now reveal some adverse and unanticipated side effects of common antibacterial drugs as they impair the ability of our immune cells to fight infections. What has escaped general attention, for example, is the finding that some drugs that are currently developed to prevent bacterial adhesion, reduce at the same time the rate by which E. coli can be picked up by macrophages. Also some antibiotics can hinder the efficiency of macrophages to clear pathogens. Research into the mechanobiological aspects of bacteria and immune cells is thus not only scientifically rewarding, but might impact our ability to deal with infections.

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IRON PHOSPHATE NANOPARTICLE DO NOT INDUCE DIRECT CYTOTOXICITY IN INTESTINAL HT29, HT29-MTX AND HCEC CELLS

LEA M. VON MOOS[1], Mirjam Schneider[1], Ioannis A. Trantakis[1], Florentine M. Hilty[2], Sotiris E. Pratsinis[3], Michael B. Zimmermann[2], Shana J. Sturla[1]
[1]Laboratory of Food and Nutrition Toxicology, Department of Health Sciences and Technology, ETH Zürich, Switzerland
[2]Laboratory of Human Nutrition, Department of Health Sciences and Technology, ETH Zürich, Switzerland
[3]Particle Technology Laboratory, Department of Mechanical and Process Engineering, ETH Zurich, Switzerland

Email: lea.vonmoos@hest.ethz.ch

The use of engineered nanoparticles (ENP) in the food and nutrition industry offers new opportunities to enhance product performance and provide health benefits to consumers. Fortifying food with highly bioavailable nanostructured iron compounds has the

potential to better combat iron deficiency, a major global public health issue, however their absorptive pathway and safety remains unclear. In this study, we tested the hypothesis that the presence of mucus and the transformational status of cells influence the potential cytotoxicity of iron phosphate nanoparticles (FePO4-NPs) to human intestinal epithelial cells in vitro. Amorphous FePO4-NPs with a specific surface area (SSA) of 100 m2/g and 190 m2/g were produced by flame spray pyrolysis (FSP). Food-grade silica nanoparticles (SiO2-NP) were used as negative control for toxicity, soluble FeSO4 as control for the biological effect of iron and fine powder FePO4 (SSA 28 m2/g) as control for the effect of larger FePO4 particles. Human colon adenocarcinoma derived (HT29, HT29-MTX) and noncancerous immortalized human colon epithelial cells (HCEC) were exposed to 0.1-500 µg/mL FePO4-NPs or control compounds for 24 or 48 h. Although exposure to high doses of SiO2-NPs (SSA 200 m2/g) and FeSO4 resulted in reduced viability of HT29 cells, and high doses of commercial fine powder FePO4 decreased viability of HCEC cells, no cytotoxicity was observed for FSP made FePO4-NPs. These results give a first indication that the tested FePO4-NPs are not acutely toxic to intestinal epithelial cells.

LIPOSOMES AS DRUG DELIVERY SYSTEM AND VACCINATION TOOL – GMP MANUFACTURING AND SCALE UP

ANDREAS WAGNER

Polymun Scientific GmbH, Donaustrasse 99, 3400 Klosterneuburg, Austria,
Andreas.Wagner@polymun.com, www.polymun.com

Over the past few years liposomal drug preparations have been increasingly used in clinical trials. Until now, several liposomal products have reached the market, many other formulations are still in the pipeline. For all these products, a simple, economic and GMP-conform production technique and facility is necessary.
Based on the ethanol injection technique, we have realised a scalable and sterile production technique. Continuous aseptic one-step operation permits the production of stable liposomes with a defined size distribution. The production plant is designed to meet several requirements, such as simplicity, robustness and easy handling of sterilisation procedures. Furthermore, the ethanol injection technique itself is distinguished by mild preparation conditions and the avoidance of hazardous solvents and forces, which may disrupt lipids as well as entrapped substances.
In the last years, we have guided many liposomal drug products from very early stage to clinical applications. Thereby many different hurdles have to be overcome. Liposomal oligonucleotide encapsulation processes with encapsulation rates up to 80 percent were developed and these products were guided from early formulation development into the clinics. Liposomal peptide formulations, to be used as vaccines were developed. These peptides were encapsulated into long term stable liposomes with loading rates up to 80 percent.
Here several points to consider right at the beginning should be listed. Product development at early stage should implement the use of high quality raw materials, robust and stable product and process conditions and robust analytical methods. E.g. the drug substance as well as excipients should be designed to allow solubilisation in a pharma compliant solvent.
In conclusion, liposome manufacture with the crossflow technique is suitable for the production of liposomal vaccines and therapeutics. The main advantage of this technique is the feasibility of manufacturing batches of 5-10 millilitres, which is directly scalable to several litres. This is of particular importance when it comes to cost-intensive drugs that are to be manufactured by novel biotechnological procedures.

NMR AND BIOPHYSICAL METHODS FOR THE CHARACTERIIZATION OF NOVEL LIPODISQ™ NANOPARTICLES FOR DRUG DELIVERY APPLICATIONS

ANTHONY WATTS

Lipodisq™ is a novel polymer-rimmed lipid nanopartcle of ~11nm in diameter, that has capcacity to deliver active compounds to cells and the stratum corneum. In addition to applications for treating melasma (Malvern Cosmeceutics Ltd., UK; www.malceutics.com/), we have been exploring their use for delivery of chemotherapy drugs to cells, and topological drug delivery. The Lipodisq™ technology is very stable and has been well characterized by us biophysically (Orwick et al., (2012) Nano Letters, 12:4687-4692; Orwick et al., (2012) Angewante Chemie, 51:1-6), including by nuclear magnetic resonance (NMR), and has further application in detergent-free membrane protein extraction and purification (Long et al., (2013) BMC Biotechnology, 13:41).

REGULATORY APPROACH TO PERSONALIZED MEDICINE

FRANK F. WEICHOLD

Advances in Nanomedicine carry the potential to support the promise of “personalized medicine” by tailoring medical treatment to the individual characteristics, needs and preferences of each patient. The concept of personalized medicine is not new: clinicians have long observed that patients with similar symptoms may have different illnesses, with different causes; and similarly, that medical interventions may work well in some patients with a disease but not in others with apparently the same disease. What is new is that advances in a wide range of fields from genomics to medical imaging to regenerative medicine, along with increased computational power and the advent of mobile and wireless capability and other technologies – including the progress made in nanotechnology - allowing patients to be treated and monitored more precisely and effectively. The discussion will lead through some examples of how FDA is addressing questions related to regulation of products that support personalized medicine within the given framework, and which challenges need to be considered when establishing preparedness to serve patients better in their individual needs. The presentation will offer the opportunity to discuss FDA’s many recent efforts to advance regulatory standards, methods and tools in support of personalized medicine and to further refine critical regulatory processes and policies in order to bring about personalized medical product development; looking toward a future where all stages of patient care—from prevention to diagnosis to treatment to follow-up—are truly personalized.

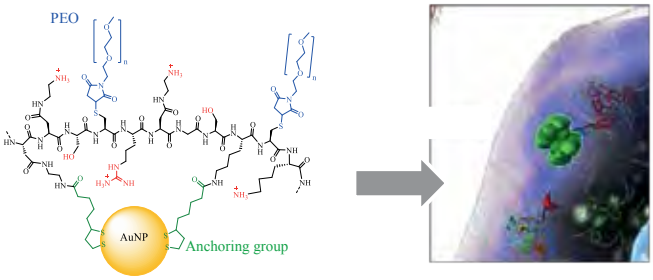
INTERFACE BETWEEN PROTEINS AND INORGANIC NANOPARTICLES

TANJA WEIL

Prof. Dr., Director of the Institute of Organic Chemistry III and Macromolecular Chemistry, Ulm University (D)

Proteins are sequence specific and geometrically defined macromolecules representing the central framework of all biological processes in Nature. Their precise physical architecture and consequent biochemical functions are unique and unrivalled in the synthetic world, providing an impetus for the incorporation of proteins into the development of contemporary hybrid materials. Unlike conventional polymers, their repertoire of chemical functionalities at discrete positions facilitates the grafting of designated synthetic moieties1 to achieve a nanoscale construct with exceptional macromolecular definition.2,3 Through these synthetic appendages, supramolecular polypeptides4 and protein-polymer biohybrids2,3, can be chemically programmed to possess new and improved physico-chemical properties while simultaneously exhibiting unique biological behavior.
We have designed multifunctional copolymers derived from proteins2 that possess attractive features such as an accurately known

length and a defined number of functional groups at distinct locations within the peptide backbone, low size-dispersity as well as intrinsic biocompatibility. Such copolymers allow the stabilization of nanoparticles such as quantum dots (QDs)5,6 or nanodiamonds7 via multivalent interactions. A pronounced responsiveness of the emission intensity of these nanoparticles was found in the presence of DNA6 or upon pH5 changes which is attractive for achieving biocompatible sensors for in vitro and in vivo applications. In addition, multiple drug molecules with different modes of action as well as cell targeting entities were attached based on sophisticated peptide chemistry. Such protein-derived nanoparticles have a great potential for in vivo therapy and pave the way to intelligent vehicles for nanomedicine applications.



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THE MORAL DIMENSIONS OF RESPONSIBILITY IN NANOMEDICINE

KLAUS-MICHAEL WELTRING
Gesellschaft für Bioanalytik Münster e. V.

Nanotechnology is a very interdisciplinary science and technology area, mainly dealt with by chemists, physicists, and material scientists. By applying nanotechnology to medicine (called nanomedicine) these scientist are all of a sudden confronted with issues such as medical needs, toxicology, animal testing, or patients looking for better disease diagnosis and treatments. This means they have to be aware and consider such questions as soon as they propose a medical application for their technology.
On the other hand, clinicians, industries, regulators and healthcare providers have to be willing to openly test and implement the improvements and benefits nanotechnology can bring to medicine. In other words, a responsible research and innovation approach is needed on both sites to bring nanomedical innovations to patients.
•In the EC Report (2013) « Options for strengthening Responsible Research and Innovation », RRI refers to ways of proceeding in RRI that allow those who initiate and are involved in the process of research and innovation at an early stage (A) to obtain relevant knowledge on the consequences of the outcomes of their actions and on the range of options open to them; and (B) to effectively evaluate both outcomes and options in terms of moral values (including but not limited to wellbeing, justice, equality, autonomy, safety, sustainability, accountability, democracy and efficiency); and(C) to use these considerations (under A and B) as functional requirements for design and development of new research, products and services ».
The application of the RRI approach to nanomedicine will be discussed.

http://ec.europa.eu/research/science-society/document_library/pdf_06/options-for-strengthening_en.pdf

SIMULATION OF PEPTIDES IN MEMBRANES AND TOXICITY PROFILES OF GOLD NANOPARTICLES

WOLFGANG WENZEL
Institute of Nanotechnology, KIT Campus North, Germany

We have recently developed very efficient methods to simulate the structure formation and function of peptides in membranes, which I will discuss in two contexts: We propose a novel concept for the folding and self-assembly of the pore-forming TatA complex from the Twin-arginine translocase and of other membrane proteins, based on electrostatic "charge zippers", which led to a novel functional understanding of the translocation processes of fully folded proteins through membranes[1]. To further our understanding of the mechanism of toxicity of nanomaterials to be used in drug delivery we investigated toxitiy profiles for ultrasmall (1.4 nm) AuNPs on the electrophysiology of HEK 293 cells expressing hERG, a standard benchmark for drug safety, depending on ligand composition. In patch clamp experiments phosphine-stabilized AuNPs irreversibly blocked hERG channels, whereas thiol-stabilized AuNPs of similar size had no effect in vitro, while neither particle blocks the channel in vivo[2]. We conclude that safety regulations may need to be re-evaluated and adapted to reflect the fact that the binding modality of surface functional groups becomes a relevant parameter for the design of nanoscale bioactive compounds.

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SHRINKAGE OF PEGYLATED AND NON-PEGYLATED LIPOSOMES IN SERUM

JOY WOLFRAM[1,2], Yong Yang[1] , Haifa Shen[1], Yuliang Zhao[2,3], Mauro Ferrari[1,4],*
[1]Department of Nanomedicine, The Methodist Hospital Research Institute, Houston, TX 77030, USA.
[2]CAS Key Laboratory for Biomedical Effects of Nanomaterials & Nanosafety, National Center for Nanoscience & Technology of China, Beijing 100190, China.
[3]Institute of High Energy Physics, Chinese Academy of Sciences (CAS), Beijing 100049, China
[4]Department of Medicine, Weill Cornell Medical College, New York, NY 10065, USA.
*Corresponding author

Keywords: Liposomes, dynamic light scattering, size, polydispersity index, zeta-potential

An essential requisite for the design of nanodelivery systems is the ability to characterize the size, homogeneity and zeta-potential of nanoparticles. Such properties play a pivotal role in overcoming biological barriers and reaching the target region [1]. The charge and size of nanoparticles can be tailored in order to create the most efficient drug delivery platforms. An important question is whether these characteristics change upon systemic injection. Here, we have studied the behavior of phosphaditylcholine/cholesterol liposome exposed to serum proteins. The results reveal a serum-induced reduction in the size and homogeneity of both pegylated and non-pegylated liposomes (Fig. 1), implicating the possible role of osmotic forces (Fig. 2). In addition, changes to zeta-potential were observed upon exposing liposomes to serum. Such changes in liposomal features suggest that the characteristics of liposomes should regularly be evaluated in serum.

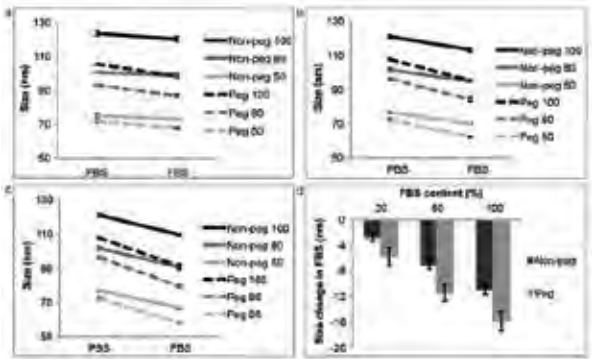

Figure 1. Size comparison of liposomes in phosphate buffered saline (PBS) and fetal bovine serum (FBS). Non-pegylated (non-peg) and pegylated (peg) liposomes were extruded through filters with 100 nm, 80 nm and 50 nm pores. (a) 20% FBS, (b) 60% FBS, (c) 100% FBS, (d) summarizing graph. For line graphs data is presented as mean ± SD of 5 measurements. For bar graphs data is presented as mean ± SD of liposomes with different sizes.

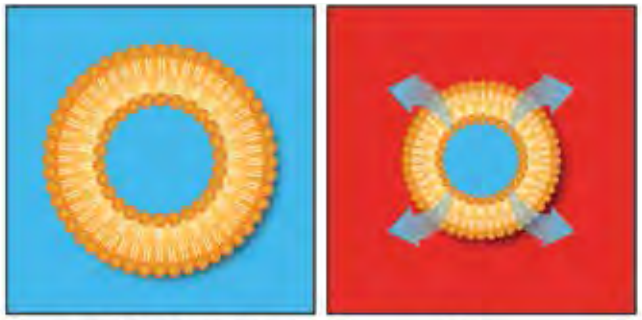

Figure 2. Schematic illustrating the shrinkage of liposomes in serum. The proposed mechanism is protein-induced osmotic pressure, causing water to escape from the core of the liposome and subsequent compression of the liposome.

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PRO-HEALING EFFECTS OF SILVER NANOPARTICLES IN BONE FRACTURES AND TENDON INJURIES

KKY WONG

Recent research interests have focused on regenerative medicine to repair body organs after degenerative diseases, injury, and trauma, with the aim to restore normal architecture and function. In this respect, the use of nanotechnology may also help provide the perfect tools to control and guide the regenerative process.
Our laboratory has previously showed that the use of silver nanoparticles could enhance skin wound healing through their effects on epidermal-derived stem cells. In our latest studies, we have been focusing on the use of silver nanoparticles in orthopedics, in particular bone fracture and tendon healing. Our results indicated that silver nanoparticles could promote the proliferation and differentiation of mesenchymal stem cells (MSCs) into as osteoblasts, thereby promoting bone fracture healing. Furthermore, we showed that silver nanoparticles could promote tendon healing through an anti-inflammatory action, leading to better collagen deposition.
This talk will thus discuss the positive impact of silver nanoparticles on both bone and tendon healing. Our new findings could provide potentially important new strategies for the field of regenerative orthopedics. It is hoped that future clinical trials could soon be realized, with the aim to bring significant benefits to patient care.

NANOPARTICLES FOR OCULAR THERAPY

TINA WONG
BSc (Hons), MBBS, FRCOphth, FRCS(Ed), FAMS, PhD,
Senior Consultant, Glaucoma Service, Singapore National Eye Centre (SGP)

By far, the major thrust of using nanoparticles for drug or gene delivery has been to enhance cellular penetration and not for sustained release. However, it has been much more challenging to apply nanotechnology to develop viable sustained-release products, in spite of the widely recognized need for such methods to improve the management of chronic diseases that require long-term medication. A number of nanoparticles have been evaluated over the years to improve loading and sustained delivery of drugs, including liposomes and polymer nanoparticles.
Presently there are only 3 products that demonstrate sustained release for up to 5 days. Currently, drugs are delivered to the anterior segment of the eye via topical eye drops only. The major issue with topical eye drops is corneal penetration of drug molecules; even for small drugs such as antibiotics, only about 2-5% of applied dose penetrates. Certainly the delivery is a bolus, and therefore short-lived (less than 24 hours) in its action. Attempts to prolong residence time of drugs on the eye have not been successful; neither have attempts to enhance penetration.
In this presentation, the conception, development and evaluation of a nanoliposome based delivery platform to provide prolonged release of latanoprost for treating glaucoma is presented. An extended release of over 120 days is reported in preclinical studies that are reiterated in a first in man study. Finally, the clinical impact of this impressive nanoliposome drug delivery system is also discussed.

PERSONALIZED PLATINUM NANOMEDCINES FOR CANCER

RICHARD WOOSTER
Blend Therapeutics, Watertown, MA (USA)

One in three people will be diagnosed with cancer while one in four will die from their disease. Platinum drugs have proven to be effective cancer drugs, for example >90% of men with testicular cancer are cured with a platinum drug. Platinum drugs are also widely used for the adjuvant treatment of common cancers such as those of the lung, colon and ovary. However for the majority of tumor types the clinical response rates for platinum therapies are low, for example the 1 year survival rate for lung cancer patients treated with platinum therapeutics is just 34%. The key limitations of the existing platinum therapies are the dose limiting toxicities that restrict dose and/or duration of therapy and the absence of personalization that targets the drugs to the patients most likely to benefit. To address these issues we have designed novel platinum nanomedicines that have improved plasma pharmacokinetic properties, accumulate in tumor tissue in xenograft models, show increased levels of DNA bound platinum, cause DNA damage and show efficacy that is improved compared to currently approved platinum drugs. In parallel to the discovery of these novel platinum medicines we have explored potential biomarkers to predict which tumors are most likely to respond. These data will be discussed towards developing personalized platinum nanomedicines for cancer patients.

UNRESOLVED ISSUES IN CANCER

JENS WÜRTHNER

Despite significant advances in cancer diagnosis and treatment over the past decades, major challenges remain. For the purpose of this overview, they are summarized into three categories: Scientific issues, clinical problems and drug development issues. Some aspects will be lined out in more detail, for example limitations of current therapeutic approaches (toxicity, resistance, tissue distribution), the recurring theme of high attrition rates, and the new challenge of marrying diagnostic development for patient selection with clinical development of new drugs. The potential impact that nano-medicines may have here, and vice versa, the impact of these challenges on the development of nano-medicines, will be discussed.

OMICS AND SEQUENCE-BASED MEDICINE

HUANMING YANG
Ph.D., BGI-Shenzhen, China

A series of important events took place in 2013, among them were the 60th anniversary of discovery of DNA double helix, the 10th anniversary of the official completion of the International Human Genome Project (HGP), and sadly passing away of Prof. F. Sanger, Father of Genomics.
"1953: Genes became information" because of the Double Helix, but the real revolution did not come until the advent of sequencing technology which was invented by Prof. Sanger in the 1970s, making life digital and dramatically expanding and deepening our views of the life world, and laying the foundation for sequence-based medicine.
The HGP has "changed biology and biotech for ever" by cultivating a new branch of science, GENOMICS, providing a new tool, SEQUENCING, and building a new culture, COLLABORATION, in exact line with two of the major trends of the world, "digitalization" and globalization.
Genomics, together with other –omics and by means of its core technology of sequencing, would not only bring more knowledge about human genome evolution and variations, especially those population- or even individual-specific variants related to diseases, but also provide a powerful tool to further study common/complex diseases by whole genome sequencing, monogenic diseases by exomic sequencing, canceromics and neurology by single cell sequencing, interaction between microorganisms and genome/environment by metagenomic sequencing, application of NIPT (Non-invasive Prenatal Test) of a certain genetic diseases by trace-DNA sequencing, as well as combined sequencing technologies and bioinformatic tools.
In combination with molecular technologies, SC/iPC, animal cloning, synthetic genomics, and other future emerging techs, omics will help reshape medicine and the world in the 21st century.

POPULATION DYNAMICS OF CANCER STEM CELLS

STEFANO ZAPPERI[2], Caterina A. M. La Porta[1], James P. Sethna[3]
[1]Department of Biosciences, University of Milano, Milano, Italy
[2]CNR-IENI, Milano, Italy and ISI Foundation, Torino, Italy
[3]LASSP, Department of Physics, Cornell University, Ithaca, New York, United States of America

Tumors are defined by their intense proliferation, but sometimes cancer cells turn senescent and stop replicating. In the stochastic cancer model in which all cells are tumorigenic, senescence is seen as the result of random mutations, suggesting that it could represent a barrier to tumor growth. In the hierarchical cancer model a subset of the cells, the cancer stem cells, divide indefinitely while other cells eventually turn senescent. It is commonly believed that cell senescence – the loss of replicative capacity of cells – acts as a barrier for tumor growth. Here we follow the evolution of senescence markers in melanoma cells and find that while most cancer cells eventually turn senescent, this is at root irrelevant for the long-term growth rate of a tumor. To demonstrate this, we construct a mathematical population dynamics model incorporating cancer stem cells which is able to reproduce quantitatively the experimental data (see Fig 1) [1]. Our results support the existence of cancer stem cells in melanoma and explain why it is difficult to fight cancer by inducing senescence in cancer cells. Only a fraction of the cells are susceptible to senescence, but those cells are irrelevant for tumor growth. A successful therapeutic strategy should instead target cancer stem cells, which are, however, likely to be strongly resistant to drug induced senescence. Finally using our model for guidance, we discuss possible pitfalls in the identification of cancer stem cells from sorting experiments [2-3]

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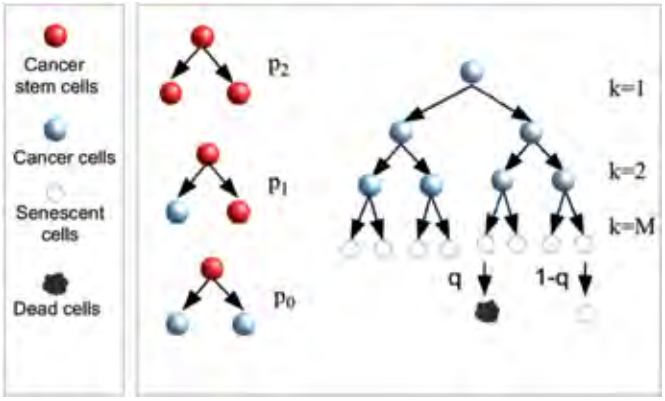

Fig 1: Branching processes for cancer growth. At each generation, CSCs can divide symmetrically, giving rise to two CSCs with probability or to two CCs with probability , or asymmetrically with probability giving rise to a CSC and a CC. Cancer cells can only divide a finite number of times (in the figure), after that they become senescent. Senescent cells die with probability at each generation.

DE NOVO DESIGN OF CANCER NANOMEDICINE AND PRECLINICAL PROGRESS

YULIANG ZHAO [1,2]
CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety,
[1]National Center for Nanoscience and Technology of China; Beijing 100190, China
[2]Institute of High Energy Physics, Chinese Academy of Sciences (CAS), Beijing 100049.

In this talk, we will discuss a novel approach which has been developed using Gd@C82(OH)22 metallofullerenol nanoparticles (f-NPs) to highly efficient inhibit tumor metastasis (inhibition rate >80% in vivo). Rather than direct cell killing, the f-NPs inhibited tumor metastases mainly through a regulation of tumor microenvironment to amend the "soil" of tumor, resulting in formation of a thick fibrous cage which serve as a"prison" capable of confining the invasive tumor cells in their primary site[1-5]. We will discuss a new approach in the management of tumor metastasis by a de novo design of cancer nanomedicine to imprisoning cancer cells but not killing with the metallofullerenol platform and their current progresses for clinical applications.
The engineered-nanoparticle-based nanomedicine formulates as "particulate medicine" [1] which is conceptually different from the conventional "molecular medicine" in various aspects: Nanomedicines possess huge surface area which forms multiple interfaces

when interacting with biological microenvironment. These nano-bio interfaces largely altered interactions of NPs with biological systems and endow nanomedicines with multiple functionalities [2,4]; To a candidate of molecular drugs, these compounds should simultaneously possess two unique nature, i.e., weak interactions and “key and keyhole” recognition between drug candidate and their receptor molecules. However, particulate medicines can exert their biological functions [1-8] as long as the weak interactions exist with their molecular targets [4], which significantly increase therapy efficacy, etc.

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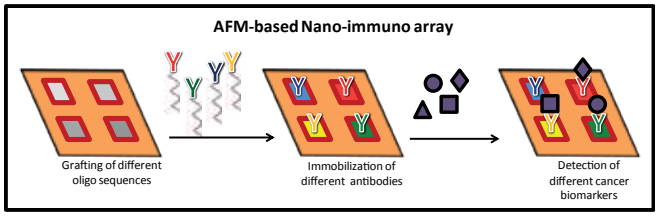
ABSTRACTS OF THE POSTERS

NANO-IMMUNO ASSAY DEVELOPMENT FOR THE DETECTION OF CANCER BIOMARKERS

ELENA AMBROSETTI[1], Alessandro Bosco[1], Elda Tagliabue[2], Ario deMarco[3], Loredana Casalis[1]
[1]NanoInnovation Lab - Elettra Sincrotrone Trieste S.C.p.A, Strada Statale 14, 34149 Basovizza, Trieste ITALY
[2]Molecular Targeting Unit, Department of Experimental Oncology and Molecular Medicine, Fondazione IRCCS-Istituto Nazionale dei Tumori, Via Amadeo 42, 20133 Milano, ITALY
[3]Center for biomedical sciences and engineering, University of Nova Gorica, Dvorec Lanthieri, Glavni Trg 8, 5271 Vipava, SLOVENIA
E-mail: elena.ambrosetti@elettra.eu

Tumors are characterized by highly proliferative cells in which genomic and proteomic modifications promote molecular mechanisms that contribute to cancer pathophysiology; these molecular components serve as novel therapeutic targets and may constitute biomarkers for cancer screening.
Therefore the detection and the quantitative analysis of molecular biomarkers is a promising strategy in diagnosis and prognosis for malignant tumor and in the monitoring of the response to therapeutic treatment.
We developed a nano-immuno array for proteomic analysis in a very small quantities of sample with potential capability of pathological screening of cancer biomarkers and profiling the expression of single cells to characterize the tumor evolution.
Nanografting, a tip assisted Atomic Force Microscopy (AFM) nanolithography technique is used to fabricate DNA nanoarrays. DNA nanospots created by nanografting are exploited in order to immobilize DNA-antibody conjugates that recognize specific proteins of interest.



The determination of the antigen content of a biological sample was obtained from the analysis of AFM topographic profiles of the nanopatches before and after the sample incubation. This method has been previously set up to develop a nano-immunosensor for the successful detection of the malignant glioma biomarker GFAP in cell lysates (Bosco, Ganau et al, Nanomedicine 2014).
We focused here on the specific biomarker Human Epidermal Growth Factor Receptor 2 (Her2), relevant antigen found in some human cancers such as breast, lung and gastric ones.
For the biorecognition we tested a monoclonal human Antibody and a camelid Nanobody, specific for different epitopes of the extracellular domain of Her2 (ECD-Her2).
By measuring spot height variation of the nanospots we detected the biomarker in the picomolar range; we were also able to optimize the device sensitivity by correlating the density of the DNA-antibody conjugates on the surface and their capability to bind ECD-Her2.
As future perspectives we are also exploring the use of immobilized aptamers, small nucleic acid sequences selected in vitro, as new tool for the recognition of specific biomarkers with potential increased sensitivity; at the same time, we are exploiting the DNA barcoding of our protein binders to move towards the multiplexing detection of different biomarkers, simultaneously on the same nanoarray, to increase the prognostic value of the test.

MICROBUBBLE DRIVEN MULTIMODAL IMAGING AND THERANOSTICS FOR GLIOMAS

PATRIZIA ANDREOZZI
IRCCS Foundation Istituto Neurologico “Carlo Besta”; University of Rome Tor Vergata; Tel Aviv Sourasky Medical Centre; Seroscience Ltd.; MedCom; ESAOTE SPA; Camelot Biomedical System S.r.l.; Nanomol Technologies SA; Praxis Biopharma Research Institute SL.
patrizia.andreozzi@ifom.eu, silke.krol@ifom.eu

The idea of TheraGlio Project is to develop a multimodal imaging system for Theranostics (therapy+diagnosis) of patients bearing malignant glioma (MG), the most common primary brain tumour. MG constitute at least 35% of all primary brain tumours and are the 3rd leading cause of death from cancer in people from 15 to 34 years of age. Surgery plays a major role in the treatment of MG, since the surgical extent of resection is clearly associated with improved patient survival. Unfortunately, a complete surgical removal of these tumours is achieved in less than 20% of cases also because of technical difficulties in identifying the tumour borders during surgery. The project presents a close collaboration between surgeons, enterprises working in the field of surgical imaging, and GMP production of pharmaceuticals and nanoparticles complemented by a team of chemists and nanotechnologists. This highly interdisciplinary consortium aims at improving the outcome of surgical intervention by providing a new generation Microbubbles (MBs), designed at the University of Rome Tor Vergata that can simultaneously act as delivery system for targeted drug-loaded nanoparticles and as a multimodal contrast agent. TheraGlio will provide a device for the integration of MR imaging, US imaging, and optical intra-operative visualization, down to the molecular level of malignant glioma into the surgical procedure. This will potentially lead to a significant improvement in defining the tumour extension, understanding the disease biology, determining the functionality of the nearby normal brain tissue, and improving the patients’ overall survival. Implementation of project results would possibly improve the outcome of other severe neoplasic conditions where surgical radicality and better drug delivery is important.
The role of the NanoMedicine laboratory (NM) of Fondazione Istituto Neurologico “Carlo Besta” is to develop the iron oxide nanoparticles targeted for glioma cells and loaded with standard chemotherapeutic drugs.
The NM lab was funded to translate in close collaboration with clinicians nanomaterial based solutions for improved imaging as well as novel and innovative drugs or targeted drug delivery systems into sophisticated treatments for brain-related diseases. The lab is situated on the IFOM-IEO-campus, which is dedicated to provide different service facilities such as a well-equipped imaging facility (two-photon microscope, confocal microscopes, epifluorescence microscopes), a cell culture facility (human cell room, animal and cell cultures room etc.), as well as an electron microscopy facility. In the NM lab the relevant equipment for nanoparticle preparation and characterization is available: Malvern zeta-sizer for measurements of size and surface charge, centrifuge and access to ultracentrifuge, spectrophotometer for particle quantification, and a fully equipped wet chemistry lab.
The research leading to these results has received funding from the European Union Seventh Framework Programme FP7/2007-2013 under grant agreement n. 602923.

GLUTATHIONE PEGYLATED LIPOSOMAL METHYLPREDNISOLONE (2B3-201) AS TREATMENT OPTION FOR CENTRAL SENSITIZATION ASSOCIATED WITH OSTEOARTHRITIS

CHANTAL APPELDOORN, Rick Dorland, Nico Kuijt, Burt van der Boom, Jaap Rip, Pieter Gaillard,
to-BBB technologies BV, J.H. Oortweg 19, 2333 CH, Leiden, NL

SUMMARY

Glutathione PEGylated liposomal methylprednisolone (2B3-201) was evaluated as treatment option for central sensitization in a rat

model of osteoarthritis (OA). The observed reduction in pain after administration of 2B3-201 was associated with a reduction of reactive microglia in the spinal cord. This is in line with the central working mechanism of 2B3-201.

INTRODUCTION

OA is the most common form of degenerative arthritis, and results in loss of joint function, disability and chronic pain. Because of alterations in the CNS and the peripheral nerves that innervate the joints, OA-associated pain gradually develops the characteristics of neuropathic pain. It is this central sensitization that is the most difficult to treat. 2B3-201 is being developed as anti-inflammatory treatment for neuroinflammation associated with MS [1,2]. 2B3-201 is targeted to the CNS, and shows an improved safety and efficacy compared to free methylprednisolone (free MP) (Figure 1). Since (late stage) OA is associated with neuroinflammation and free MP (Solu-Medrol) is indicated for OA, we want to evaluate the efficacy of 2B3-201 in an animal model of OA.

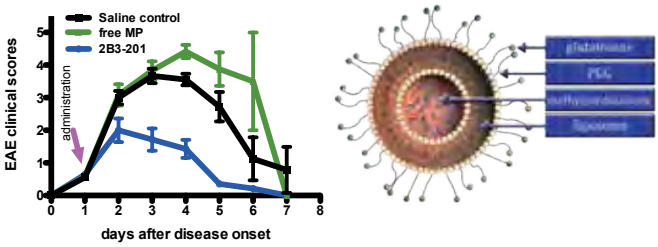


Figure 1: Glutathione PEGylated liposomal methylprednisolone (2B3-201, 10 mg/kg) showed an improved efficacy in an animal model of MS compared to free methylprednisolone (free MP, 10 mg/kg) or saline. Figure on the left is from Gaillard et al., 2012 [1].

EXPERIMENTAL METHODS

The efficacy of 2B3-201 was investigated by WuXi AppTec (Shanghai, China) in a rat model that reflects the neuropathic components of OA pain: the injection of monosodium iodoacetate (MIA; 3 mg in 25 µl saline) into the knee. The change in hind paw weight distribution (WD) was used as functional outcome and was calculated by determining the difference in the amount of weight between the left and right limbs, corrected for body weight. Treatment groups (n=8 rats) received 2B3-201 (20 mg/kg i.v.), free MP (20 mg/kg i.v.), empty liposomes (i.v.), or celecoxib (100 µmol/kg bid p.o. for 4 days) 5, 12 or 19 days after induction of OA. Rats receiving 2B3-201 or celecoxib at the late stage of the disease were further analyzed for joint pathology and spinal cord immuno-histochemistry.

RESULTS AND DISCUSSION

2B3-201 was well tolerated in this study. 2B3-201 showed a similar effect compared to free MP in reducing OA-associated pain (figure 2). In the early phase of the disease, 2B3-201 already showed an effect 1 day after treatment start, while celecoxib only showed a limited effect in reducing OA pain after 4 days of treatment (figure 2). The effect of 2B3-201 was sustained in weeks 2 and 3, while celecoxib did not show an effect at the later phases of OA. After 3 weeks of weekly treatment with 2B3-201 or celecoxib, no disease modification occurred: hind paw WD returned to baseline after each treatment cycle and the results between groups treated for 3 weeks and groups treated at week 3 only were comparable.

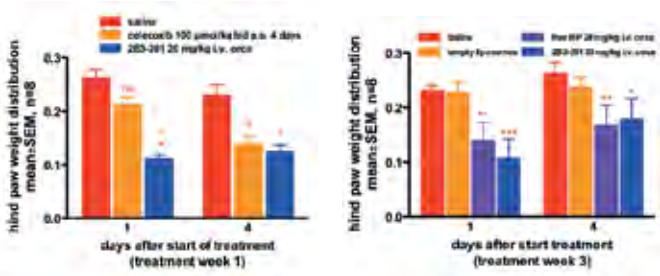


Figure 2: Efficacy of 2B3-201 in reducing osteoarthritis-associated pain. 2B3-201 is equally effective as free MP at the same dose level of 20 mg/kg. Celecoxib was only effective in the first treatment week, not at later stages of the disease.

2B3-201 and celecoxib groups treated at week 3 only were selected for further analysis. MIA significantly increased the arthritis score in the joint, which was not affected by treatment with 2B3-201 or celecoxib. This finding is in line with the aim to treat central sensitization. Activation of microglia was observed in both the ipsilateral and contralateral sides in the dorsal horn of the spinal cord. 2B3-201 significantly inhibited the activation of microglia. Celecoxib also inhibited the activation of microglia, even though no reduction in pain sensation was observed. Administration of MIA did not result in an increased number of reactive astrocytes. This could be due to the relative early time point, since reactive astrocytosis is only seen from day 28 onwards [3].

CONCLUSION

2B3-201, like free MP, was more effective than celecoxib in reducing the osteoarthritis-associated pain. The reduction in pain was associated with an inhibition of reactive microglia in the spinal cord. Further studies are warranted to evaluate the benefit of the central working mechanism of 2B3-201 in osteoarthritis.

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TARGETING THE CANCER STEM CELLS AND CANCER STEM-LIKE CELLS ISOLATED FROM CELL LINE BY LIPOSOMAL NANOPARTICLES CONJUGATED WITH MONOCLONAL ANTIBODIES

L. ARABI[1], A. Badiee[1], M. Jafaari[1]
[1]Nanotechnology Research Center, Department of Pharmaceutical Nanotechnology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran Arabil881@mums.ac.ir, leilaa.arabi@gmail.com

OBJECTIVES

Recently, the theory of cancer stem cells (CSCs), the existence of a distinct subpopulation of cancer cells that possesses the capacity to self-renew and to cause the heterogeneous lineages of cancer cells has presented new targets and orientations for tumor therapy(1,2). A growing body of evidence supports a role for these cells in tumor recurrence and metastasis (3-5). CSCs have a number of properties permitting them to survive conventional cancer chemotherapy and radiotherapy (6). Based on the above information, the development of alternative therapeutic approaches using nanotechnology-derived targeted drug delivery systems specifically targeting CSCs seems necessary. The present study was designed for the isolation and characterization of putative CSC populations in established C26 murine colon carcinoma cell line and targeted drug delivery to these cells via liposomal nanoparticles conjugated with CD44 and CD133 monoclonal antibodies (mAbs).

MATERIALS AND METHODS

The major difficulties in researching CSCs are isolation and purification of these cells. We analyzed the expression of define phenotypic profiles, including CD133+, CD44+ and EpCAM+ reported as CSC¬specific in human primary colorectal cancer (CRC)(7,8), on established murine CRC cell line (C26) by flow cytometry. Then isolation or enrichment of the putative CSC population was carried out by Magnetic Activated Cell Sorting (MACS) based on the expression of CD133 cell surface proteins. After sorting, these populations were evaluated for CSC properties in comparison to their negative counterparts or to the parental cell line. Stemness-related gene expression (Oct-4, SSEA-1), spheroid formation ability in serum-free medium and tumorigenicity upon injection in mice were assessed. On the targeting part of study, nanoliposomes consisted of HSPC, cholesterol, mPEG2000-DSPE were prepared by thin film method plus extrusion. mAbs attached to the distal end of NHS functionalized mPEG3400¬DSPE was post inserted into nanoliposomes. Cell interaction and MTT test was carried out in order to evaluate the rate of cellular uptake and the cytotoxicity of formulations.

RESULTS AND DISCUSSION

The flow cytometry results showed that the percent of CD44. CD133, EpCAM were 99.9%, 0.9%, 0.1%

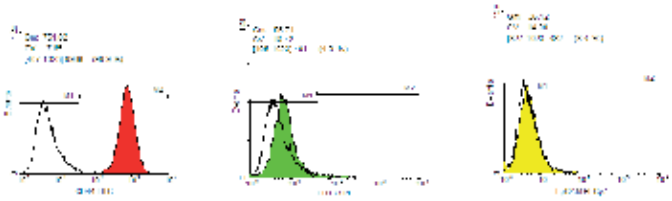
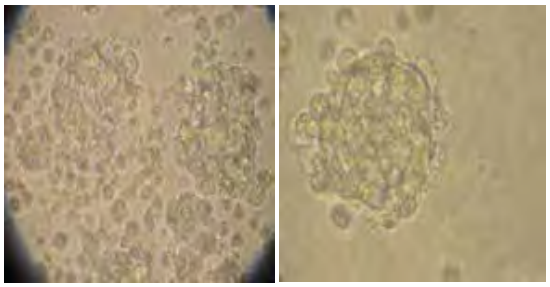


Figure1: Expression of CD44. CD133, EpCAM markers in C26 cell line by flow cytometry

After isolation of cells according to the CD133 marker by MACS , the sorted cells could form spherical clones in serum-free culture media, but the rate of clonegenesis of CD44+CD133+ and CD44+CD133_ cells was similar.



CD44+CD133_ cells CD44+CD133+ cells Figure 2: Colony formation ability of different cell populations

The isolated cells according to the CD133 marker were enriched at the same time for the EpCAM marker. It could be as a result of the simultaneous expression of these two markers in the cells. In vivo, CD44+CD133+ cells showed greater ability to form tumor (about 1•105cells were sufficient) in comparison to CD44+ CD133- population (3•105 cells were needed).This step should be repeated in order to confirm the significant difference in tumorigenicity of subpopulations. Cell interaction results after 3hr incubation of cells with formulations showed significant cellular uptake of mAb modified liposomes in comparison with PEGilated liposomes. This results show that mAb modified liposomes are more efficient intracellular drug delivery formulations than stealth liposomes.

CONCLUSION

This study indicated that C26 cell line probably contained some distinct subpopulation with stem cell properties and combination of some of these cell surface proteins could be cancer stem cell markers for colon carcinoma. These results could provide an important research tool for testing and developing novel targets for cancer therapy and mAb modified liposomes against CSC markers could be as a promising and efficient platform for an active targeted delivery system to cancer cells and CSCs.

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BIODEGRADABLE MAGNETIC NANOCAPSULES OF IXABEPILONE: IN VITRO STUDY

[2]**AVGOUSTAKIS KONSTANTINOS**, [1]Koutsospyrou Nefeli, [1]Andreas Sergides, [1,2]Eirini Ioannou, [1]Athina Angelopoulou, [1]Bakandritsos Aristides

[1]Department of Pharmacy, [2]Department of Materials Science, University of Patras, Patras 26500, Greece.

INTRODUCTION

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

METHODS

2.1 Synthesis of Polymers

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

2.2 Preparation and characterization of nanocapsules

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

2.3 Cell studies

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

RESULTS

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

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

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

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

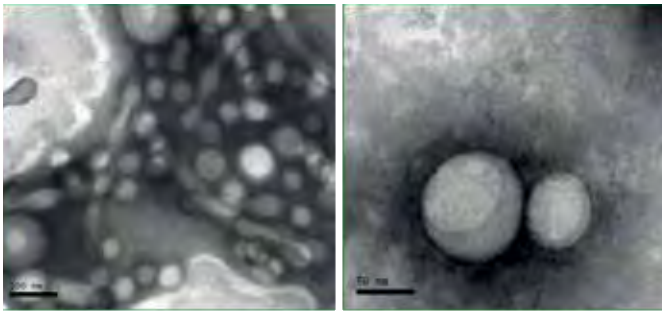

Figure 1. TEM image for PLA(20)-PEG(5)-IXA nanocapsules.

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

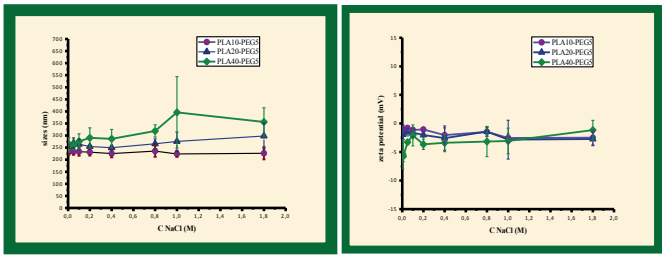

Figure 2. Hydrodynamic diameter and ζ-potential of nanocapsules in the presence of NaCl (at 25 oC).

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

CONCLUSIONS

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

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Acknowledgement: This research has been co-financed by the European Union (European Social Fund-ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF)-Research Funding Program : Synergasia. Investing in knowledge society through the European Social Fund..

CERIA COATING OF SILICA NANOPARTICLES REDUCES THEIR ATTENUATION EFFECTS ON VASODILATOR FUNCTION, EX VIVO

MAY AZZAWI*, Asima FAROOQ, Teba MOHAMED, Debra WHITEHEAD†
Corresponding authors:
*Dr. May Azzawi, The School of Healthcare Science, Faculty of Science and Engineering, Manchester Metropolitan University, Manchester, M1 5GD, UK.
Tel +44 0161 247 3332, Email m.azzawi@mmu.ac.uk
†Dr. Debra Whitehead, The School of Biology Chemistry & Environmental science, Faculty of Science and Engineering, Manchester Metropolitan University, Manchester, M1 5GD, UK.
Tel +44 161 247 3341, Email d.whitehead@mmu.ac.uk

BACKGROUND AND AIM

Ceria nanoparticles (CeNPs) have attracted considerable interest in the treatment of a number of conditions associated with increased production of reactive oxygen species (ROS), due to their unique antioxidant properties (Korsvik et al, 2007). We have previously demonstrated the attenuation in vasodilation of aortic vessels after uptake of silica nanoparticles (SiNPs), which improved after co-incubation in SOD, suggesting a role for ROS in quenching nitric oxide (NO) (Farooq et al, 2012). In the present study, we examined whether coating of SiNPs with ceria, would reduce/prevent SiNP induced attenuation in vasodilation, hence increase their biocompatibility.

METHODS

SiNPs were synthesised via a sol-gel precipitation method and the ceria nanoparticulate shell was grown on the SiNPs surface as previously described by Oh et al. (2010). Nanoparticles were characterised using transmission electron microscopy (TEM). Thoracic aortic arteries from male Wistar rats (150-250 g) were excised after humane killing following institutional approval and in accordance with European Commission Directive 86/609/EEC guidelines. Aortic rings were mounted in an organ-bath system and tension recorded using Labchart 6 (Powerlab, AD Instruments, UK). Responses to endothelium-dependent dilator agonist were examined by adding cumulative doses of acetylcholine (ACh; 0.01-100 μM), before and after incubation with NPs (1.96×1012 NPs mL-1) for 30 min. Data are expressed as mean ± standard error of mean (SEM) with 'n' representing the number of vessels. Dilator responses are expressed as percent relaxation. Statistical significance is taken as $P<0.05$.

RESULTS

The SiNPs were monodispersed with an average diameter of 47±8 nm (Figure 1A). The addition of ceria around the SiNPs increased the diameter to 50±4 nm with the individual CeNPs size of 3.8±1.2 nm (Figure 1B, C). The energy dispersive spectroscopy (EDS) of SiNPs confirms the presence of silica. The CeSiNPs contain both silica and ceria. Nanoparticle uptake was identified within the cytoplasm of endothelial cells. We demonstrate that while SiNPs significantly attenuate endothelial-dependent (acetylcholine-ACh) vasodilation, their surface modification with CeNPs leads to significant improvement in dilator responses (n=5, $p<0.001$, at most ACh concentrations).

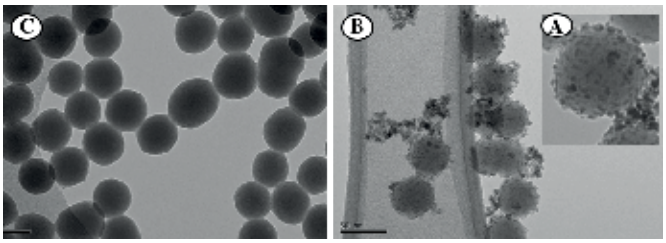

Figure 1: TEM image of A) SiNPs (47±8 nm); B) CeSiNPs (50±4 nm), C) enlarged image of CeSiNP.

CONCLUSION

These findings have implications in the fabrication of biocompatible nanoparticles for medical intervention. Furthermore, CeSiNPs may represent novel therapeutic tools for the protection and treatment of conditions where attenuated dilator responses are observed.

Key words: nanoparticles; silica; ceria; free-radical; vasodilation.

ACKNOWLEDGEMENTS: We thank Dave Maskew, MMU, for technical support; Dr. Aleksander Mironov, EM facility, Faculty of Life Sciences, University of Manchester, for his assistance in TEM.

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QUANTITIVE SIZE AND STRUCTURE CHARACTERIZATION OF NANO-DRUGS

MORIA BARLEV-GROSS[1], Erez Koren[1], Avigael Gotlib[1], Yaelle Schlit[2], Sivan Peretz-Damari[3], Einat Nativ-Roth[3], Oren Regev[3], Uri Raviv[2] and Yechezkel Barenholz[1]

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

[2]The Institute of Chemistry, The Hebrew University of Jerusalem, Israel

[3]Department of Chemical Engineering, Ben-Gurion University of the Negev, Beer-Sheva, Israel

The design of nano-drugs of precise size and shape/structure distributions is a crucial parameter for nano-drug performance, as it influences its physical properties including in vivo pharmacokinetics, biodistribution and active pharmaceutical ingredient (API) release. In this work, we have prepared liposome-based nano-drugs in controlled size and shape distributions with identical lipid compositions. We started with nano-liposomes of identical size-distribution, but by remote-loading with increasing drug levels, their shape changed from spherical to prolate ellipsoid with increasing axial ratio. The higher the drug-to-lipid ratio, the larger the axial ratio was. These nano-liposomes were characterized using a variety of techniques, including dynamic light scattering (DLS), quantitative cryo-transmission electron microscopy (cryo-TEM), and solution X-ray scattering analysis*. Our studies also show the maximum amount of drug that can be loaded per nano-liposome. Our results indicate that cryo-TEM combined with the quantitive X-ray scattering analysis (rather than the DLS technique) is the preferred approach to accurately characterize non-spherical particles, and that the assumption used in commercial DLS, that all particles are spherical, can be misleading when exploring certain nano-scale particles that deviate from spherical shape. Our cryo-TEM analysis determined shape parameters including axial ratio distributions, liposomal volume distributions as well as the physical shapes of the drug in the liposomes. In addition, small-angle X-ray scattering (SAXS) measurements described the dimensions of intra-liposomal drug crystals with high correlation to the amounts of encapsulated drug inside the nano-liposomes. The SAXS and the quantitative cryo-TEM analyses were highly consistent with one another.

Understanding how size and shape can affect the biodistribution of intra-vascular injected particles is of major importance, both for the rational design of drug delivery systems and for regulatory standardization.

LABORATORY OF NANOSTRUCTURES FOR PHOTONICS AND MEDICINE AS A LEADER IN NANOPARTICLES PRODUCTION AND CHARACTERIZATION

WOŹNIAK BARTOSZ, J. Rogowska-Tylman, S. Kuśnieruk, T. Chudoba, J.Wojnarowicz, E. Pietrzykowska, W. Łojkowski

Laboratory of Nanostructures for Photonics and Medicine, Institute of High Pressure Physics, Polish Academy of Sciences, Sokołowska 29/37 01-142 Warsaw, Poland

e-mail: _bwozniak@unipress.waw.pl

Nowadays, the aim of tissue engineering is to replace conventional treatments like organ transplants, bone grafting or artificial implants. To repair lost or damaged tissues nanomaterials-based devices are used. Laboratory of Nanostructures for Photonics and Medicine specializes in the production of nanoparticles and nanocoatings intended for the use in medicine such as nanohydroxyapatite or nano zinc oxide particles. Nanohydroxyapatite is the main mineral of which dental enamel and dentin in human body are composed. Synthetic nano HAp main advantages are good biocompatibility with surrounding tissues. However, its bioresorbability is limited. In our laboratory we achieved a novel nano HAp structure with a much higher degradation rate than commercially used nanoparticles. Addition of nano HAp particles during manufacturing process of biomaterials can significantly increase biocompatibility and bioresorbability of future devices. Moreover, nano HAp can induce bone growth around dental implants or into polymer scaffolds during bone healing process. On the other hand, nano zinc oxide particles show antimicrobial properties which can be used in decreasing antibiotic resistance therapies or to decrease the problem of inflammation around an implantation site.

Laboratory of Nanostructures for Photonics and Nanomedicine also specializes in nanomaterials characterization. Parameters such as size distribution, phase composition, density, surface area and zeta potential can be studied using world's most advanced equipment. Materials testing is perfomed according to ISO standards by a well qualified group of laboratory staff.

LC-100, A NOVEL PEGYLATED LIPOSOMAL DOXORUBICIN NANO-DRUG WITH GREATER SAFETY AND THERAPEUTIC EFFICACY THAN DOXIL®

YAELLE BAVLI[a], Tal Berman[a], Ilan Winkler[b], Doron Friedman[b] and Yechezkel Barenholz[a,b]

[a]The Laboratory for Membrane and Liposome research, IMRIC, The Hebrew University - Hadassah Medical School, Jerusalem, Israel

[b]LipoCure Ltd, Jerusalem, Israel

INTRODUCTION

Doxil® (pegylated liposomal doxorubicin = PLD) is the first FDA approved (1995) nano-drug and is still in extensive clinical use. PLD is based on three principles: (i) prolonged drug circulation time and avoidance of the RES due to the use of pegylated nano-liposomes; (ii) high and stable remote loading of doxorubicin driven by a transmembrane ammonium sulfate gradient, which allows for drug release at the tumor site; and (iii) having the liposome lipid bilayer in a "liquid ordered" phase. Due to the EPR (enhanced permeability and retention) effect, Doxil is "passively targeted" to tumors. Doxil/Lipodox (approved generic) has a characteristic "coffee bean" shape due to its intraliposome long, stable crystals which impose on the nano-liposome a transformation from a sphere to a prolate ellipsoid shape (Barenholz JCR 2012).

Berman et al. abstract describes the design, R&D and characterization of LC-100; a spherical ~85 nm PLD in which the doxorubicin was remotely loaded by a transmembrane gradient of ammonium-methanesulfonate. The doxorubicin-methanesulfonate salt in the intraliposome aqueous phase doesn't form a long crystal and maintains the liposome in spherical shape. Moreover, the drug release rate of LC-100 was significantly faster than of Doxil/Lipodox in tumor microenvironment conditions.

LC-100 and Lipodox were compared in two studies: 1. Severity of

palmar-plantar erythrodysesthesia (PPE) induced by liposomal doxorubicin products; 2. Evaluation of anti-tumor potential using a breast cancer (MDA-MB-231) xenograft model.

EXPERIMENTAL METHODS

1. PPE:

Forty male Sprague-Dawley rats were injected intravenously with LC-100 or Lipodox®, twice weekly for 40 days (total of 12 injections) at 1mg/kg. The clinical symptoms observed on rats were scored according to a six-point severity grading system on six different areas of the body. The maximum lesion score at any one scoring time point is thus 36.

2. Breast cancer xenograft model:

Efficacy of LC-100 was tested in a breast cancer xenograft model. In short, female Athymic Nude mice bearing 130 ± 15% mm3 tumor volume (derived from the MDA-MB-231 cell line) were injected intravenously at 4mL/kg once a week for 3 weeks with saline, Lipodox, free doxorubicin, or LC-100 (n=8 in each group). The solid tumor length (longest axis) and width (shortest axis) were measured using a digital Vernier caliper.

RESULTS

1. PPE:

The PPE model demonstrates the superiority (two tailed, t-test, p=0.0007) of LC-100 over Lipodox in minimizing the severity of the dermal lesions following twice weekly I.V. administrations for 6 weeks (Fig 1).

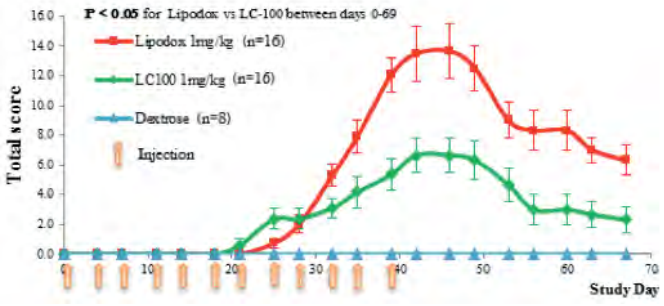


Figure 1 describes the total scoring average (±SE) during the study (period of injection followed by 4 weeks of recovery). P<0.05 for Lipodox versus LC-100 between days 0-69 (Two-tailed T-test).

2. Breast cancer xenograft model:

Tumor growth results revealed 69.3% and 55.2% inhibition for LC-100 and Lipodox, respectively following once weekly administration for 3 weeks. The mice bearing mammary tumors and injected with LC-100 had a better survival rate and a superior delay in tumor growth (higher tumor growth inhibition) when compared with mice injected with Lipodox and free doxorubicin (Fig 2).

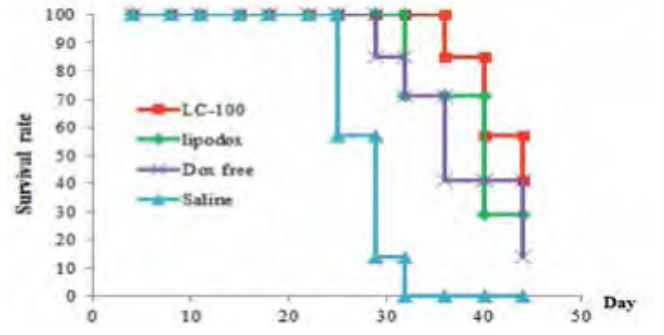


Figure 2 depicts survival of mice as a function of the dose of drug administered. The event was counted as "death" when mice died spontaneously or if tumor volume reached 2500mm3 (the mice were sacrificed) (n=8 in each group).

CONCLUSION

The results of our in vivo studies clearly show that LC-100 met our expectations and that changing the physical state of the intra-liposome drug had a major effect on the PLD performance by decreasing the toxicity resulting in PPE, while maintaining or even improving the therapeutic efficacy.

STUDIES ON THE AGGREGATION OF LIPOSOMES; MECHANISM OF PARTICLE FORMATION AND IN VIVO EVALUATION OF IT IN COMPLEMENT ACTIVATION RELATED PSEUDOALLERGY (CARPA)

K. BENEDEK[1, 3]*, T. Mészáros[1], A. Kovács[1], R. Urbanics[2], J. Szebeni[1] and L. Rosivall[1]

[1]Nanomedicine Research and Education Center Semmelweis University Budapest Hungary; [2]Seroscience KFT, Budapest, Hungary

[3]iGORi Thousand Oaks CA USA,

* to whom correspondence should be sent (kbenedek@igori.com)

Aggregation in a drug product is one of the main concerns of pharmaceutical industry. Aggregation could occur as a result of various internal events during production. The aggregation of PEGylated liposomes could also take place but the process might be very slow under general formulation conditions. The most sensitive evaluation of the immune toxicology of a drug is the complement activation related pseudoallergy (CARPA) method. It is suggested that in PEGylated liposome formulation the CARPA reaction initiated by aggregates. However there is no direct evidence to prove that.

The particle formation behavior of PEGylated liposomes were studied by applying various ionic strength solutions, under salting out conditions. It is illustrated in Figure 1 that empty and filled liposomes could form large particles by the method, indicating that the effect is associated with the liposome in general and not with the drug.

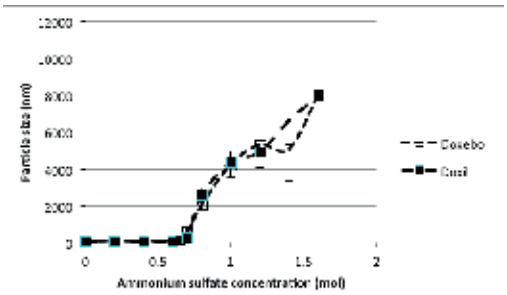


Figure 1, Increasing particle size formation as a function of salt concentration. Particle size was measured by the Malvern Nanosizer DLS instrument.

Preparation of liposome aggregates at will allowed a study on the mechanism of aggregation and the consequences aggregates in vivo. The liposome suspension was mixed with the ammonium salt solution and the final salt concentration turned to 2 Molar.

This suspension was subjected to the CARPA assay. The man-made liposome particles initiated a dose dependent CARPA reaction. The assay consisted of the measurement of the systemic arterial pressure (SAP) and pulmonary arterial blood pressure (PAP). The data are displayed in Figure 2.

The experiment proved the direct relationship between liposome particles and the in vivo CARPA system as illustrated on Figure 2. It shows that aggregates are making the CARPA reaction. The control solvent showing no reaction at all and the response of the starting Doxebo is negligible.

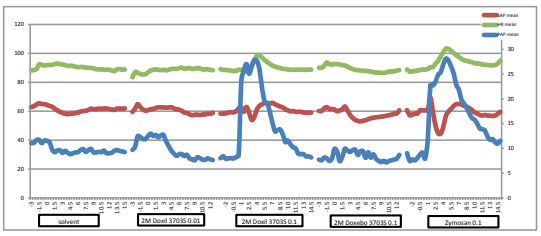


Figure 2, CARPA reaction of aggregates produced by various samples. The group of data is related to the media, the second group are the reaction to the low dose 2M Doxil, the third group associated with the high dose 2M Doxil, the fourth group related to the high dose Doxebo and last the fifth group of data were generated by Zymosan control.

The understanding of the particle formation in drug development is one of the most important information needed for formulation. The understanding of the thermodynamics and kinetics of particle formation of the formulation components is the key to safety and long shelf life.

IMPACT OF SURFACE MODIFICATION ON TOXICOLOGICAL BEHAVIOR OF CARBON-NANOTUBES

FODOR BERTALAN, E. Kiss-Tóth Dojcsak, J Emmer, E. Kiss-Tóth, Z Lovrity
University of Miskolc, Department of Nanobiotechnology and Regenerative Medicine, HUNGARY

INTRODUCTION

the increasing number of synthetic and environmental nanomaterial has an emerging importance of characterization their biological behavior. The nanotoxicology is a promising discipline focusing on the investigation of relationship of nanoparticles and the biological systems. The carbon-nanotubes (CNT's) are producing in an excessively amount and widely used in the different field of chemistry, biology and medical sciences. They have several advantageous chemical properties but their impact on the living cells is very controversial yet.

AIM

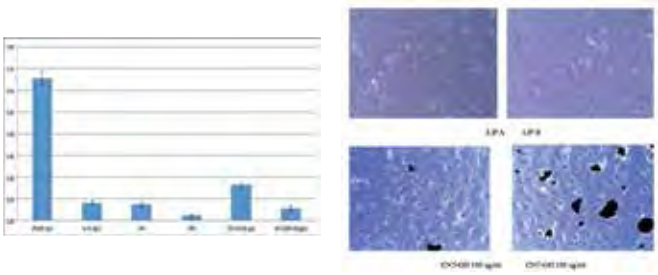
our purpose was to investigate the effect of nanotubes on the function of different types of blood cells and endothelial cells. We compared the impact of surface modification (OH vs. COOH) of CNT's on it's immunotoxicological behavioral characteristics.

METHODS AND MATERIALS

we investigated single walled CNT-s functionalized in different ways. (CNT-OH, CNT-COOH) The CNT solutions (100 mg/ml final concentration) were applied on the human blood samples. e studied the involvement of JAK/STAT signaling pathway in the process of "nanomaterial-derived" activation of human NK cells and monocytes. We determined the apoptosis induction capability of CNT's on peripheral blood cells by Annexin V labeling methods. Finally we compared the gene expression rate of E-selectin in HUVEC cells treated with OH and COOH CNT's to the liposome induced endothelial cells.

RESULTS

The expression rate of surface CD11c and CD123 (dendritic cell markers) was a slightly elevated in presence of CNT, independently the type of functionalization. The CNT-OH had significantly greater basophilic activating capacity than the CNT-COOH according to the CD63 antigen expression. We didn't find any sign of activation of JAK/STAT signaling pathway in NK cells and monocytes in presence of CNT's. The amount of E-selectin mRNA significantly elevated in the CNT-OH treated cells compared to CNT-COOH and liposome induced HUVEC cells.



DISCUSSION

Nanotoxicological characterization often has more difficulty, but increasingly has importance as an essential process for environmental and occupational health aspects, both in terms of preparing potential nanomedical products. Our presentation aims to raise awareness of these proceedings. The CNT has a number of beneficial properties but unfortunately in most cases it has serious problems in biological applications. Our work pointed out, that the surface modification of nanomaterials (CNT also) determines the basophil activation. It could lead to a severe allergic reaction in the human body. In spite this we didn't observed dramatically changing in the antigen presenting DC cells and monocytes nor the rate of complement activation. The OH modification of CNT's surface means increased activation capability to this nano material compared to COOH modification. In every flow cytometric analysis, related to CNT we observed difficulty to interpret these data, because we detected a large amount of non-specific fluorescent signals (probably due to aspecific absorption to the CNT's wall) what can lead to misinterpret the immunotoxicological results.

Acknowledgements: This research was carried out as part of the TÁMOP-4.1.1.C-12/1/KONV project in the framework of the New Hungarian Development Plan. The realization of this project is supported by the European Union, co-financed by the European Social Fund.

A PHOTOSENSITIZER DELIVERED BY BISPECIFIC ANTIBODY RETARGETED HUMAN T LYMPHOCYTES BOOSTS CYTOTOXICITY AGAINST CARCINOMA CELLS UPON LIGHT IRRADIATION

ANDRÉ-RENÉ BLAUDSZUN[1], Gerhard Moldenhauer[2], Marc Schneider[3] and Anja Philippi[1]
[1]Environment and Bio Group, KIST Europe Forschungsgesellschaft mbH, Saarbrücken, Germany
[2]Division of Translational Immunology, German Cancer Research Center, Heidelberg, Germany
[3]Department of Pharmaceutics and Biopharmacy, Philipps-University Marburg, Marburg, Germany

Photodynamic therapy (PDT) is an emerging treatment modality for cancer. In PDT a light-sensitive photosensitizer (PS) is administered and after a latency period the malignant lesions are selectively irradiated with light of a specific wavelength, thereby exciting the photosensitizing agent. In the presence of oxygen, activated PS-molecules generate singlet oxygen and reactive oxygen species, which provoke lethal oxidative damage in aberrant cells. However, tumor selectivity after systemic application of PS is limited and healthy tissues also accumulate PS. The general distribution of the photosensitizing agent leads to adverse effects, like eye and skin photosensitivity. In order to circumvent these obstacles, tumor-targeting PDT strategies are under investigation.
Here, we report on the feasibility of a cell-based drug targeting concept, using bispecific antibody (bsAb, EpCAM×CD3) redirected human T lymphocytes as selective transport vehicles for the model photosensitizer 5,10,15,20-tetrakis(3-hydroxyphenyl)-porphyrin (mTHPP) in vitro. This photosensitizing agent is the parent porphyrin of mTHPC, which is the active pharmaceutical ingredient of Foscan. In the context of adoptive cell transfer (ACT), the concept aims at enhancing the selectivity and efficacy of PDT, while simultaneously decreasing adverse effects. Most notably, the approach intends to combine the phototoxicity of PS-molecules delivered by redirected CD4+ and CD8+ T cells (drug effect) with the cytotoxicity of retargeted CD8+ T cells (T cell effect) in a synergistic manner (Figure 1.).

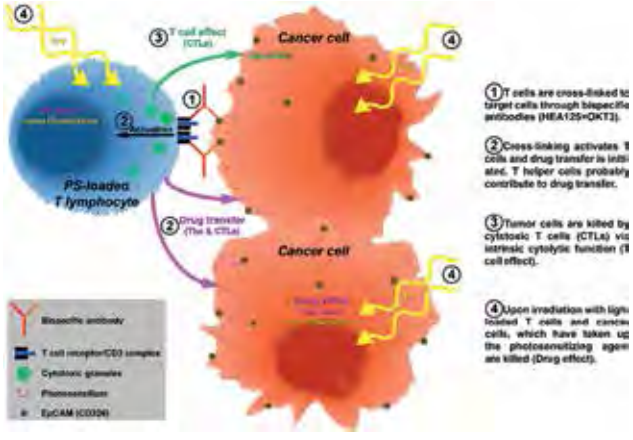


Figure 1. Schematic illustration of T cells as living target site specific drug delivery system

We have demonstrated that ex vivo activated human polyclonal T lymphocytes take up water-soluble complexes composed of hydrophobic mTHPP and poly(styrene sulfonate) sodium salt (PSS) after short-term incubation (Figure 2).

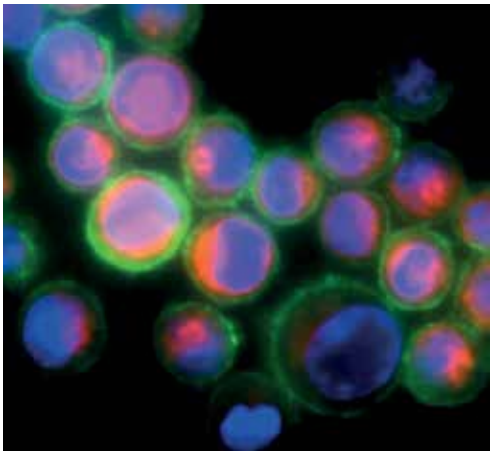


Figure 2. Fluorescence microscopy image showing human T cells loaded with polymer-photosensitizer complex PSS/mTHPP (red fluorescence). Cell nuclei were stained with Hoechst dye (blue). Cell membranes were labeled with FITC-conjugated anti-CD3 antibodies (green) (A. Philippi and A.-R. Blaudszun, Eur. J. Nanomed. 2014; 6(1): 9-10)

In absence of light and when drug loading occurred at a tolerable concentration of PSS/mTHPP-complexes, viability and cytotoxic function of carrier cells were not impaired. When "drug-enhanced" T cells were co-cultivated with EpCAM expressing human carcinoma cells, mTHPP was transferred to target cells. Interestingly, in the presence of bsAb, which cross-links effector and target cells thereby inducing the cytolytic activity of cytotoxic T lymphocytes, significantly more PS was transferred. Therefore, the bsAb does not only provide tumor cell specificity but also indirectly enhances drug specificity. Consequently, redirected drug-loaded T cells were more effective in killing A549 lung carcinoma and SKOV-3 ovarian carcinoma cells than retargeted unloaded T lymphocytes upon irradiation of co-cultures. Notably, the additive approach using redirected unloaded T cells in combination with separately applied PSS/mTHPP equal to the amount of complex carried by T cells was less efficient, as well. Thus, the combined cytotoxicity of transferred photosensitizer molecules and T lymphocytes exhibited synergistic antitumor effects. Our findings support the feasibility of the envisioned T cell-based targeted PDT approach.

IDENTIFICATION AND CHARACTERIZATION OF NOVEL DRUG TARGETS FOR PERSONALIZED BREAST CANCER NANOMEDICINE

INES BLOCK[1,2], Carolin Müller[1,2,3], Daniel Sdogati[1,2], Aleksandra Jaskot[1,2], Birgyl Kruezi[1,2], Henriette Pedersen[1,2], Pernille Lund Hansen[1,3], Mads Thomassen[1,4], Torben Kruse[1,4], Petra Kioschis[1,5], Jan Mollenhauer[1,2]

[1]Lundbeckfonden Center of Excellence in Nanomedicine NanoCAN, Institute for Molecular Medicine, University of Southern Denmark, Odense, Denmark

[2]Molecular Oncology, Institute for Molecular Medicine, University of Southern Denmark, Odense, Denmark

[3]Clinical Physiology and Nuclear Medicine, Odense University Hospital, Odense, Denmark

[4]Clinical Genetics, Odense University Hospital, Odense, Denmark

[5]Institute of Molecular and Cell Biology, University of Applied Sciences, Mannheim, Germany

Personalized cancer nanomedicine aims at the design of novel nanodrugs that would match the molecular fingerprint of an individual patient`s tumor. Expression profiling and next generation-sequencing data represent rich resources for discovering new starting points for such approaches. Here, we selected a set of 140 genes, which have been proposed to show potentially relevant alterations in breast cancer by genome-wide next-generation sequencing. We constructed a panel of isogenic breast cancer cell lines and systematically analyzed the normal and the mutant gene variants for their effects on breast cancer cells.

After completing about half of the primary screen, several novel growth modulators for breast cancer were identified. To this end, we will present data on detailed follow-up analyses of two of these novel genes, which represent novel breast cancer tumor suppressors. At least one of these may provide starting points for the consecutive design of synthetic lethal screens for personalized nanodrugs.

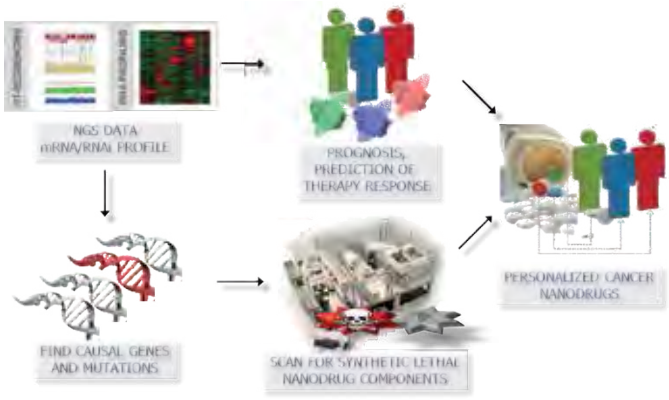


Fig. 1: Strategy of the project. Based on expression profiling and next generation-sequencing (NGS) data we aim at the systematic identification of causal genes and mutations in breast cancer using recombinant breast cell lines. Newly identified breast cancer driver genes may further provide promising scenarios for consecutive synthetic lethal screens to recover novel starting points for the development of personalized nanodrugs.

ETCHABLE PLASMONIC NANOPARTICLE PROBES TO IMAGE AND QUANTIFY CELLULAR INTERNALIZATION

GARY B. BRAUN[1,2]*, Tomas Friman[1,2], Hong-Bo Pang[1], Alessia Pallaoro[3], Tatiana Hurtado de Mendoza[1], Anne-Mari A. Willmore[4], Venkata Ramana Kotamraju[1,2], Aman P. Mann[1], Zhi-Gang She[1], Kazuki N. Sugahara[1], Norbert O. Reich[3], Tambet Teesalu[1,2,4], Erkki Ruoslahti[1,2]*

[1]Cancer Research Center, Sanford-Burnham Medical Research Institute, La Jolla, CA 92037, USA.

[2]Center for Nanomedicine, Sanford-Burnham Medical Research Institute at University of California, Santa Barbara, CA 93106, USA.

[3]Department of Chemistry and Biochemistry, University of California, Santa Barbara, CA 93106, USA.

[4]Laboratory of Cancer Biology, Institute of Biomedicine, Centre of Excellence for Translational Medicine, University of Tartu, Tartu, 50411, Estonia.

There is considerable interest in using nanoparticles as labels or to deliver drugs and other bioactive compounds to cells in vitro and in vivo. Fluorescent imaging, commonly used to study internalization and subcellular localization of nanoparticles, does not allow unequivocal distinction between cell surface-bound and internalized particles, since there is no methodology to turn particles 'off.' We have developed a simple technique to rapidly remove silver nanoparticles outside living cells leaving only the internalized pool for imaging or quantification. The silver nanoparticle (AgNP) etching is based on the sensitivity of Ag to a hexacyanoferrate/thiosulfate redox-based destain solution. In demonstration of the technique we present a new class of multicolored plasmonic nanoprobes comprising dye-labeled AgNPs that are exceptionally bright and photostable, carry peptides as model targeting ligands, can be etched rapidly and with minimal toxicity in mice and that show tumour uptake in vivo.

We describe an etching method we believe to be a powerful tool for studies of nanoparticle uptake into cells, combined with fluorescence tracking that exploits plasmonic enhancement from silver cores. Simply put, most nanoparticles used in the literature have no

'off-switch', leading to complications in background descrimination in biological assays. We present new nanomaterials that consist of etchable, dye-labeled, peptide-carrying silver for fluorescence and darkfield imaging, flow cytometry, and elemental analysis. The etch we describe is a non-membrane permeable combination of thiosulfate/hexacyanoferrate anions that were found to be non-toxic and highly efficient for silver dissolution. We also leverage etching as an aid for coat composition analysis during synthesis.
Silver is gaining prominance in biological assays due to its intriguing and complex plasmonic resonance properties that operate through the concentration of electromagnetic radiation near the surface, enhancing chromophores and increasing photostability by lifetime modulation, while the Ag core exhibits resonant scattering. Future applications of these tunable materials could utilize multi-photon excitation for 3-D localization in tissue or exploit non-linear electronic processes. Many coatings may be designed around the etchable plasmonic core. Broadly useful, metal nanoparticles can be detected against a low biological background through elemental analysis, enabling pharmacokinetic profiling of prototype nanomedical vectors.
This class of etchable and bright nanoparticles should make possible and fuel a multitude of studies aimed at cell biology and nanomedicine.

MINIATURIZED ASSAYS FOR POINT-OF-CARE THERAPEUTIC DRUG MONITORING

DIANA BURGHELEA, Denis Prim, Marc Pfeifer, Jean-Manuel Segura
Institute of Life Technologies, University of Applied Sciences Western Switzerland Valais, Route du Rawyl 64, CH-1950 Sion, Switzerland

Many modern therapeutics involved for instance in the treatment of infections, cancer or in post-transplant therapies require Therapeutic Drug Monitoring (TDM) owing to their narrow therapeutic range. Currently this process is demanding for the patient, as several milliliters of blood are required, slow and costly, as the sample need to be transferred to a central laboratory, and suffer of limited efficacy, as the results are difficult to interpret for a non-specialist. To overcome these problems, we aim at providing a simple, rapid and sensitive solution by developing a compact and cost-effective Point-Of-Care (POC) drug quantification device based on miniaturized competition immunoassays. First results have demonstrated the feasibility of downsizing Fluorescence Polarization immunoassays and shown that the two prototypical drugs tobramycin and tacrolimus, an antibiotic and an immunosuppressant, can be quantified using minute amounts (only 20 µl) of human blood. For tobramycin, the assay could be further miniaturized down to just one µl of human serum while preserving its performance. Moreover, the assays could be transposed onto custom-made FP instruments as a first step towards a Point-Of-Care Therapeutic Drug Monitoring device.

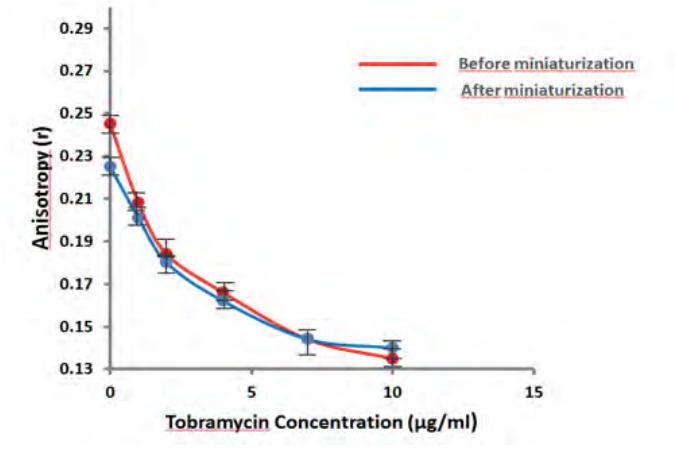


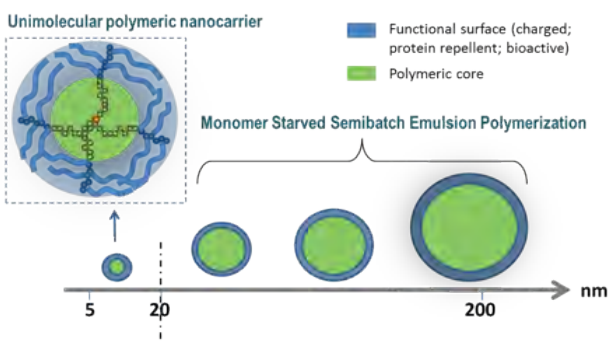
Figure 1: Quantification of tobramycin by a miniaturized Fluorescence Polarization Immunoassay using 1 µl of human serum

UNIMOLECULAR AND MULTIMOLECULAR POLYMERIC NANOCARRIERS FOR TARGETING PODOCYTES IN KIDNEY GLOMERULUS

FRANCESCO CELLESI[1,2,3], Riccardo Bruni[1,2,3], Claudio Colombo[3], Li Min[1,2,3], Shojiro Watanabe[2],Davide Moscatelli[3], Piergiorgio Messa[2], Maria Pia Rastaldi[2]
[1]Fondazione CEN - European Centre for Nanomedicine, Piazza Leonardo da Vinci 32, 20133 Milan, Italy;
[2]Renal Research Laboratory, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Via Pace 9, 20122 Milan, Italy;
[3]Dipartimento di Chimica, Materiali ed Ingegneria Chimica "G. Natta". Politecnico di Milano, Via Mancinelli 7, 20131 Milan, Italy

Chronic kidney diseases (CKD) are recognized as a major health threat worldwide, due to the exponential epidemics of nephropathies associated to obesity, diabetes and hypertension[1], with frequent progression to terminal renal failure and higher risk of cardiovascular events even in subjects with mild kidney dysfunction[2]. The majority of CKD are characterized by defects of the glomerular filtration barrier, where podocytes guarantee selective filter permeability[3]. Several experimental results have suggested that all drugs currently in use to treat or slow progression of glomerular damage, such as steroids, immunosuppressive agents, ACE-inhibitors, have a direct action on podocytes. These therapies are charged by severe side effects, particularly when a systemic prolonged administration is required. Therefore, there is an urgent need of more specific therapies and of a cell-targeted administration of novel and traditional drugs.
The aim of this work was to evaluate the potential of biodegradable polymeric nanocarriers for targeting podocytes in the kidney glomerulus. Multimolecular poly(ε-caprolactone)-based nanoparticles were synthesized according to a Monomer Starved Semibatch Emulsion Polymerization Process (MSSEP)[4]. Depending on the chemistry of the monomers and process conditions, nanoparticles were produced with a fine control of key properties such as size (tunable size range 20-200 nm), and surface chemistry. In order to expand the particle size range below 20nm, i.e. a range compatible with physiological glomerular filtration in kidneys, ultrasmall polymeric nanomaterials were prepared using controlled living polymerization techniques (figure 1A). Unimolecular nanostructures composed of a (bio)degradable hydrophobic core and a dense hydrophilic corona were synthesized from star-shaped poly(ε-caprolactone) co-polymerized with PEG-based macromonomers.
The effects of the exposure of podocytes to these nanomaterials were evaluated in vitro. Tests revealed a marked capacity of podocytes to internalize nanoparticles (figure 1B), suggesting that these cells may be efficiently treated by intracellular drug release nanosystems. Cytotoxicity, changes in cell morphology and cytoskeleton, cellular uptake mechanisms, intracellular compartmentalization, were assessed by incubating podocytes with nanomaterials of different size and surface chemistry (i.e. pegylated, non peglylated, positive/negatively charged surfaces).
By selecting the best nanocarrier sytems in terms of podocyte uptake and cytotoxic profile, these polymeric nanomaterials may find promising applications as new drug delivery systems for targeted therapies in kidney diseases.

A)



B)

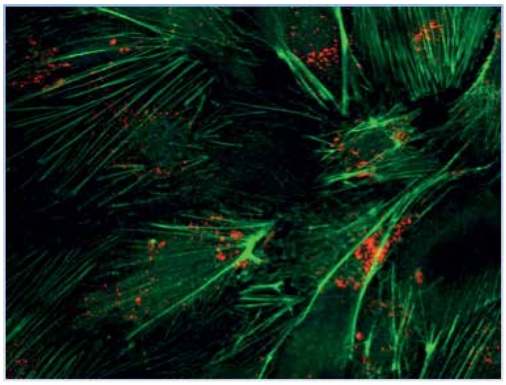


Figure 1. A) Library of engineered nanocarriers, size range 5-200nm, presenting a functional surface (i.e. charged, or PEGylated, bioactive surface). While 20-200nm sized polymeric nanoparticles were produced by MSSEP, particles <20nm were synthesized form single multi-arms polymers. B) Fluorescence microscopy image showing a marked internalization of polymeric nanoparticles (negatively charged, size 30nm, red stained) by murine podocytes (green: actin cytoskeleton revealed by phalloidin staining) .

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TIMP-1 LOADED NANOPARTICLES: A THERAPEUTIC STRATEGY FOR NEUROPROTECTION

M. CHATURVEDI[1], Y. Molino[2], S. Bojja[3], M. Khrestchatisky[4] and L. Kaczmarek[1]
[1]Laboratory of Neurobiology, Nencki Institute, Warsaw, Poland
[2]Vect-Horus S.A.S., Marseille, France.
[3]Indian Institute of Chemical Technology, Hyderabad, India
[4]UMR 7259 - CNRS - Aix-Marseille Université, Marseille, France

The aim of the study was to develop sustained release TIMP-1 loaded NPs for delivery across blood brain barrier (BBB) and evaluate them for neuroprotective effects. Here, we have developed TIMP-1 loaded sustained release poly lactic-co-glycolic acid (PLGA) nanoparticles (NPs) which can cross BBB. To summarize the results, we have shown that in vitro TIMP-1 loaded NPs have neuroprotective effects.

INTRODUCTION

There is a marked, deleterious increase in expression of Matrix Metalloproteinase-9 (MMP-9) during numerous pathologic conditions such as ischemic stroke, epilepsy and various excitotoxic/neuroinflammatory processes. Therefore, inhibition of MMP-9 is considered as a potential therapeutic target for neuroprotection. Currently available chemical inhibitors of MMP-9 are poorly specific and have many off-targets leading to unanticipated side effects. As development of specific inhibitors is always a challenging task therefore, we planned to evaluate neuroprotective effects of an endogenous inhibitor of MMP-9, Tissue Inhibitor of Matrix Metalloproteinase-1 (TIMP-1), which is a 28 KDa protein. However, the major obstacles of using TIMP-1 as a neuroprotective agent are its in vivo short half-life and low brain permeability. Hence, we planned to explore a nanotechnological approach for delivery of TIMP-1, by using poly lactic-co-glycolic acid (PLGA) based Nanoparticles (NPs), so in the future it can be developed as a neuroprotective agent.

RESULTS

Here, we have developed TIMP-1 loaded PLGA NPs which can deliver TIMP-1 in a sustained release manner and can cross the blood brain barrier (BBB). These NPs were coated with polysorbate 80 (Ps80) to improve their BBB penetration. These NPs were characterized by SEM, DLS, PDI, Zeta potential, protein loading and drug release. We evaluated these NPs for their in vitro and in vivo BBB penetration by using primary rat brain endothelial cell model and by tail vein injection in mice respectively. The in vitro and in vivo results have shown that NPs are non-toxic to endothelial cells and they have BBB penetration. Finally, we evaluated their neuroprotective effects on organotypic hippocampal slice culture using propidium iodide staining and LDH assay which have shown that TIMP-1 and TIMP-1 loaded have neuroprotective effects against Kainic Acid (KA) induced excitotoxicity. Moreover, we have shown through gelatinase assay that these effects are mediated through MMP-9 inhibition. Currently, we are exploring in vivo neuroprotective effects of TIMP-1 NPs.

MONOCLONAL ANTI-PEG ANTIBODIES FOR MEASUREMENT AND TEMPERATURE-SELECTIVE CAPTURE OF PEGYLATED STEALTH NANOPARTICLES

BING-MAE CHEN[1], Yu-Cheng Su[1,2], Talal Salem Al-Qaisi[1,3], Kuo-Hsiang Chuang[4], Tian-Lu Cheng[5] and Steve R. Roffler[1]
[1]Institute of Biomedical Sciences, Academia Sinica, Taipei, 11529, Taiwan
[2]Institute of Microbiology and Immunology, National Yang-Ming University, Taipei, 11529, Taiwan
[3]Program in Molecular Medicine, National Yang-Ming University and Academia Sinica, Taipei 11221, Taiwan.
[4]Graduate Institute of Pharmacognosy, Taipei Medical University, Taipei 11031, Taiwan
[5]Institute of Medicine and Department of Biomedical Science and Environmental Biology, MedicoGenomic Research Center, Kaohsiung Medical University, Kaohsiung, 80708, Taiwan.

Poly(ethylene glycol) (PEG) is often attached to nanoparticles to confer "stealth" properties to increase serum half-life and enhance bioavailability. Accurate and sensitive quantification of PEG stealth nanoparticles is critical for product development, pharmacokinetic measurements and efficacy studies.1

We generated a panel of monoclonal antibodies that bind to the repeating ethylene oxide subunits of the PEG backbone (AGP3, AGP4, E11, 3.3 and 6.3).2-4 AGP3 and AGP4 are mouse IgM antibodies while E11, 3.3 and 6.3 are mouse IgG antibodies. These antibodies can bind to PEG coated on surfaces, proteins or nanoparticles. Figure 1 shows an example of 3.3 and AGP4 binding to PEG molecules coated in the wells of 96-well microtiter plates. These antibodies appear to bind more strongly to longer or branched PEG molecules. In addition, AGP4 appears to bind even short PEG molecules (700 Da PEG).

We now report that AGP4 can bind very short PEG molecules as demonstrated by binding to a protein modified with a carbohydrate (Globo H) via a short PEG spacer (PEG4 with 4 repeating ethylene oxide subunits) (Fig 2, top panel). By contrast, AGP4 could not bind to Globo H attached directly to the same protein without a PEG4 spacer (Fig. 2, bottom panel). AGP4 may therefore be useful for detecting nanoparticles or block copolymers that contain very short PEG chains.

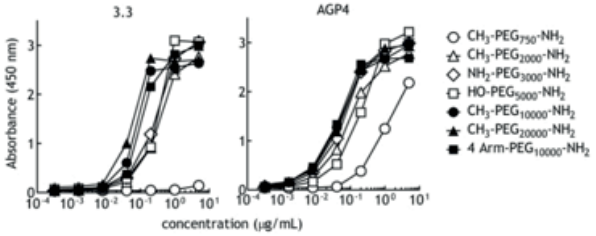

Figure 1. Anti-PEG antibody binding to PEG. The binding of 3.3 and AGP4 anti-PEG antibodies to amino-PEG molecules coated in 96-

well microtiter plates was determined by direct ELISA. PEG masses are in Da.

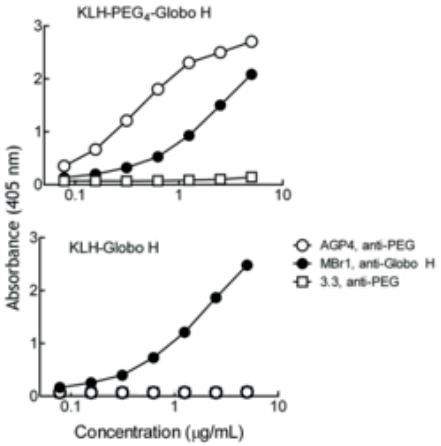

Figure 2. AGP4 can bind PEG4. AGP4 binds to KLH-PEG4-Globo H (4 repeating ethylene oxide subunits) but not KLH-Globo H.

A sandwich ELISA was developed in which AGP4 anti-PEG antibody was coated in 96-well ELISA plates to facilitate capture of PEGylated nanoparticles and proteins. After capture, the concentration of PEGylated nanoparticles can be determined by adding biotinylated AGP4 or 3.3 anti-PEG antibodies (3.3-biotin or AGP4-biotin) followed by addition of streptavidin-horse radish peroxidase and ABTS substrate. This sandwich ELISA allowed quantification of PEG-Qdots down to about 40 pM and Lipo-Dox (PEGylated liposomal doxorubicin) with about a 1 ng/mL detection limit (Fig. 3). The assay was unaffected by the presence of 50% human serum or 20% free PEG molecules.

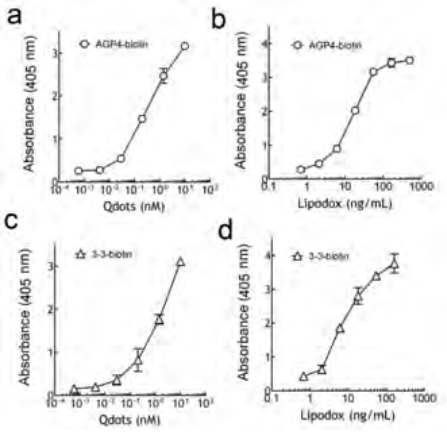

Figure 3. Anti-PEG ELISA measurement of stealth nanoparticles. Microtiter plates coated with AGP4 anti-PEG antibody were used to capture PEGylated Qdots (left panels) or PEGylated liposomal doxorubicin (right panels). Nanoparticle binding was detected with AGP4-biotin (upper panels) or 3.3-biotin (lower panels) followed by streptavidin-HRP.

We generated another monoclonal antibody (15-2b) that binds to the terminal methoxy end of mPEG molecules. We compared biotinylated 15-2b (15-2b-biotin) and 3.3-biotin for the detection of PEGylated liposomal doxorubicin in a sandwich ELISA using AGP4 as the capture antibody. Figure 4 shows that 15-2b appeared to provide more sensitive detection of Lipodox as compared to 3.3-biotin. 15-2b may be useful for sensitive measurement of mPEG-modified nanoparticles.

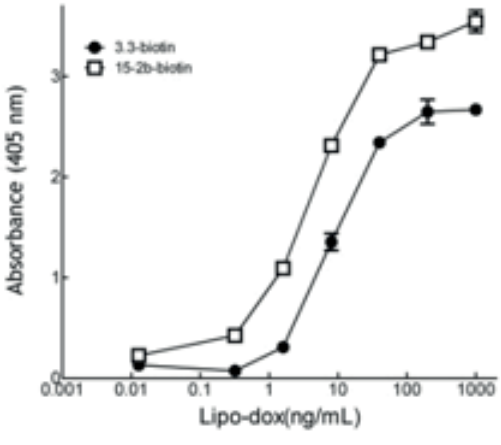

Figure 4. Comparison of anti-mPEG and anti-PEG antibodies for measurement of Lipodox concentrations. Plates were coated with AGP4 anti-PEG antibody. Serial dilutions of Lipodox were detected with anti-PEG 3.3-biotin or anti-mPEG 15-2b-biotin followed by streptavidin-HRP.

We mimicked the germinal center reaction in 3.3 anti-PEG hybridoma cells to generate new anti-PEG monoclonal antibodies (2B5 and 1E3) that can bind to PEG in a temperature-selective fashion. The 2B5 variant bound PEG with greater apparent affinity than the parental 3.3 antibody whereas 1E3 bound about as well as 3.3 at 4oC, but both variant antibodies displayed progressively reduced binding to PEG at 25oC or 37oC (Fig. 5a). The melting temperatures of 1E3 and 2B5 antibodies (73.6°C and 74.2°C, respectively), as determined by differential scanning calorimetry, were actually higher than the parental 3.3 antibody (70.8°C), indicating that 1E8 and 2B5 antibodies were thermostable (Fig. 5b).

We explored whether 2B5 anti-PEG antibody could be employed for mild affinity purification of PEGylated compounds. PEG-Qdots in cold PBS (4oC) were loaded onto a column packed with agarose beads with 3.3 or 2B5 antibodies covalently linked to their surface. After washing the columns with cold PBS, PEG-Qdots were eluted with citrate buffer (pH 3.0) or with 37oC PBS. Both 3.3 and 2B5 antibodies could capture PEG-Qdots at low temperature. Elution with 37oC PBS released PEG-Qdots from the 2B5 column, whereas elution of the PEG-Qdots from the 3.3 column required acid elution (Fig. 6). These results indicate that 2B5 may be useful for the mild purification of PEGylated compounds by simply thermal cycling between 4oC and 37oC. Antibodies able to bind PEG in a temperature-dependent fashion may be useful for novel applications such as temperature tunable capture of nanoparticles, creation of temperature controllable gated nanodevices and for gentle bio-separation of PEGylated compounds based on temperature-dependent elution.

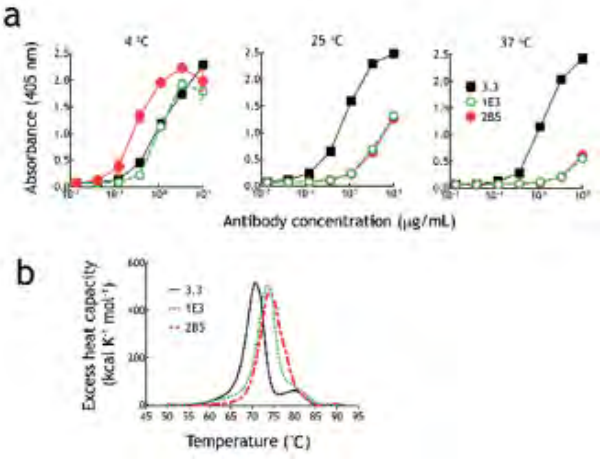

Figure 5. Temperature-dependent binding and stability of anti-PEG antibody variants. a) Graded concentrations of purified 3.3, 1E3, or 2B5 antibodies were added to microplate wells coated with linear amino-PEG (MW10,000 Da) at the indicated temperatures. After 1 h, the wells were washed and antibody binding was determined by adding HRP-conjugated donkey anti-mouse IgG Fc antibodies, followed by ABTS substrate. The mean absorbance values (405 nm) of triplicate determinations are shown. Bars, SD. b) Thermal unfolding of 3.3 (black line), 1E3 (short dashed green line) and 2B5 (long dashed red line) as measured by differential scanning calorimetry in PBS at a heating rate of 1 °C/min.

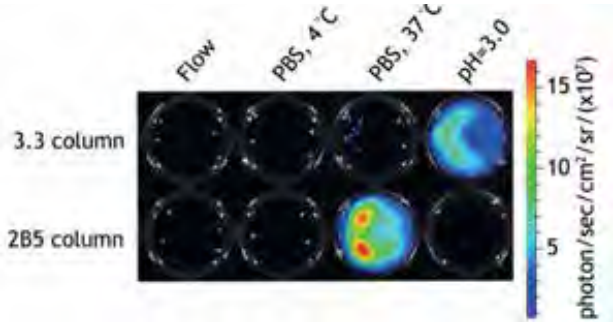

Figure 6. Mild affinity purification of PEGylated compounds. 3.3 or 2B5 antibodies were immobilized on CNBr-activated Sepharose and packed into columns. PEG-Qdot655 were passed into the columns and then the columns were washed with cold PBS (4oC) and eluted with 37oC PBS or citrate buffer (pH=3.0). The fluorescence of PEG-Qdot655 was detected on an IVIS 200 optical imaging system (Xenogen).

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DEVELOPMENT AND CHARACTERIZATION OF LIQUID CRYSTALLINE SYSTEMS FOR INCORPORATION OF OCTYL P-METHOXYCINNAMATE: EVALUATION OF IN VITRO SKIN PERMEATION AND RETENTION

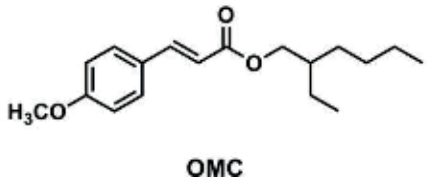
CHORILLI, M.[1]; PRADO, A. H.[1]; CALIXTO, G. M. F.[1]; BERNEGOSSI, J.[1]; RIGON, R. B.[1]; BORGES, M. C.[1]; PECCININI, R. G.[1]

[1]Universidade Estadual Paulista – Faculty of Pharmaceutical Sciences of Araraquara

Excessive exposure of human skin to ultraviolet radiation (UV) type A (UVA) or B (UVB) from the sun is an important risk factor for sunburn and the development of skin cancer. Furthermore, they may cause erythema, eye diseases, changes in DNA, premature skin aging and immunosuppression[1]. To protect the skin from these effects we use sunscreens.

An ideal sunscreen should absorb UV radiation in a broad spectrum and physically cover, adhere well to the skin and resist removal by water. Moreover, to be effective, must remain in the outermost layer of skin with minimal permeation into the systemic circulation[2].

The octyl p-methoxycinnamate (OMC), Figure 1, cinnamate most used worldwide[3], is a UVB sunscreen organic, insoluble in water and soluble in ethanol, propylene glycol and mineral oil[4]. However, a major problem for the use of this filter is the possibility of absorption and systemic effects[5]. Studies have observed the effect of the OMC in the systemic circulation presenting evidence of hormonal changes[6, 7].



OMC

Figure 1. Octyl p-methoxycinnamate structure[8].

However, the performance of a sunscreen formulation depends not only the physicochemical properties of the filters, but also the vehicle used. Thus, in order to increase retention of sunscreen on the skin, many studies involving the development of more suitable carriers for incorporation of filters have been conducted[2, 9]. Among these carriers, the nanostructured materials has attracted significant attention because they may present advantages in terms of retention on the skin and absence of penetration through the epidermal layer[1]. An example is represented by the liquid crystals (LCs) that meet the current expectation formulation for topical use. The aim of this study was to develop and characterize liquid crystalline systems containing octyl p-methoxycinnamate and evaluate your in vitro skin permeation and retention.

The formulations were developed using 0.5% dispersion of polycarbophil as the aqueous phase (A) glycol copolymer silicone fluid - DC¨ 193 as the oil phase (O) and Procetyl AWS¨ - ethoxylated propoxylated cetyl alcohol as surfactant (T). The OMC was added to the oily phase in the proportion of 5%. Formulations 22, 23, 24 and 25 were chosen comprising 50% A, 40% T and 10% O; 40% A, 40% T and 20% O; 30% A, 40% T and 30% O; and 20% A, 40% T and 40% O, respectively (Figure 2), to continue the characterization tests. The tests were performed with (22F, 23F, 24F and 25F) and without (22, 23, 24 and 25) sunscreen OMC.

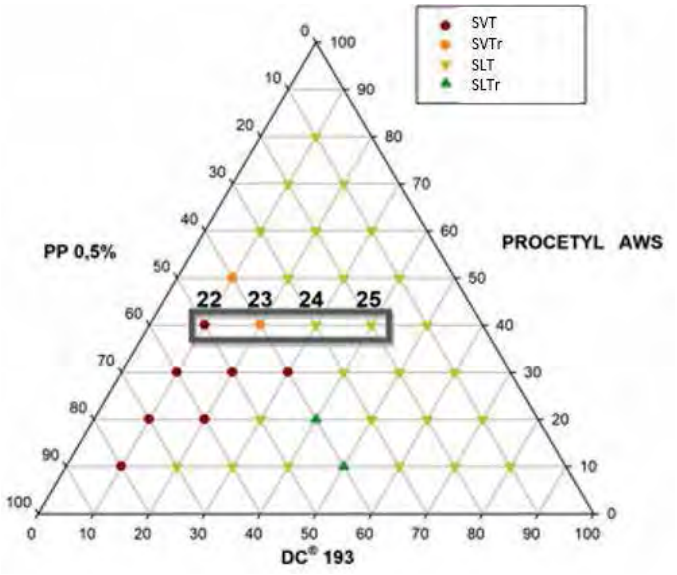


Figure 2. Ternary diagram with visual characterization of systems represented by Transparent Viscous System (SVT), Translucent Viscous System (SVTr), Transparent Liquid System (SLT) and Translucent Liquid System (SLTr).

The analysis of polarized light microscopy showed the presence of liquid crystal of cubic and hexagonal phases in formulations 22 and 23 respectively. The rheological tests showed that the incorporation of the OMC in formulations 23, 24 and 25 can facilitate application of the product on the skin. The oscillatory tests showed that only 22F formulation presented elastic property.

The texture profile testing showed that the incorporation of the sunscreen system may favor the retention of the formulation 22 in the skin, since increased adhesiveness. Moreover, the incorporation of filter promoted an increase in hardness and compressibility. However, the analysis of bioadhesion presented as the most bioadhesive for formulation 22F with significant results when compared to other formulations, which suggests greater retention on the skin.

The in vitro permeation test was conducted in a diffusion cell system with the model of pig ear membrane. In turn, the retention test was performed in vitro using the technique of tape-stripping. The samples were quantified by high pressure liquid chromatography using the equipment Waters Acquity UPLC¨ with Column HSS C18 SB 1.8um (2.1x100mm), UV system operating at 310 nm and a mobile phase consisting of acetonitrile and water acidified (80:20) at flow rate 0.5 mL/min.

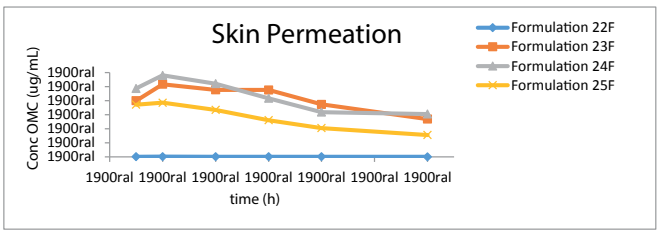


Figure 3. Test of in vitro skin permeation.

The data obtained for the permeation of formulation 22F showed low permeation to the OMC incorporated (Figure 3) and high retention skin (Figure 4).

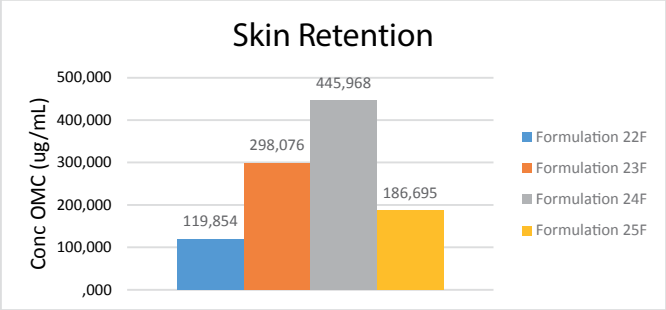


Figure 4. Test of in vitro skin retention.

From the analysis it can be concluded that the system comprises 40% T, 10% and 50% A is promising for incorporation of the sunscreen OMC, since there was the formation of cubic phase liquid crystal, rheological and mechanical characteristics that indicate an increased length of stay in the skin, ease of implementation and spreading of the product, in addition to low permeation and skin retention high in vitro tests performed.

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A THERMOSTABLE NANOVACCINE FORMULATION BASED ON PROTAMINE NANOCAPSULES

N. CSABA, J.V. Gonzalez-Aramundiz, M. Peleteiro, A. Gonzalez, M.J. Alonso

[1]CIMUS-University of Santiago de Compostela, Campus Vida 15782, Santiago, Spain
[2]Immunology, Biomedical Research Center (CINBIO), Univ. of Vigo, 36310, Vigo, Spain; noemi.csaba@usc.es

INTRODUCTION

The goal of the present work has been the design, optimization and in vitro/in vivo evaluation of protamine nanocapsules as highly versatile thermostable vaccine delivery platforms. These reservoir-type nanocarriers are constituted of an oily core and a surrounding polymeric shell. The oily core is based on the combination oils and surfactants with immunoadjuvant properties.[1] These combinations, completed by the outer protamine shell, are expected to promote intracellular delivery and release of antigens to immunocompetent cells and trigger an efficient immune response as well as providing enhanced stability for the associated antigens.

METHODS

Nanocapsules were prepared by the solvent displacement technique[2] using different combinations and amounts of oils and surfactants (experimental design with 2 levels and 4 factors). The resulting formulations were characterized regarding their physicochemical characteristics, morphology and colloidal stability by dynamic light scattering, laser-Doppler anemometry, TEM etc. The association efficiency, release and structural integrity of the associated antigens were analyzed by ELISA and Western Blot. Cytotoxicity was evaluated in RAW 264.7 macrophages by the real-time measurement of cell growth and morphology using the xCELLigence® system. The internalization of fluorescent nanocapsules (covalent TAMRA-protamine labelling) was studied by confocal microscopy and by FACS analysis in different phagocytic cell lines and primary cultures. Cytokine secretion was evaluated in human peripheral blood monocytes by flow cytometry using FlowCytoMixTM. Immune responses to antigen-loaded nanocarriers were evaluated by ELISA following parenteral and nasal administration.

RESULTS

Protamine nanocapsules prepared by the solvent displacement technique have adjustable particle size (from 50nm to 500 nm) with homogeneous distribution and positive surface charge. These carriers are able to associate the recombinant Hepatitis B surface antigen (rHbsAg, ~ 80% association efficiency) and the influenza haemagglutinin antigen (HI, ~70% association efficiency). Protamine nanocapsules are efficiently internalized in macrophage cell lines and primary cultures. No toxic effects on cells were observed up to high nanocapsule concentrations. The cytokine expression pattern of human peripheral blood monocytes shows that nanocapsules can efficiently stimulate these cells and induce the secretion of cytokines such as IL-8, IL-1β, TNFα (proinflammatory), IL-4, IL-6 (Th2 profile) and IL2, TNFβ (Th1 profile).

Nanocapsules loaded with HI antigen elicit protective immune responses comparable to the same dose Alum-adjuvanted HI (commercially available as Fluval-P) after subcutaneous injection. Protective IgG levels can also be obtained with a significantly lower dose of encapsulated HI. Nanocapsules loaded with the rHbsAg antigen elicit protective serum IgG levels following intramuscular, intranasal and combined i.m/i.n administration schedules. The analysis of IgG subtypes and IgG1/IgG2a ratios indicate that parenteral administration of the nanocapsules elicits predominantly Th2-type response, while in the case of nasal administration this balance is shifted towards a more Th1-type response. Overall, these results suggest the potential of protamine nanocapsules as vaccine adjuvants for parenteral and nasal administration and also offer the possibility of combining both modalities to achieve mixed Th1/Th2 responses.

Regarding stability and preservation of the antigens bioactivity, antigen-loaded nanocapsules suspensions show satisfactory stability under long-term storage (4ºC) and in biological media (37ºC, pH 7.4). The formulations can easily be converted to a dry powder formulation by freeze-drying, without the need of using additional cryoprotectant excipients. Freeze-dried nanocapsules can be stored at room temperature and can be easily reconstituted by manual shaking. Most importantly, western blot analysis of the associated antigen showed the complete preservation of its bioactivity for more than one year.

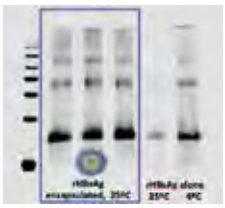

Figure 1. Structural integrity of nanoencapsulated antigen is preserved after one year storage

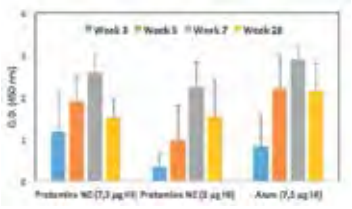

Figure 2. Nanocapsules show comparable efficacy to Alum-adjuvanted antiged following s.c. administration

CONCLUSIONS

Protamine nanocapsules represent a highly versatile delivery platform with easily adjustable composition and physico-chemical properties. They offer the possibility of simultaneously formulating antigens with adjuvant lipophilic compounds and provide these with high biological and physical stability. The in vivo results indicate that these formulations could be explored for new vaccination strategies with needle-free boosting schedules. These findings, together with the excellent long-term protection of the antigens bioactivity suggest that protamine nanocapsules may be a promising option for the development of new thermostable nanovaccines.

ACKNOWLEDGMENTS

Authors thank the financial support received from the Xunta de Galicia and the Spanish Ministry of Science and Education.

ENHANCED BRAIN TARGETING OF ENGINEERED SOLID LIPID NANOPARTICLES

R. DAL MAGRO[1], F. Ornaghi[1], I. Cambianica[1], S. Beretta[1], F. Re[1], A. Brambilla[1], F. Barbero[2], C. Musicanti[2], A. Cagnotto[3], E. Donzelli[4], A. Canta[4], M. Masserini1, G. Cavaletti4, P. Gasco[2], G. Sancini[1]

[1]Department of Health Sciences, University of Milano-Bicocca, via Cadore 48, 20900 Monza, MB, Italy.

[2]Nanovector S.r.l., via Livorno, 60 - 10144 Torino, TO, Italy.

[3]Department of Molecular Biochemistry and Pharmacology, Mario Negri Institute for Pharmacological Research, via La Masa 19, 20156 Milano, MI, Italy.

[4]Department of Surgery and Translational Medicine, University of Milano-Bicocca, via Cadore 48, 20900 Monza, MB, Italy.

INTRODUCTION

The blood-brain barrier (BBB) plays an important role in maintaining the homeostasis of the central nervous system and in protecting the brain from potentially harmful endogenous and exogenous compounds. Nevertheless it represents also the major obstacle for the diagnosis and therapy of brain diseases. One of the most promising strategies to overcome the limited BBB penetration of drugs and contrast agents is based on nanoparticles (NP). Lipid based NP, basically liposomes and solid lipid nanoparticles (SLN), have several advantages in terms of biocompatibility, non-immunogenicity, non-toxicity; they can be used as carrier systems [1], and they have a high blood circulation residence time [2]. Moreover their surface can be easily modified with ligands which mediate a site-specific targeting.

GOAL OF THE WORK

The objective of present investigation was to study the effect of surface characteristics of SLN covalently coupled with the monomer of ApoE-residues (141-150) (mApoE-SLN) in promoting BBB crossing and brain targeting using both in vitro and in vivo models.

METHODS

Radiolabelled or fluorescent dye-loaded SLN, covalently coupled by DSPE-PEG(2000)-Maleimide with the monomer of ApoE-residues (141-150) [3] and functionalized with phosphatidic acid (Aβ ligands) [4], were used in the present work and produced by Nanovector s.r.l. (Torino, Italy). In vitro evaluations were performed using cultured human cerebral microvascular endothelial cells (hCMEC/D3) obtained from Institute Cochin (INSERM, Paris, France). SLN cell uptake was monitored by confocal-laser-scanning microscopy and cell-associated fluorescence was quantified by FACS analysis. Radiochemical technique was used in order to assess the ability of ApoE monomer to enhance SLN transcellular transport across the hCMEC/D3 BBB model [5]. The in vivo biodistribution of SLN, loaded with DiR (near-infrared fluorescent cyanine dye), was evaluated by means of Fluorescent Microscopy Tomography (FMT 1500, Perkin Elmer). BALB/c male mice were intravenous (IV), intratracheal (IT) or intraperitoneal (IP) administered with 50 ul of SLN formulation and tomographic data analyses were achieved using the TrueQuant software supplied (Perkin Elmer). The total amount (in picomoles) of fluorophore in the brain region was calculated by the provided software using previously generated standards of the appropriate dye [6].

RESULTS AND DISCUSSION

We demonstrated that surface functionalization of SLN with ApoE monomer plays a major role in promoting their cellular uptake within hCMEC/D3. Cell associated fluorescence was about two-fold higher in presence of SLN-mApoE compared to unfunctionalized SLN (SLN-cys) and the same trend was observed by CLSM analysis. The ability of SLN to cross the in vitro hCMEC/D3 BBB model was assessed using dual-radiolabelled formulations. With respect to SLN-cys, the presence of monomer ApoE significantly enhanced their permeability through the cell monolayer; moreover PE values obtained with the two radiotracers were equivalent for the same SLN formulation, and about 6-fold higher for SLN-mApoE (Fig. 1). These results suggest that, at least at the dose tested, SLN cross intact the cell monolayer.

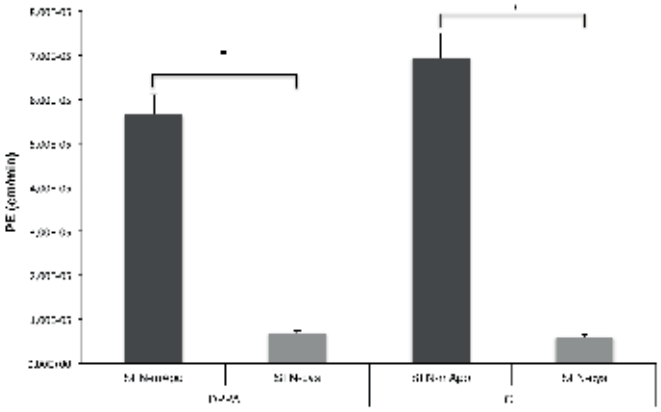

Fig.1 Transport of dual-radiolabelled SLN across hCMEC/D3 monolayer (= p<0,01)*

In vivo results confirmed the role of monomer ApoE in sustaining the delivery of SLN to the central nervous system. In particular we demonstrated that, compared to the most common routes for drug administration (IP and IV injections), IT instillation represents the best method to guarantee the biodistribution of SLN-mApoE in the brain district and to favour their retention up to 24 hours after the administration (Fig. 2).

SLN-mApo IN BRAIN (% of administered dose)

	IP	IV	IT
3 hours	0,00%	0,05%	8,93%
24 hours	0,00%	0,03%	1,10%

Fig. 2 Per cent of SLN-mApo in brain after IP, IV or IT administration

Bronchoalveolar lavage fluid (BALF) analysis does not evidence any pro-inflammatory reaction in lungs of SLN-mApoE IT-treated mice with no alteration of the alveolar-capillary barrier permeability.

CONCLUSIONS

The results here obtained suggest that the SLN formulation herein analysed could represent a suitable tool for sustaining drug delivery to the brain.

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IMMUNE TOXICOLOGICAL BEHAVIOUR OF LIPOSOMES WITH DIFFERENT SURFACE CHARGE AND PEGYLATION IN PORCINE AND RAT MODELS OF COMPLEMENT ACTIVATION-RELATED PSEUDO - ALLERGY

LÁSZLÓ DÉZSI[a], Tamás Mészáros[a], Tamás Fülöp[a], Gábor Szénási[b], Rudolf Urbanics[c], Csenge Vázsonyi[a], Erik Őrfi[a], László Rosivall[b], Réka Nemes[d], Robbert Jan Kok[d], Josbert M. Metselaar[e], Gert Storm[d,e], János Szebeni[a,b,c,f]

[a]Nanomedicine Research and Education Center, Semmelweis University, Budapest, Hungary

[b]Department of Pathophysiology, Semmelweis University, Budapest, Hungary

[c]Seroscience Ltd., Budapest, Hungary

[d]Dept. Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands

[e]Dept. Targeted Therapeutics, MIRA Institute, University of Twente, PO Box 217, 7500 AE Enschede, The Netherlands

[f]Dept. Nanobiotechnology and Regenerative Medicine, Faculty of Health, Miskolc University, Hungary

INTRODUCTION

One potential problem with the intravenous use of controlled drug delivery systems is their recognition by the immune system as foreign. The response is a hypersensitivity reaction, which recently described as complement (C) activation-related pseudoallergy (CARPA). CARPA is characterized by severe cardiopulmonary changes that in some cases can cause cardiac (anaphylactic) shock and death. Because of its potential fatal outcome, the phenomenon is considered as a safety issue and its preclinical assessment has been recommended by the European Medicines Agency in the development of liposomal drugs. The in vivo assays in which CARPAgenic properties of nanomolecules applied i.v. are not standardized, and they also substantially differ among animal species, e.g. pigs or rats. A further issue is that surface properties of liposomes might exert major impact on CARPA1-6. Therefore, the aim of the present study was to compare the hemodynamic and hematological changes in porcine and rat models of CARPA in response to two different liposomes. The surface modifications yielded a negatively charged (AmBisome) and a neutral PEGylated (Chol-2K-PEG) liposome.

MATERIALS AND METHODS

Pigs: Domestic male Yorkshire pigs (20-25 kg) were sedated with Calypsol/Xilazine (10 and 2 mg/kg respectively) and then anesthesized with isoflurane (2-3% in O2). Animals were breathing spontaneously. Pulmonary arterial blood pressure (PAP) was measured using a Swan-Ganz catheter introduced into the pulmonary artery via the right external jugular vein, while systemic arterial pressure (SAP) was measured in the femoral artery. The left femoral vein was cannulated for blood sampling. Samples were injected in the animals in bolus (< 10 sec) via the left external jugular vein. Hemodynamic changes were continuously monitored. The mean PAP, SAP, and heart rate (HR) data are evaluated before the test material infusion then every minute for 3 min, until 15 min after start of the injection, or every 5 min for longer observation reactions.

Rats: The experiments were performed in male Wistar rats weighing 400-600 g. Animals were anesthetized with thiobutabarbital (Inactin, 120 mg/kg i.p.). A tubing was inserted into the trachea and the rats spontaneously breathed. The left common carotid artery, the left femoral artery and vein were cannulated. Arterial blood pressure was continuously recorded via the femoral artery catheter. SAP and HR were checked before blood sampling. Blood samples (0.5 ml each) were collected into Eppendorf tubes containing 10 µl lepirudin (Refludan, 1mg/ml) before (time 0) and at different time points (1-3-5-10-30 min) after the injection. Aliquots of 100 µl blood were drawn into tubes with K2-EDTA for hematological analysis. Blood was centrifuged at 1500 rpm for 10 min at 4°C; plasma was stored at -80°C until analysis.

Complement activation: The total complement activation was determined using the classical C hemolytic (CH50) assay. A fixed volume of optimally sensitized SRBCs was added to serum with appropriate dilution. After incubation, the mixture was centrifuged, and hemolysis was quantified by measuring the absorbance of the hemoglobin released into the supernatant at 540 nm. The amount of complement activity was determined by examining the capacity of test serum to lyse antibody coated SRBCs.

Thromboxane B2 levels: Plasma TXB2 (the stable metabolite of plasma TXA2) levels were measured with an ELISA kit (Cayman Chemicals).

Test items: To induce CARPA zymosan the direct complement activator was applied. AmBisome and Chol-2K-PEG (Chol-PEG) served as liposomal complement activators. Their doses refer to their phospholipid (PL) content.

RESULTS

Pigs: Cardiopulmonary effects of bolus administration of 0.01 mg/kg AmBisome showed a 3-fold (200%) rise in PAP with a 50% drop in SAP. In addition, it has been found that pigs are one order of magnitude more sensitive to AmBisome (0.01 mg PL/kg) that to Chol-PEG (0.1 mg PL/kg). A CARPA reaction similar to that of AmBisome could be elicited by a 50-fold higher dose of zymosan (0.1 mg/kg).

Rats: Fig. 1 left panel shows that i.v. administration of AmBisome in rats lead to a gradual decrease of SAP by 40% after 5 min (left A), that was associated with thrombocytopenia (left

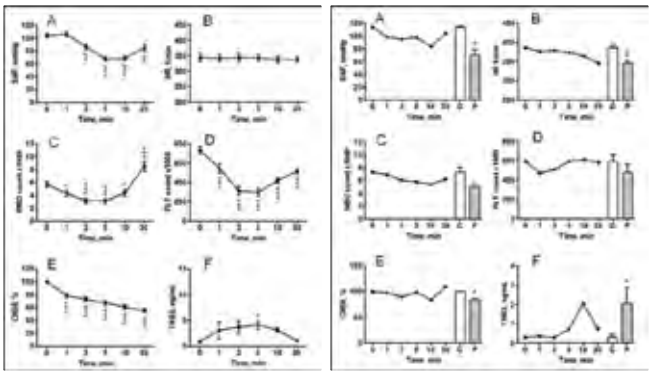

*Figure 1. Physiological changes in rats injected i.v. with 22 mg PL/kg AmBisome (left panel) or 300 mg PL/kg Chol-PEG (right panel). Values shown are Mean ± SE for 8 or 3 animals, respectively. The curves were constructed from the 0, 1, 3, 5, 10 and 30 min readings of SAP and HR after injection, as well as of other parameters measured from blood samples taken at the same time points. *, **, ***: p<0.05, 0.01, 0.001 vs. the time 0 value, respectively. Line graphs: one-way ANOVA with repeated measures; bar graphs: paired Student's t-test.*

D) and reduction of hemolytic activity (left E). A significant (50%) initial leukopenia at 5 min, switching to leukocytosis at 10 min (left C). The HR did not change (left B), and plasma TXB2 rose minimally (left F). Chol-PEG liposomes of 60 mg PL/kg did not show any physiological effects.

In Fig. 1 right panel the effects of 300 mg PL/kg Chol-PEG are shown. Minor changes observed resembled the effect of 2.2 mg PL/kg AmBisome. Bars inserted into small panels, comparing changes at peak relative to baseline, revealed significant alterations in SAP (right A), HR (right B), hemolytic activity (right E), and TXB2 (right F). The effect of 10 mg/kg zymosan, except for somewhat less hematological and more expressed TXB2 changes, was essentially identical to that seen with 22 mg PL/kg AmBisome (data not shown).

CONCLUSIONS

This study confirmed the previous evidence that pigs provide a highly sensitive model of CARPA and also confirmed previous claims that rats are less sensitive for liposome-induced reactions than pigs. However, the present study went far beyond as it provided head-to-head comparison of the reactions in pigs and rats. The difference in sensitivity turned out to be in the range of 100 to 10000, based on the dose-effect relationship information in this study. For example, in the case of AmBisome-induced drop of SAP there was a 2200-fold higher effective dose in rats than in pigs. These figures provide strong indication that the rat is not a sensitive model for

immune toxicity screening or quantitative evaluation of the risk of CARPA. However, because the physiological changes in rats are essentially the same as those seen in pigs and man, rats still provide a good model for studying the mechanism of CARPA.
In addition to the difference in CARPA sensitivity, this study showed that the reactions to liposomes are of a very specific nature depending on the type of surface modification, being charged (AmBisome), or decorated by PEG (Chol-PEG) and that this difference is consistent among distinct species. AmBisome, a highly charged nonPEGylated liposome was stronger inducer of CARPA in both species than its identically sized (~100 nm), uncharged PEGylated counterpart, Chol-PEG liposomes.

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Supported by National Office for Research and Technology, Grant No. TECH_08_D1 (NANOMEDI); EU project FP7-NMP-2012-LARGE-6-309820 (NanoAthero), Phospholipid Research Center, Heidelberg, Germany, Grant to Dr Metselaar.

A RAPID SCREENING ASSAY TO DETERMINE THE ANTI-MYCOBACTERIAL PROPERTIES OF NANOPARTICLES FOR THE TREATMENT OF TB

SAMANTHA DONNELLAN[1], Vicki Stone[1], Karen Stevenson[1], Lang Tran[2]
[1]Heriot Watt University, Edinburgh, Scotland, smd31@hw.ac.uk
[2]Moredun Research Institute, Pentlands Science Park, Midlothian, Scotland

There is an urgent need to develop effective treatments for the disease tuberculosis (TB), caused by the organism Mycobacterium tuberculosis (Mtb). The burden of the disease is enormous, with a third of the world's population estimated at being infected with latent TB, and it continues to kill up to two million people annually. Mortality rates of multi-drug resistant TB are increasing and there are the continuing problems of treating HIV infected patients who are prone to secondary infection, especially TB. The objective of this project is to create a rapid assay for investigating the anti-mycobacterial activity of different nanomaterials. A related surrogate organism is being used, Mycobacterium avium subsp. paratuberculosis (Map) causing paratuberculosis in ruminants, as it can be handled in a lower containment level facility (L2), making it a relevant and cheaper alternative to Mtb to develop and optimise the assay. Map has a cell wall similar in structure and chemical composition to that of Mtb which hinders the entry of drugs and is resistant to a similar spectrum of drugs. Map has been transformed with a plasmid carrying the gene for Green Fluorescent Protein (GFP) to create a reporter strain (Map-GFP), thus allowing both growth and viability to be tracked by fluorescence. The anti-mycobacterial properties of nanopreparations of silver, copper (II) oxide and the first line anti-TB drug rifampicin are being investigated. Suspensions of pre-weighed nanoparticles (NPs) are prepared in media, employing water bath sonication for 16 minutes, and serial dilutions of the particulate solutions are made and added to Map-GFP. Growth is monitored over 7 days and a dose response is measured, showing the effects of the different nanoparticles at various concentrations. Further data analyses are underway, using different NPs and comparing the differences in potencies of NPs of differing sizes. The project will progress to use a macrophage infection model, the immune cell where Mtb resides, with the aim of killing the mycobacterium in its host environment. This work has many stages involved and will contribute towards the development of nanomedicines for the treatment of TB.

IMAGING AND TARGETING OF LIVER AND KIDNEY FIBROSIS

JOSEF EHLING[1], Matthias Bartneck[2], Janka Bábíčková[3], Felix Gremse[1], Zhuojun Wu[1], Rene Botnar[4], Marc van Zandvoort[5], Peter Boor[3], Frank Tacke[2], Fabian Kiessling[1], Twan Lammer[s1,6,7]
[1]Department of Experimental Molecular Imaging, RWTH Aachen University, Aachen, Germany, D
[2]Department of Medicine III, RWTH Aachen University, Aachen, D
[3]Department of Medicine II, RWTH Aachen University, Aachen, D
[4]Division of Imaging Sciences and Biomedical Engineering, King's College London, London, UK
[5]Institute for Molecular Cardiovascular Research, RWTH Aachen University, Aachen,D
[6]Department of Controlled Drug Delivery, University of Twente, Enschede, NL
[7]Department of Pharmaceutics, Utrecht University, Utrecht, NL

Correspondence: jehling@ukaachen.de; tlammers@ukaachen.de

INTRODUCTION

Fibrosis refers to the excessive deposition of extracellular matrix (ECM) proteins in response to chronic injuries and it results in a pathological obliteration of organ architecture and function. Two of the most commonly affected organs are the liver and the kidney, affecting millions of people worldwide. Remarkably, hardly any diagnostic and therapeutic probes are available for the diagnosis and treatment of liver and kidney fibrosis. The aim of our efforts is to establish novel contrast agents and imaging techniques to visualize and quantify liver and kidney fibrosis, and to design and evaluate targeted therapeutic interventions.

RESULTS AND DISCUSSION

Functional imaging of blood vessels in mice suffering from liver and kidney fibrosis was performed by contrast-enhanced micro-CT (µCT), providing information on the relative blood volume (rBV) [1]. rBV values were repetitively determined during disease progression. Areas of sprouting angiogenesis were observed in fibrotic livers (Fig. 1A) and the rBV significantly increased from early- to late-stage disease (from +11% at week 2 to +40% at week 8). In line with this, infiltrating inflammatory macrophages highly expressed VEGF. A novel targeted therapeutic, which blocks the chemokine C-C-motif ligand 2 (CCL2; a key factor for recruiting inflammatory macrophages) was employed to block macrophage infiltration into the liver, and was shown to significantly inhibit fibrosis-associated angiogenesis (from rBV=25% in untreated to rBV=18% in anti-CCL2-treated mice) [2]. In kidney fibrosis, vessel rarefaction was observed (Fig. 1B) and the rBV significantly decreased during disease progression (from -33% at day 3 of induced fibrosis to -66% at day 10). To visualize and quantify fibrosis progression in relation to vascular alterations, novel elastin- and collagen-specific molecular imaging agents were employed. Using the elastin-specific contrast agent ESMA [3] and T1-weighted MR imaging, a strong perivascular T1 signal enhancement was observed in fibrotic livers (Fig. 1C) [4]. Similarly, a fluorophore-labeled collagen-specific contrast agent (CSPI) was used to monitor perivascular collagen deposition in fibrotic kidneys, using in vivo µCT-FMT in combination with ex vivo two-photon laser scanning microscopy (Fig. 1D).

CONCLUSION

Novel contrast agents, imaging techniques and targeted therapeutics have been developed for monitoring and treating liver and kidney fibrosis. Liver fibrosis was characterized by the infiltration

of inflammatory and angiogenesis-promoting macrophages, which could be inhibited by blocking CCL2. Kidney fibrosis showed a progressive reduction of blood vessels and a strong perivascular collagen formation, which could be targeted using a novel collagen-specific probe. Our findings contribute to the development of novel probes and protocols for facilitating the diagnosis and treatment of liver and kidney fibrosis.

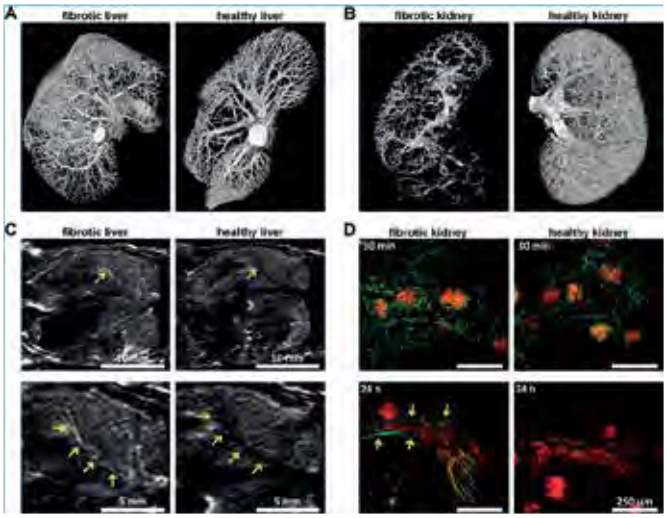

Figure 1: A+B) High-resolution contrast-enhanced µCT imaging of functional blood vessels in fibrotic and healthy livers (A) and kidneys (B). C) Elastin-based molecular MRI of liver fibrosis 2 hours after the i.v. administration of ESMA resulted in strong perivascular T1 signal enhancement in fibrotic but not in healthy control livers (see arrows). D) Imaging and targeting of perivascular collagen fibers in kidney fibrosis using the fluorophore-labeled contrast agent CSPI via 2-photon microscopy. Vessels are stained using rhodamine-lectin.

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CARBON-BASED NANOMATERIALS INHIBIT THE DRUG METABOLIZING ACTIVITY OF CYP3A4

RAMY EL-SAYED,[1] Mi-Young Lee,[2] Kunal Bhattacharya,[1] Souren Mkrtchian,[2]
Magnus Ingelman-Sundberg,[2] Bengt Fadeel[1]
[1]Nanosafety & Nanomedicine Laboratory, Division of Molecular Toxicology, Institute of Environmental Medicine, Karolinska Institute
[2]Section of Pharmacogenetics, Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

Carbon-based nanomaterials including carbon nanotubes (CNT) and graphene oxide (GO) are under intensive academic investigations because of their exceptional properties compared to other organic and inorganic nanoparticles and these materials are currently being considered for various applications in nanomedicine. However, these materials may induce inflammatory reactions and close attention to safety issues is needed [Bhattacharya et al. Adv Drug Deliv Rev. 2013]. Notably, single-walled CNTs, as well as other nanoparticles, show high accumulation in the liver upon intravenous administration [El-Sayed et al. Nano Lett. 2013]. The liver expresses phase I drug metabolizing enzymes know as cytochrome P450 (CYPs) that play a major role in the metabolism of xenobiotic compounds including drugs. In the present in vitro study we demonstrate how SWCNT and GO inhibit the conversion of the model compound, testosterone to 6β-hydroxytestosterone by CYP3A4. Moreover we show that pre-adsorption of a 'corona' of bovine serum albumin (BSA) on the surface of the nanomaterials can mitigate this effect. We have also shown that covalent functionalization with polyethylene glycol (PEG) limits the formation of the protein corona. Current studies are focused on cellular uptake of SWCNT and GO by liver cell lines. Together, these results provide evidence that carbon-based nanomaterials may impact on CYP function.

Supported by: Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning, and the European Commission (FP7-MARINA, Grant Agreement No. 263215; FP7-NANOREG, Grant Agreement No. 310584; FP7-NANOSOLUTIONS, Grant Agreement No. 309329; GRAPHENE Flagship Project, Grant Agreement No. 604391).

ALDOXORUBICIN LOADED – PAS MASKED HUMAN FERRITIN NANO-CAGES FOR TUMOR TARGETING

GIULIO FRACASSO[1], Marco Colombatti[1], Cristina Anselmi[1], Silvia Zanini[1], Marcella Pinto[1], Elisabetta Falvo[2], Miriam Carbo[3] and Piepaolo Ceci[2]
[1]Department of Pathology and Diagnostics, Immunology Section, University of Verona, Italy
[2]CNR–National Research Council of Italy, Institute of Biology, Molecular Medicine and Nanobiotechnology, Rome, Italy
[3]Department of Biochemical Sciences, University of Rome "Sapienza," Italy

Targeted delivery of anti-cancer therapeutics to cancer cells ("site-specific drug delivery") aims at enhancing accumulation of the drugs within the tumor while guiding them away from potentially endangered healthy tissues. Selective delivery to disease sites is particularly critical for many current cancer therapeutics that are distributed non-specifically in the body, thereby affecting both normal and cancer cells.
Among the currently investigated nano-carriers for targeted delivery, protein-cage molecules based on heavy chain ferritins (HFts) are attracting growing interest due to their exceptional characteristics: [1]

- HFts protein-cage are of human origin and therefore the risks of immunoresponse and inflammatory response which may hamper the patients' treatment are substantially reduced;
- HFts exploit transferrin receptor 1 (TfR1), also called CD71, for their internalization.It is long known that TfR1 is up to 100 fold more expressed in cancer than in normal cells.
- HTf nano-cages may be loaded with drugs/trackers following two strategies: cross-linking to the exterior surface and/or positioning of the drug/tracker in their internal cavity (about 8 nm in diameter).

In the present work we report the use of protein nanosystems based on the heavy chain of the human protein ferritin loaded with a Doxorubicin analogous (Aldoxorubicin, INNO-206) for tumor cell intoxication; ferritin H-chain was genetically linked to a repetitive sequence of the tripeptide Proline-Alanine Serine (PAS peptide, PA-Sylation), which is endowed with high masking properties. This new masking shell shows some advantageous features: [2]

- The coating process can be controlled more accurately;
- The de-masking process of the NPs can be induced by metalloproteinases by including a specific peptide spacer recognized by tumor MMP (see MMP2 and MMP9). [3]
- We can also increase the rate of NPs de-masking with respect to the conventional NPs-PEG coating and therefore the releasing rate of the loaded drug.

In the first series of experiments we demonstrated the efficacy of the enzymatic cleavage of the linker peptide; the HFt-PAS nanocarriers loaded and unloaded with Aldoxorubicin were treated with collagenase to induce the releasing of the masking shell of PAS peptides. The release was evidenced by SDS-PAGE electrophoresis. Then we verified by FACS analysis that the binding capability of our PAS-coated HFts was reduced with respect to the uncoated one. As depicted in Figure 1a-1b we observed a 6 fold reduction of the MFI values after incubation of PC-3 cells at +4°C with the

same amount of HFts and PAS-HFts. Moreover the binding capability of collagenase treated PAS-HFts was increased approximately 4 fold (Fig 1c) thus demonstrating that the masking properties of PAS could be modified.

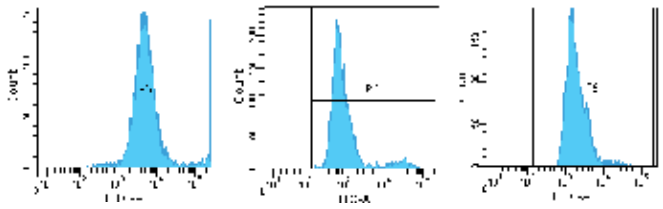

Fig.1a Fig.1b Fig.1c

FIGURE 1. FACS analysis of the binding capability of HFts, HFt-PAS and collagenase treated HFt-PAS on PC-3 cells.

Finally the killing efficacy of our Aldoxorubicin loaded nano-cages was investigated on different prostate cancer (LNCaP, PC-3 and DU145) and pancreatic cancer (PaCa44, Capan-1 and MiaPaca 2) cell lines. In all our cytotoxicity assays no signs of toxicity were measured for the drug-unloaded nanosystems. As reported in Figure 2 when Paca44 cells were pulsed for 48 hrs with our NPs or Doxorubicin alone and then the viability assayed after a lag phase of 48 hrs (by XTT assay) the IC50 measured for the drug alone or the drug-loaded NPs were superimposable. (IC50 is about 200 nM). The anti tumor efficacy and the non specific toxicity in xenograft mouse models will be investigated in the next future.

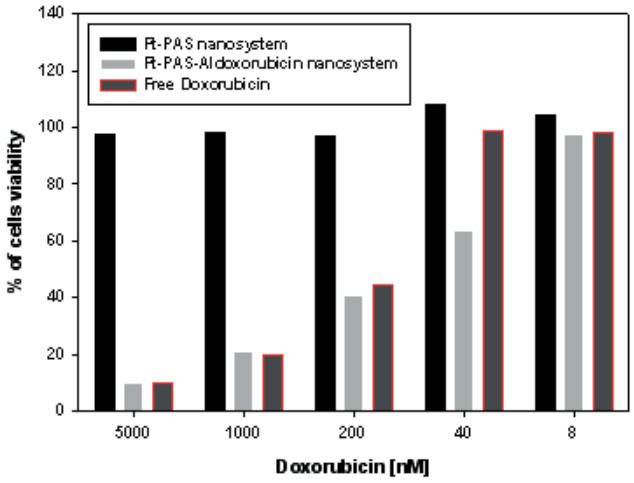

FIGURE 2. Cytotoxic efficacy of HFt-PAS, Aldoxorubicin-loaded HFt-PAS and Doxorubicin alone on Paca44 pancreas tumor cells.

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NOVEL POMEGRANATE OIL NANO-EMULSIONS FOR THE PREVENTION AND TREATMENT OF NEURODEGENERATIVE DISEASES: THE CASE OF GENETIC CJD

RUTH GABIZON[1], Michal Mizrahi1, Yael Friedman-Levy[1], Liraz Larush2, Kati Frid[1], Orli Binyamin1, Dvir Dori[1], Nina Feinstein[1], Haim Ovadia[1], Tamir Ben-Hur[1], Shlomo Magdassi[2]

[1]Neurology, Hadassah Univerisity Hosital, Israel; [2]Casali Institute of Chemistry, Hebrew University, Israel

BACKGROUND

Since disease progression in neurodegenerative diseases is associated with irreversible brain damage, prevention of disease onset and aggravation by safe compounds in at risk individuals should be the main target in treatment development. This is the case for carriers of mutations in the PrP protein, causing genetic Creutzfeldt-Jacob disease (gCJD).

METHODS

To this effect and since sensitivity to oxidative stress plays a major role in prion and other neurodegenerative diseases, we treated our TgMHu2ME199K mice, which model for patients expressing the E200K PrP mutation in gCJD, with Pomegranate seed oil (PSO) in its natural form or in a novel emulsified formulation (Nano-PSO). PSO comprises large concentrations of a unique polyunsaturated fatty acid, punicic acid, considered as one of the strongest natural antioxidants. Young and asymptomatic mice, as well as sick TgMHu2ME199K mice were treated for months with PSO or Nano-PSO . Results: We show here that administration of Nano-PSO significantly delayed disease onset in asymptomatic TgMHu2ME199K mice and postponed disease aggravation in already sick mice. Biochemical and pathological analysis revealed that while Nano-PSO did not affect PrP accumulation, it reduced lipid oxidation and neuronal loss and restored neurogenesis, indicating a strong neuroprotective effect.

CONCLUSIONS

Nano-PSO formulations maybe beneficial to subjects suffering from diverse neurodegenerative conditions, including prion diseases. They may be used as stand alone drugs or in combination with disease specific reagents.

APIDSOL: NANOSIZED MICELLE FORMULATIONS FOR LOCAL DRUG DELIVERY

DORIS GABRIEL

ApidSOL technology is based on co-polymers of methoxy poly-ethylene glycol (mPEG) and hexyl substituted poly-lactide acid (hexPLA) (Figure 1A). In an aqueous environment, amphiphilic mPEGhexPLA polymers (ApidSOL) spontaneously form nanosized micelles in a self-assembly process. During the self-assembly process of the co-polymers, poorly water-soluble active pharmaceutical ingredients (APIs) are incorporated in the lipophilic core of ApidSOL micelles (Figure 1B) [1, 2].

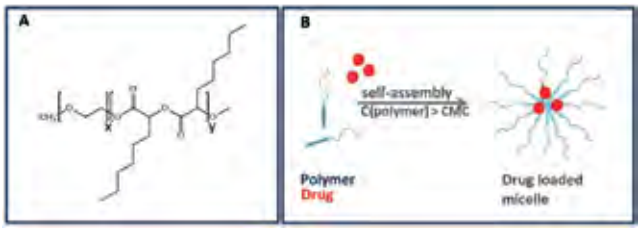

Figure 1: A] ApidSOL technology uses co-polymers of methoxy poly-ethylene glycol (mPEG) and hexyl substituted poly-lactide acide (hexPLA), B] in a self-assembly process poorly water- soluble APIs are incorporated into the micelle core.

So far over 100 bioactive molecules have been successfully incorporated into ApidSOL micelles and the API's apparent solubility was thereby increased up to 1000-fold. mPEGhexPLA polymers are degraded to PEG, a polymer frequently used in pharmaceutical formulations and 2-hydroxyoctanoic acid, a derivative of fruit acids, which are commonly found in food. Preclinical toxicity studies showed good biocompatibility after topical administration to the eyes or to the skin [3, 4].

Three distinct mechanisms enable ApidSOL technology to achieve efficient loco-regional delivery of lipophilic and poorly soluble drugs: 1] incorporation of the API into the hydrophobic micelle core increases solubility and actual bioavailability 2] particle sizes in the lower nano-range (20 - 50 nm) together with stealth surface properties minimize interactions with the biological matrix and facilitate rapid transport into biological structures 3] dissociation of the carrier leads to the release of the drug cargo inside the biological target site and to the in situ formation of a drug depot.

While local tissue targeting is generally achieved by topical application of the drug loaded ApidSOL formulations, above mentioned mechanisms help to obtain high local drug concentrations, to increase therapeutic efficacy and to reduce systemic side effects. ApidSOL technology is useful in a wide range of clinical indications

where high local drug concentrations are beneficial for optimal therapeutic outcome: examples range from dermatology [3] to ophthalmology [4, 5], otology, urology and gastroenterology. Apart from applications in drug delivery, ApidSOL micelles could also efficiently deliver imaging agents such as iodine, gold, iron or fluorophores to obtain X-ray, NMR or fluorescent imaging probes.
The present paper aims to give an overview on applications of ApidSOL for tissue selective drug delivery in the field of dermatology and ophthalmology. Formulation process, nanovector characteristics and delivery/therapeutic efficacy will be presented based on selected active pharmaceutical ingredients (Figure 2).

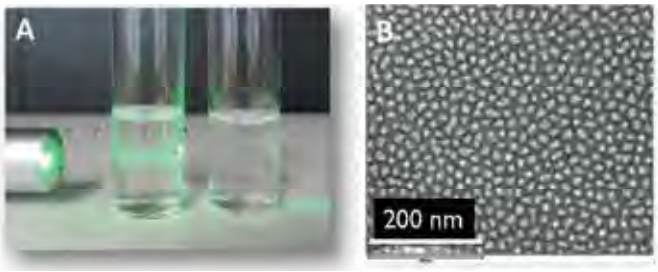

Figure 2: A] 0.5% Cyclosporine A ApidSOL micelle formulations are perfectly transparent colloidal solution B] Transmission electron micrograph of ApidSOL micelles shows spherical nanoparticles with homogeneous size distribution.

Furthermore, new perspectives of using ApidSOL for the delivery of imaging agents will be discussed.

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INVESTIGATION REGARDING QUALITY OF DENTAL ENAMEL. A NEW APPROACH AND EXPLANATION BY USING RAMAN AND AFM TECHNIQUE

EDUARD GATIN[1, 2], Ruxandra Sfeatcu[2], Catalin Luculescu[3], Adriana Balan[4], Roxana Ilici[2], Lidia Ciobanu[5], Stefan Iordache[3], Ioan Patrascu[2]
[1]Materials Department, Faculty of Physics, University of Bucharest P.O. Box MG - 11, Magurele – Bucharest, Romania;
[2]University of Medicine "Carol Davila", Faculty of Dentistry, Calea Plevnei 19, Sector 5, Bucharest, Romania
[3]INFLPR, National Institute for Laser, Plasma and Radiation Physics, P.O. Box MG- 36, Magurele – Bucharest, Romania
[4]University of Bucharest, 3Nano-SAE Research Centre, P.O. Box MG-38, Magurele, Romania
[5]„St. Pantelimon" Hospital, Sos Pantelimon Nr. 340 - 342, Sector 2, Bucharest, Romania

Tooth discoloration or spots may develop on the coronal surfaces of human teeth. This affection found on the teeth is similar in composition and structure to that reported to occur in different populations. The tooth discoloration is a special type of dental plaque characterized by its simple flora and its tendency to calcify [1].
The dentine-predentine junction enamel where it becomes acid soluble, and the edge of the preosseous matrix of bone are stained dark blue, the rest of the calcified tissues being unstained [2].
Development of dental caries is associated with the loss of minerals and change in the enamel structure. The present study emphasises a similar mechanism favoring the appearance of tooth discoloration and a possible real time evaluation of dental enamel for children tooth eruption.
In this study, we have measured and compared Raman spectra of not affected tooth enamel area and those of spots affected tooth for the same patient [3,4].
Changes of the Raman spectra for the investigated tooth area were correlated to the enamel surface roughness investigation, performed by AFM technique. Investigation was completed by SEM investigation, observing tendency for eroded enamel for the black stain affected area [4,5].

Acknowledgment: Part of financial support was obtained from B&W Tek Company, USA.

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LIPID MICROSPHERES AS CARRIERS FOR CERIUM OXIDE NANOPARTICLES: A PRELIMINARY INVESTIGATION

AGOSTINA FRANCESCA GRILLONE[a,b], Eugenio Redolfi Riva[a,b], Barbara Mazzolai[a], Virgilio Mattoli[a], Gianni Ciofani[a]
[a]Istituto Italiano di Tecnologia, Center for Micro-BioRobotics @ SSSA, Viale Rinaldo Piaggio 34, 56025 Pontedera (Pisa), Italy
[b]Scuola Superiore Sant'Anna, The BioRobotics Institute, Viale Rinaldo Piaggio 34, 56025 Pontedera (Pisa), Italy

INTRODUCTION

In recent years, devices consisting of micro- and nanoparticles able to influence drug biodistribution, to stabilize the active principle, and to support the release in a controlled manner have attracted particular attention in the pharmaceutical research. Among them, an example is represented by lipid-based system [1]. Lipid microparticles are made of a solid matrix dispersed in an aqueous solution and stabilized by the presence of surfactants or polymers. The advantages of using these materials reside in their low toxicity due to the use of biocompatible lipids well tolerated by the organism, in their small size, in the possibility of tuning drug release and targeting, and in the possibility to incorporate both lipophilic and hydrophilic drugs [2].
In this work, we assess the possibility to exploit lipid microparticles as delivery systems for cerium oxide nanoparticles (nanoceria), a potential pharmacological agent widely investigated in all those pathologies where oxidative stress plays a key role, thanks to their self-regenerating anti-oxidant properties [3].

EXPERIMENTAL METHODS

Lipid microparticles were prepared using an oil-in-water homogenization at high temperature. Briefly, 150 mg of nanoceria were added to 350 mg of cetylpalmitate (CP), melted at temperature above the melting point (70º C). Nanoceria-loaded CP was quickly added to the aqueous phase, composed by 5 ml of polysorbate 80 (20 mg/ml in deionized water), under high-speed homogenization with a homogenizer (T10 basic, UltraTurrax) for 5 minutes at 6000 rpm. Finally, formed microparticles were allowed to solidify at 4°C. Obtained particles have been characterized with atomic force microscopy (Innova SPM, Bruker) and their cytocompatibility was

evaluated with the metabolic assay WST-1 on human umbilical vein endothelial cells (HUVECs), incubated for three days with 0-100 µg/ml of particles.

RESULTS

Figure 1 reports AFM imaging, representative of the whole sample, highlighting the formation of well-defined spherical solid lipid microparticles, of size in the range of 0.5-1 µm.

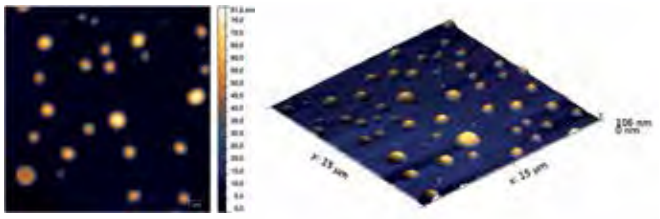

Figure 1: AFM image of nanoceria-loaded lipid microparticles: single scan (left) and 3D rendering (right)

Results of metabolic WST-1 assay, reported in Figure 2, highlight absence of significant toxic effects up to 20 µg/ml of lipid microparticles in the cell culture medium, and up to 3 days of incubation.

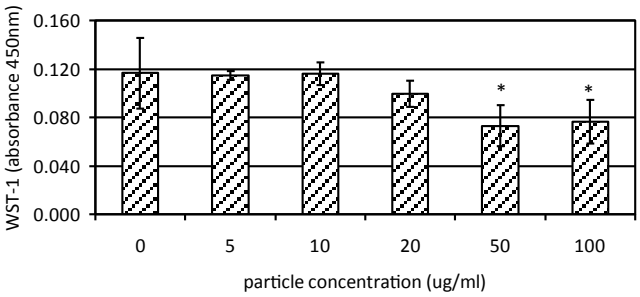

Figure 2: WST-1 assay results

CONCLUSION

Our results, even if preliminary, demonstrate the possibility to exploit solid lipid carriers for the encapsulation of cerium oxide nanoparticles. Future mandatory investigations will aim at obtaining smaller structures (in the range of 50-100 nm) and at the assessment of the unaltered catalytic activity of the entrapped nanoceria. Moreover, the possibility to functionalize the surface of the lipidic structures with appropriate ligands for a targeted delivery will be also considered.

Acknowledgments: Authors would like to thank Dr. Serena del Turco (CNR, Institute of Clinical Physiology, Pisa, Italy), who kindly provided human umbilical vein endothelial cells for cytocompatibility experiments.

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ATOMIC FORCE MICROSCOPY-BASED FORCE SPECTROSCOPY STUDY OF FIBRINOGEN-ERYTHROCYTE INTERACTIONS: ASSOCIATION WITH CARDIOVASCULAR RISK ON HEART FAILURE PATIENTS

GUEDES, ANA FILIPA[1]; Carvalho, Filomena A.[1]; Lousada, Nuno[2]; Sargento, Luís[2]; Santos, Nuno C.[1]

[1]Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal; [2]Unidade de Insuficiência Cardíaca, Departamento de Cardiologia, Hospital Pulido Valente, Centro Hospitalar Lisboa Norte, Lisbon, Portugal

The significance of hemoheological disorders on the development of heart diseases is presently well known, but their role in pathogenesis is still unclear. A better understanding of the role of fibrinogen on erythrocyte aggregation in heart failure patients might be relevant for potential future drug interventions to reduce aggregation and enhance microcirculatory flow conditions.

The aims of this study were to evaluate the fibrinogen-erythrocyte interaction in chronic heart failure (CHF) outpatients and to study the relationship between hemorheological and hemodynamic factors.

Thirty outpatients with CHF, followed-up in a Heart Failure Unit, and 15 healthy blood donors were included in the study. Patients were grouped according to two etiologies: ischemic or non-ischemic. Fibrinogen-erythrocyte interactions were evaluated using atomic force microscopy (AFM)-based force spectroscopy. Hemorheological parameters (erythrocyte aggregation, erythrocyte deformability and whole blood viscosity) were evaluated both for patients and healthy controls.

CHF patients had higher fibrinogen-erythrocyte binding forces than the control group (60.6 ± 6.6 pN vs. 40.4 ± 3.0 pN; p=0.038), despite lower binding frequency (13.0 ± 2.4 % vs. 27.3 ± 4.2 %; p=0.003). Ischemic patients had higher binding forces than healthy donors (74.9 ± 10.7 pN vs. 40.4 ± 3.0 pN; p=0.004), but lower binding frequency (11.7 ± 2.1 % vs. 27.3 ± 4.2 %, p=0.002). Furthermore, ischemic patients presented higher forces than non-ischemic patients (74.9 ± 10.7 pN vs. 45.4 ± 5.6 pN; p=0.021). Non-ischemic patients also had a lower binding frequency than donors (14.3 ± 4.3 % vs. 27.3 ± 4.2 %, p=0.040). When compared with the control group, CHF patients had higher erythrocyte deformability at lower shear stress values (0.3 to 1.2 Pa; p<0.001), but lower deformability at higher shear stress (12 to 60 Pa; p<0.05). There were no significant changes on erythrocyte aggregation among these groups. CHF non-ischemic patients presented whole blood viscosity (at hematocrit 45%) higher than the control group (7.63 ± 0.13 mPa.s vs. 6.80 ± 0.11 mPa.s; p<0.001, at a shear rate of 22.5 mPa.s, and 4.73 ± 0.07 mPa.s vs. 4.18 ± 0.09 mPa.s; p<0.001, at a shear rate of 225 mPa.s). Ischemic patients' data were similar to the control group. CHF non-ischemic patients had significantly higher whole blood viscosity than ischemic patients (p<0.01).

We can conclude that fibrinogen-erythrocyte interactions are modified in CHF patients. The hemorheologic parameters of erythrocytes from CHF patients are altered, leading to changes on the whole blood flow. These results may be relevant to conclude on the degree of pathophysiological relevance of fibrinogen and erythrocyte aggregation, since an increment on both might induce a state of hyperviscosity and of microcirculatory slow flow. As fibrinogen is an essential protein in this mechanism, it might become an important therapeutic target in the reduction of CHF events.

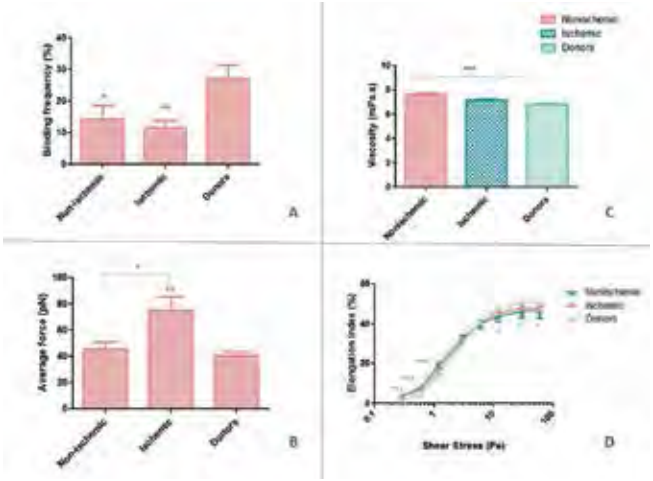


*Figure 1 - Variation of the fibrinogen-erythrocyte (un)binding frequency between ischemic and non-ischemic chronic heart failure patients and healthy donors (A). Force data for the same system is also presented (B). Results were obtained using AFM-based force spectroscopy. Whole blood viscosity of ischemic or non-ischemic patients and control group (after correction of hematocrit to 45%) was represented at shear rate 22.5 mPa.s (C). Erythrocyte deformability (elongation index) was measured at shear stress values between 0.3 and 60 Pa, for the same groups. *, p<0.05; **, p<0.01;***, p<0.001.*

INFLUENCE OF THE NANOPARTICLE PEG CORONA ON THE AFFINITY OF TARGETING LIGANDS

ROBERT HENNIG, Klaus Pollinger, Anika Veser, Miriam Breunig and Achim Goepferich
University of Regensburg, Department of Pharmaceutical Technology, 93053 Regensburg, Germany

The conjugation of polyethylene glycol (PEG) molecules to the surface of nanoparticles, known as PEGylation, is the most prominent measure to reduce serum protein adsorption. This way, the blood circulation time of colloids can be prolonged and they can be sterically stabilized and thus protected against aggregation. To amplify the specific interactions of the nanoparticles with their target cells, ligands can be introduced on the distal end of the PEG chains, which are able to bind specific receptors on the cell surface. In doing so, a precise cell response, like nanoparticle uptake, can be triggered. However, it is often overlooked that the attachment of a ligand to a PEG chain can have a dramatic effect on the ligand's affinity to the receptor, since the PEG molecules often hinder the interaction of the ligand with the receptor binding pocket. Using the angiotensin II receptor type 1, a G-protein coupled receptor that was recently discovered for nanoparticle therapy of the infarcted heart, we investigated to what extent PEGylation alters the affinity of ligands. Furthermore, we determined how nanoparticles can regain a high receptor binding affinity by exhibiting multivalent interactions with their target cells.

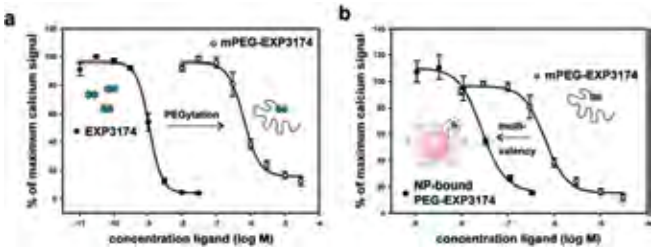


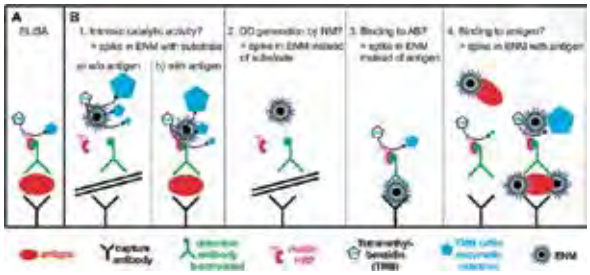
Figure 1: Comparison of ligand affinities. (a) Upon PEGylation of the ligand, its affinity is drastically reduced. (b) By exhibiting multivalent interactions nanoparticles regained a high affinity.

We investigated the receptor affinities of two receptor ligands, the agonist angiotensin II and the antagonist EXP3174, before PEGylation, after PEGylation and when attached to a PEGylated nanoparticle. Interestingly, the affinity loss upon PEGylation was greatly different for both molecules. The affinity of the physiological peptide angiotensin II dropped by a factor of 5 whereas the affinity of the non-peptide small-molecule EXP3174 was tremendously reduced by a factor of approx. 600 after PEGylation. This is due to the fact that both ligand molecules bind to separate sites of the receptor protein and that the PEG tether interferes with the binding pocket differently. However, when conjugated to PEGylated nanoparticles, the affinity of the agonist-modified nanoparticles increased 36-fold whereas the affinity of the antagonist-modified colloids increased 250-fold compared to their PEGylated counterparts. This phenomenon depends on the receptor response after ligand binding. Due to the rapid internalization of the ligand-receptor complex, the multivalent interactions of the agonist-modified nanoparticles are less extensive. Conversely, since antagonists do not provoke receptor internalization, they can rest at the cell membrane and establish tight ligand-receptor interactions although each monovalent interaction is weaker. This has two major implications for choosing the proper ligand for active targeting: (1) the location of the binding pocket of the ligand is of utmost importance for high monovalent affinities, (2) the receptor response upon ligand binding further decides if nanoparticles are capable of establishing a high avidity to the cell. Our findings underscore the importance of affinity testing of ligands before and after PEGylation to identify potent molecules for nanoparticle targeting.

A COMPREHENSIVE EVALUATION PLATFORM TO ASSESS NANOPARTICLE TOXICITY IN VITRO

CORDULA HIRSCH, Matthias Roesslein, Peter Wick, Harald F. Krug
Empa, Swiss Federal Laboratories for Materials Science and Technology, Lerchenfeldstrasse 5, 9014 St. Gallen, Switzerland
cordula.hirsch@empa.ch

The unique properties of engineered nanomaterials (ENM) render them suitable for various consumer as well as industrial applications. The same properties however, may also change their reactivity towards biological systems. Even though studies on biological effects of ENM are available, standardized and validated test systems are still missing. For instance differences in cell type, assay, dose range as well as suspension method for ENM testing make it impossible to compare existing studies [1]. Furthermore, interference reactions of nanomaterials with the test systems or assay reagents frequently lead to false results [e.g. 2 – 9]. Hence, standardized, robust and comprehensively validated tools to assess toxicological effects of ENM are urgently needed.
Here we briefly present the concept of our in vitro evaluation system which addresses four key aspects of cytotoxicity: viability, inflammation, genotoxicity and oxidative stress. To implement this testing platform we exploit well known and for standard chemicals well established assay systems. In a first set of experiments special emphasis is put on the recognition of interference reactions of ENM with these systems themselves and a thorough characterization of the nanoparticles of interest. This is mainly done under cell free conditions. Methods that are found to be least susceptible to such interference phenomena enter a second evaluation round based on classical 2D cell culture systems. During this phase of testing, the robustness, reliability and reproducibility of all methods are carefully reassessed and harmonized for ENM applications. Here we make use of interlaboratory comparisons.
An overview on ENM interference testing, major difficulties, optimization procedures as well as first toxicity results will be presented. In summary our platform not only facilitates reliable and reproducible testing of ENM toxicity but also offers a guideline how to optimize the in vitro assay(s) of interest for ENM suitability.



Schematic overview of potential ENM interference sites during an ELISA procedure.

A) Sandwich ELISA. B) Considerations of potential ENM interference: 1. Do ENMs possess intrinsic catalytic activity? Do they process the substrate by themselves? 2. Does the presence of ENMs per se change the optical density (OD)? 3. Do ENMs bind to the antibodies (AB) used? If yes, does the mere presence of ENMs result in a (false positive) signal? 4. Do ENMs bind to the antigen? Does this binding prevent antigen binding to the AB (false negative result)? Or rather increase antigen affinity towards the AB (false positive result)?

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PEGYLATED NANOPARTICLES FOR BIOMEDICAL APPLICATIONS

CRISTIAN IACOVITA[1,2], Rares Stiufiuc[1,2], Adrian Florea[1], Gabriela Stiufiuc[2,3], Romulus Tetean[3] and Constantin Mihai Lucaciu[1]
[1]"Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania
[2]"Octavian Fodor" Regional Institute of Gastroenterology and Hepatology, Cluj-Napoca, Romania
[3]"Babes-Bolyai" University, Cluj-Napoca, Romania

The importance of nanomaterials in the emergent field of nanomedicine is highly increased in the last years. Among the numerous nanomaterials developed so far, noble metal and magnetic nanoparticles constitute important platforms owing to their interesting material properties never before observed [1,2].
As a consequence, there is a strong demand for developing synthesis strategies capable of producing ready to use biocompatible nanoparticles in a facile, rapid, and cost friendly manner. On the other hand, polyethylene glycol (PEG) is nowadays one of the most used biopolimers, being a principal component in different classes of therapeutic agents which are already in clinical use. It is inexpensive, versatile, and FDA approved for clinical use in USA [3]. Two major advantages can be highlighted in the case of PEGylated nanoparticles with respect to the nonfunctionalized ones: a considerable increase in the circulation time and a much better hydrophilicity.
In this poster we present a simple, green, cost effective and extremely fast synthesis method for preparing stable biocompatible silver and gold colloids by the reduction of either silver nitrate or gold(III) chloride hydrate with PEG at alkaline pH in aqueous media [4,5]. In this synthesis method the unmodified PEG has a dual function, acting as reducing and stabilizing agent. The addition of sodium hydroxide shifts the solution pH towards the alkaline environment, thus reducing the reaction time from several hours to a few seconds. The key element of our method is in the presence of additional -OH groups generated in the solution by sodium hydroxide, enhancing the speed of chemical reduction of silver ions from Ag+ to Ag0. In the case of gold ions (Au3+) the initiation of the reduction process (Au3+ to Au0) requires an addition increase of the solution temperature close to 100°C. The transmission electron microscopy (TEM) images show that both the silver and gold nanoparticles are spherical in shape being covered by a thin PEG layer. By employing PEG of different chain lengths, we were able to tune the zeta potential of the nanoparticles in an interval ranging between −28mV and −2mV in a very controllable and reproducible manner. The variation of the zeta potential could represent a major advantage in the case of their potential use in the biomedical applications since this class of PEGylated nanoparticles could strongly reduce their association with nontargeted serum and tissue proteins
The above method has been applied, into a some extent, for the synthesis of cobalt ferrite (CoFe2O4) nanoparticles. In this case the magnetic precursors (ferric and cobalt chloride) together with the PEG and sodium hydroxide have been steered for 1 h at 80°C. However, the formation of magnetic nanoparticles requires a strong alkaline environment (pH higher than 12), which was achieved by significantly increasing the amount of sodium hydroxide in the synthesis process. TEM images exhibit agglomeration of magnetic nanoparticles having almost spherical shape and a mean diameter below 50 nm. In order to improves the spherical form of the magnetic nanoparticles we have directly dissolved the magnetic precursor into PEG200 in the presence of sodium acetate [6] and kept the mixture inside the oven at 240°C for at least 4 h. This time, the PEGylated magnetic nanoparticles exhibit a well-defined spherical shape and an acceptable poly-dispersity. The magnetic measurements show that in both cases the magnetic nanoparticles are superparamagnetic at room temperature, a very important characteristic for hyperthermia applications.

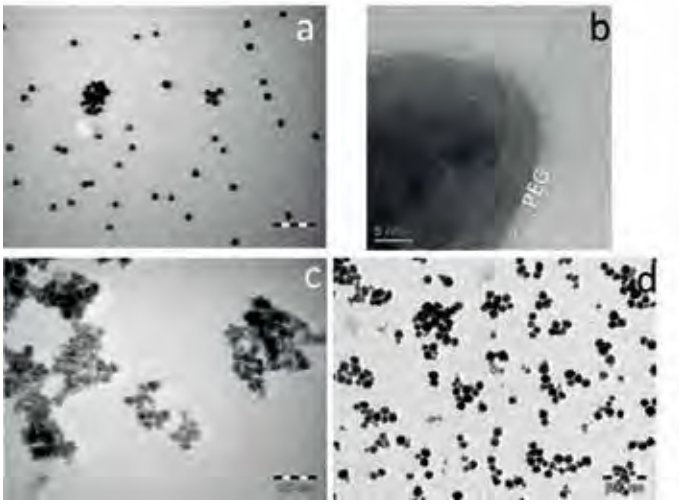

Figure 1: TEM images of a) monodispersed Pegylated Au NPs; b) a single Pegylated Ag NP, PEG layer is clearly visible as a thin layer surrounding the AgNP; c) agglomerations of cobalt ferrite MNPs and d) individual cobalt ferrite MNPs of spherical shape.

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CHALLENGES CONCERNING UPTAKE, INTRACELLULAR TRANSPORT AND METABOLISM OF NANOPARTICLES FOR CLINICAL USE

TORE GEIR IVERSEN[1], Anne Grete Gargul[1], Inger Oulie[1], Tore Skotland[1] and Kirsten Sandvig[1]
[1]Center for Cancer Biomedicine, Faculty Division, Norwegian Radium Hospital, University of Oslo, Norway

Nanoparticles (NPs) have proven promising both as delivery vehicles for therapeutics and as imaging agents for various diseases such as cancer. Importantly, as most molecular targets of the drugs are inside the cell, the NPs should be taken up by the diseased cells in order for the drugs to efficiently reach their target. Thus, thorough investigation of cellular uptake and intracellular transport of the NPs in different cells are warranted (1). We have been studying the uptake and intracellular transport of quantum dot (QD) NPs coupled to different protein ligands (ligand-QDs) that bind to cell receptors in different cell lines. Intracellular transport pathways were perturbed following uptake of the ligand-QDs (2). Moreover, we demonstrated that one of these ligand-QDs was also endocytosed by a mechanism different from the ligand itself (3). Even these relatively small NPs (diam. < 30 nm) seem to accumulate in the cells. Thus, it is important that NPs injected into the body are degraded by the cells into non-toxic metabolites.
Super-paramagnetic Iron oxide NPs (SPIOs) have been safely used as contrast agents in magnetic resonance imaging (MRI) because of their dissolution in the body (4; 5). As part of a clinical cancer vaccination project, we aim at tracing subcutaneously injected mature dendritic cells labelled with internalized SPIOs that migrate to the lymph nodes by MRI. Thus, dendritic cells isolated from cancer patients were exposed to the SPIOs ex vivo for various times. Uptake

and intracellular localization of SPIOs in the dendritic cells were studied by confocal fluorescence microscopy, and we observed uptake and localization of the SPIOs within endosomes and lysosomes (Fig.1a).

a. b.

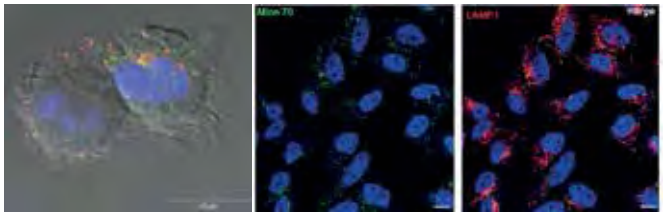

Figure 1. SPIO NPs (Mion 70) internalized into dendritic cells (a.) and HeLa cells (b.) at 37°C for 24 h and 6 h, respectively. The cells were then fixed and prepared for immunofluorescence microscopy labeling them with antibodies against dextran and LAMP-1 and with the appropriate secondary antibody-fluorophore conjugates. Mion 70 nm (green) and lysosomal marker LAMP-1 (red). Yellow in the merged images indicates colocalization. Bars 10µm.

Moreover, cellular uptake and stability of SPIOs with different size and surface properties were also studied in HeLa cells. We observed and quantified the uptake of the SPIOs after increasing time of endocytosis. A major fraction of the internalized SPIOs localized to lysosomes (Fig.1,b), where they might be degraded. Incubation of the different dextran-coated SPIOs in low pH buffers confirmed that the iron oxide core of the SPIOs dissolved in low pH buffers in the presence of iron complexing substances. Cell biological research is crucial to assess the impact of size, surface properties, and chemical composition of NPs on their interaction with, and behavior inside or outside cells.

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IN VIVO STUDIES OF CYTOTOXICITY OF DEXTRAN-DOXORUBICIN NANOPARTICLES AGAINST MURINE BREAST CANCER

MAGDALENA JANCZEWSKA[1], Tomasz Ciach[1], Paweł Włodarski[2], Radosław B. Maksym[2]

[1]Warsaw University of Technology, Faculty of Chemical and Process Engineering, Biomedical Engineering Laboratory, Warynskiego Street 1, 00-645 Warsaw, Poland

[2]Medical University of Warsaw, Department of Histology and Embryology, Chalubinskiego Street 5, 02-004 Warsaw, Poland

Today, in the age of cancer being undeniable a major issue in medicine, nanotechnology arises as a new treatment strategy. A vast scientific, medical and financial contribution made during last years, resulted in development of new drugs having high specificity to the tumor. Even though, the western civilization still cannot manage to cure many types of cancer, and their treatment is mainly based on early diagnosis. That is why more and more scientists turn their steps towards nanotechnology.

Nanoparticles are described as submicron structures able to chemically or physically entrap drug or diagnostic agent. [1] Due to the small diameter they are able to avoid opsonization leading to fast clearance by RES system. Moreover, all cellular pathways take place in nano size range. Small size also favors accumulation of nanoparticles almost exclusively in tumor area. According to the literature, vessels created during angiogenesis are relatively fragile and particles of diameter below 100 nm are able to passively cross their barrier, increasing drug concentration in the malignant cells and simultaneously reduce the side effects. This phenomena is known as Enhanced Permeation and Retention (EPR) effect. [2,3] Another important feature of a nanoparticle is its surface charge which is expressed by zeta potential measurement. Biocompatibility and biodegradability of nanoparticles is an important issue when designing a drug carrier. To make sure that particles can be fully metabolized by human cells, the nanoparticle skeleton is made of natural polymer approved by FDA and already used in medical applications – dextran.

Targeting properties are investigated by conducting in vivo experiments. There are two common ways of drug administration described in the literature: intravenous and intraperitoneal. Intravenous injection enables accurate comparison of therapeutic effect of applied nanoparticles with traditional chemotherapy. It provides certainty that the delivery system is present in the bloodstream. However, studies comparing both routes of administration have shown that intraperitoneal application provides higher blood levels of nanosystem throughout the experiment. [4] Intraperitoneally injected particles go through diaphragm and are absorbed into lymphatic capillaries. Finally, through lymph nodes nanoparticles reach the bloodstream. This route eliminates the risk of opsonisation and rapid clearance by the MPS system and allows administration of higher doses of the drug. [5]

The aim of this paper is to discuss in vivo experiments of dextran – doxorubicin nanoparticles when compared to traditional chemotherapy with free doxorubicin. To obtain nanoparticles, dextran is oxidized and modified chemically to form pH-dependent bounding of coiling agent – dodecylamine and anticancer drug – doxorubicin. That leads to self-assembly of polysaccharide chain into nanoparticle by hydrophilic – hydrophobic interactions in aqueous solution. Synthesized particles have size range of 70 – 100 nm and slightly negative zeta potential. Nanoparticles were purified and lyophilized and stored in 4 Celsius degree. In vivo experiment was carried out on murine model of breast cancer (4T1 cell line). Balb/c female mice were injected subcutaneously with 1 mln of cells. Mice weight range was 19-22 g and tumor size varied between 5-7 mm. The experiment was carried out for 20 hours and then drug concentration in murine organs was depicted using IVIS scan. Results are presented on fig. 1.

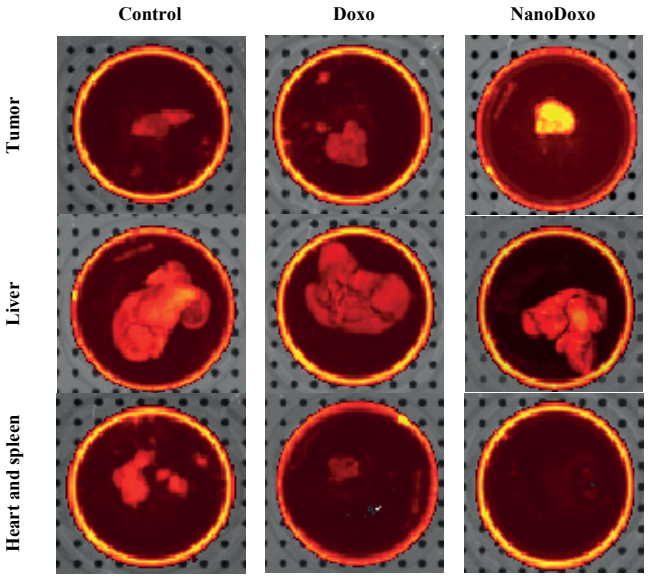

Fig. 1. Doxorubicin distribution in the key organs after 20 h from injection. Comparison of targeting of free doxorubicin and doxorubicin containing nanoparticles. IVIS scan Ex/Em 465/600 nm.

Images confirmed significant increase of doxorubicin concentration in tumor area when administrated inside nanoparticles. Quantitative measurement of fluorescence showed almost fivefold higher concentration of drug in the tumor tissue. What is particularly important, the use of nanoparticles allowed to minimize cardiotoxicity , often unpleasant side effect of therapy with doxorubicin. Simultaneously, showed relatively unchanged, comparing to free drug, liver toxicity. Presented results are promising, however, they need to be confirmed on the larger scale and in vivo drug release needs to be investigated.

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TOXICOLOGICAL ANALYSES OF UNLOADED AND CHEMOTHERAPEUTICS-LOADED SPION FOR CANCER THERAPY

C. JANKO, H. Unterweger, J. Zaloga, S. Dürr, S. Knaup, J. Matuszak, I. Cicha, RP. Friedrich, R. Tietze, S. Lyer, C. Alexiou
Department of Otorhinolaryngology, Head and Neck Surgery, Section for Experimental Oncology and Nanomedicine (SEON), University Hospital Erlangen, Glückstraße 10a, 91054 Erlangen

Chemotherapy is a major treatment strategy for cancer. Since conventional chemotherapeutics are systemically applied intravenously, extremely high doses are necessary to reach effective therapeutic doses in the tumor region. Consequently, severe systemic side effects may occur in the patients.
The non-specific toxic effects of chemotherapeutic drugs in healthy tissues can be strongly reduced by the site directed tumor therapy using Magnetic Drug Targeting (MDT). For that, a chemotherapeutic agent (e.g. mitoxantrone, cisplatin) is loaded on superparamagnetic iron oxide nanoparticles (SPION). These particles are applied into the tumour supplying vascular system and enriched in the tumour region employing an external magnetic field [1]. Thus, high local concentrations of the chemotherapeutic agent can be achieved in the tumour region while the rest of the body is preserved from the toxic effects [2].
For the future translation of SPION from bench to bedside, in addition to the physicochemical characterization of the nanoparticles extensive toxicological analyses must be performed to exclude possible risks for the patients. For that, the combination of complementary techniques as flow cytometry, impedance measurement (xCELLLigence system), and transmission/ fluorescence microscopy of SPION treated cells enables us to draw a comprehensive picture of the nanoparticle mediated effects. So far, our preliminary toxicological analyses in vitro showed that SPION loaded with chemotherapeutics (cisplatin, mitoxantrone) induce massive cell death in tumor cells whereas unloaded SPIONs are well biocompatible (Figure 1) [2, 3].
Our future aim is to broaden our toxicological assay basis for the comprehensive pre-clinical toxicological characterization of our nanoparticle systems to finally aspire the translation into clinical applications.

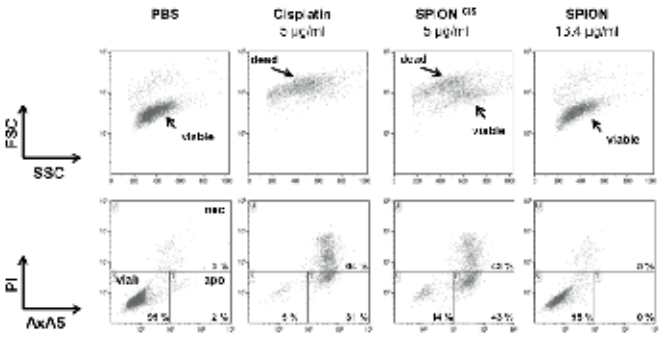

Figure 1: Flow cytometric determination of cytotoxic effects of unloaded SPION, cisplatin-loaded SPION (SPIONCIS) or fluid cisplatin on Jurkat cells (t=48h). PBS treated cells served as controls.

Upper row: Forward (FSC) and side scatter (SSC) provide information about cellular size and granularity. Untreated cells and cells treated with unloaded SPION both show viable cell morphology. Treatment with SPIONCIS or fluid cisplatin leads to altered cellular morphology due to blebbing processes during apoptosis and plasma membrane rupture during necrosis.
Lower row: AnnexinA5-Fitc/Propidium iodide (AxA5/PI) staining provides information about the cell death pathway. Thus, AxA5-/PI- cells are viable, AxA5+/PI- cells are apoptotic and PI+ cells are necrotic. Untreated cells and SPION treated cells remain viable during the observation period. In contrast, treatment with SPIONCIS or fluid cisplatin induces apoptosis and secondary necrosis.
Note: Since cisplatin has to be released from SPIONCIS, a delayed cell death induction can be observed compared to fluid cisplatin.

Acknowledgements: We thank the Bavarian State Ministry of the Environment and Consumer Protection (74-U8793-2012/7-35) for their financial support.

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IN VITRO ANTIOXIDANT AND HEPATOPROTECTIVE POTENTIAL OF AZOLLA MICROPHYLLA PHYTOCHEMICALLY SYNTHESIZED GOLD NANOPARTICLES ON CYPRINUS CARPIO L.

SELVARAJ KUNJIAPPAN, Ranjana Chowdhury, Chiranjib Bhattacharjee
Department of Chemical Engineering, Jadavpur University, Kolkata-700032, India

Hepatic disorder remains a serious health problem and even death, and is caused by excess consumption of alcohol, high doses of acetaminophen, chemotherapeutic agents, hepatitis viral infection, dantrolene sodium, valporic acid, peroxidised oil and isonicotinic acid hydrazide, etc. Medicinal plants are frequently considered to be less toxic and free from side-effects than synthetic drugs. The search for bioactive compounds of plant origin with potent hepatoprotective activity has become a central focus for study of hepatoprotection today. Azolla is a genus of small aquatic fern that is freely found in the temperate and tropical regions of the world. Azolla microphylla is one of the species from the genus Azolla; it is pteridophyte plantae belongs to the Salvinacea family. It is phytochemically reported to be rich in proteins, vitamins, alkaloids, fla-

vonoids and anthroquinone glycosides. Flavonoids have long been known to exhibit a strong antiproliferative, hepatoprotective and free radicals scavenging activities during various biological functions, and prevent cell damage and cell lysis. For the first time rutin and quercetin were isolated and purified from Azolla microphylla in our laboratory. The antioxidant potential of the plant hepatoprotectors is an important factor in the phototherapy of acetaminophen induced liver disfunction because acetaminophen toxic (N-acetyl-p-benzoquinoneimine) metabolite could lead to oxidative stress by enhancing free-radical oxidation processes and destroys the hepatocellular antioxidant protection system. Phytochemically synthesized green gold nanoparticles provide strong platform in medical diagnostics and therapeutics. The present study aims to evaluate the in vitro antioxidant and hepatoprotective effects of gold nanoparticles (GNaP), biosynthesized through the mediation of methanol extract of Azolla microphylla, on acetaminophen-induced hepatocyte damage in common carp fish (Cyprinus carpio L.). The molecular size, shape and involvement of bioorganic compounds in green synthesized gold nanoparticles were analyzed by UV-Spectroscopy, FTIR, FESEM, UHRTEM, XRD, EDX and TG-DTA. The fish was anaesthetized and dissected to remove the liver and homogenized to isolate primary hepatocytes. The biosynthesized gold nanoparticles (100, 150, 200µg/mL) and Azolla microphylla methanol extract powder (100, 200, 400µg/mL) were added to the primary hepatocytes in different conditions; treatment-I (before 12mM acetaminophen), treatment-II(after 12mM acetaminophen) and treatment-III(both before- and after- 12mM acetaminophen) with the incubation of the hepatocytes. The primary hepatocyte cultures, 6×105 cells/well in a 24 well plates, 12mM acetaminophen exposed to damage and rupture the plasma membrane, a cell leakage in culture medium at the indicated concentrations of lactate dehydrogenase (LDH), catalase (CAT), glutamate oxalate transaminase (GOT), glutamate pyruvate transaminase (GPT) and malondialdehyde (MDA) significantly increased the levels by almost 50-80%, and significantly decreased the levels of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) almost 60-75%. Methanol extract of A. microphylla phytochemically biosynthesized gold nanoparticles (100, 150, 200µg/mL) and A. microphylla methanol extract powder (100, 200, 400µg/mL) were added and significantly improved the viability of cells in a culture medium. It was observed that it significantly reduced the levels of lactate dehydrogenase (LDH), catalase (CAT), glutamate oxalate transaminase (GOT), glutamate pyruvate transaminase (GPT) and malondialdehyde (MDA), and significantly increased levels of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). Comparatively, methanol extract of A. microphylla mediated biosynthesized gold nanoparticles showed more potent action than methanol extract of A. microphylla. The observed hepatoproductive and antioxidant effects of methanol extract of Azolla microphylla phytochemically biosynthesized gold nanoparticles might be due to its several flavonoids on the surface of the gold nanoparticles. This study validated the hepatoproductive and antioxidant potential of the A. microphylla mediated biosynthesized gold nanoparticles.

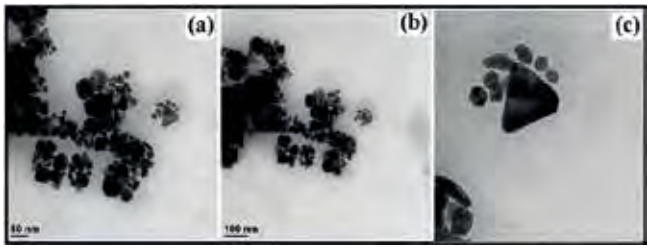

Figure 1 HRTEM image of green synthesized gold nanoparticles

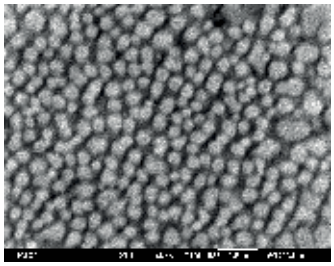

Figure 2 FESEM image of green synthesized gold nanoparticles

DEVELOPMENT OF A SAFE AND ROBUST TARGETED NANOMEDICINE STRATEGY FOR RNAi BASED CANCER THERAPEUTICS

DALIT LANDESMAN-MILO[1,2§], Meir Goldsmith[1,2§], Sharon Avkin[3§], Boaz Nachshon[4], Vered Morad[4], Sigalit Leibovitch[4], Lael Werner[4], Asaf Ori[3], Anna Gelfand[3], Elena Safarian[3], Hagar Kalinski[3], Eyal Shalom[3], Igor Mett[3], Hagit Ashush[3], Elena Feinstein[3]* and Dan Peer[1,2]*

[1]Laboratory of NanoMedicine, Dept. of Cell Research and Immunology and Dept. of Materials Science and Engineering, Tel Aviv University, 69978, Israel.

[2]Center for Nanoscience and Nanotechnology, Tel Aviv University, 69978, Israel.

[3]QBI Enterprises Ltd, Weizmann Science Park, Ness Ziona 70400, Israel.

[4]Harlan Biotech Israel Ltd, Kiryat Weizmann, Rehovot 76326, Israel.

[§]These authors have equally contributed to this work

*To whom the correspondence should be addressed

Utilizing RNA interference (RNAi) as a novel therapeutic modality has an enormous potential to bring the era of precision medicine from a notion to reality. Several clinical trials already demonstrated promising results. Yet, the targeting of RNAi payloads into specific tissues and cells is still an obstacle. Attempts to overcome the systemic, local and cellular barriers that prevent RNAi are usually based on nucleating the RNAi with a positive molecule that when released generate undesirable adverse effects. It has been recently established by us and others that the pro-inflammatory response is the result of the cationic nature of the transfection reagents that agonize toll-like receptors 4 and 2.

In order to enable successful utilization of RNAi payloads as potential novel therapeutics as well as to utilize the advantages of targeted drug delivery, we set out to develop a safe, non-cationic based RNAi delivery strategy to invasive tumors that frequently overexpress a collagen internalizing receptor Endo180.

Herein, we report on a novel, safe, targeted nanoparticles (NPs) coated with proprietary anti-Endo180 mAb as a targeting vehicle of RNAi payloads towards tumors expressing Endo180, Endo180-HA-NPs. These lipid-based NPs are composed of naturally occuring lipids with a stabilized coating, the glycosaminoglycan hyaluronan (HA), which also acts as a scaffold for mAb binding.

We demonstrated a specific and potent knockdown in vitro using Endo180-HA-NPs entrapping siRNAs as well as preferential in vivo accumulation of siRNAs in human lung adenocarcinoma (A549) tumor xenografts expressing Endo180. In mouse efficacy studies with established A549 tumor xenografts, their significant growth inhibition was demonstrated using Endo180-HA-NPs entrapping siRNA targeting Polo-like kinase 1(PLK1). Finally, healthy animals treated with Endo180-HA-NPs entrapping siRNAs did not display signs of toxicity, and no cytokine induction or interferon response were observed when the Endo180-HA-NPs were incubated with human peripheral mononuclear cells (PBMCs). In addition, no complement activation was observed when tested on human plasma. Taken together, these results demonstrate that Endo180-HA-NPs might be a new RNAi delivery strategy to highly invasive tumors and ultimately might become a new therapeutic modality for advanced cancer.

PEPTIDE LINKED NANOPARTICLES TO CROSS THE BLOOD-BRAIN BARRIER

MIGUEL A. LÁZARO[1], Pau Brugada[1,2], Primiano de Mauro[1,2], Anna Cascante[1], Xavier Rivero[1], Eduard Diviu[1], Salvador Borros[1,2]*

[1]Sagetis-Biotech, Barcelona, Spain; [2]Grup d'Enginyeria de Materials (GEMAT), Institut Quimic de Sarria, Universitat Ramon Llull, Barcelona, Spain; E-mail: salvador.borros@iqs.url.edu

INTRODUCTION

The blood-brain barrier (BBB) is the major problem in drug delivery to the brain because limits drug penetration. In fact it could be said that the delivery of small molecules and genetic material across the BBB is not fully resolved at the moment. Nowadays, the use of

biodegradable polymeric nanoparticles has a great interest as non-invasive method for the delivery of drugs to the brain. It is know that some peptides can cross the BBB due to receptor-mediated transport (transcytosis). One strategy to improve the drug delivery to the brain is the use of these specific peptides linked to the surface of the nanoparticles [1].
The aim of our project is to deliver small molecules or genetic material encapsulated into a polymeric nanoparticle to the brain. These nanoparticles will be linked to peptides to facilitate the transcytosis. The present work is focuses on the in vitro analysis of this nanoparticles.

RESULT

The nanoparticles were obtained with a block-copolymer of polyester and PEG to increase the stealth and help the transport in the blood. In addition, the nanoparticles were derivatized with specific peptides to improve the active transport across the BBB. Peptide linked nanoparticles were obtained by precipitation of a solution of polymer in water. The suspension was purified and concentrated by tangential flow filtration (TFF) to obtain the working samples. Nanoparticles were characterized by dynamic light scattering (DLS) and particles sizes smaller than 200 (PDI < 0.1) and negative surface charge were obtained. The amount of peptide in the nanoparticles was determined by amino acids analysis in a UPLC after peptide hydrolysis with HCl (6M).
Different peptides that could be able to cross the BBB have been designed and synthetized by our company. Also an in vitro model of BBB for checking the pass of these peptides and the nanoparticles has been developed. The fluorescent derivatives of our proprietary peptides were used for experiments of transport across the BBB model (figure 1). Angiopep (ANG) has been shown to cross the BBB by receptor-mediated transcytosis via low-density lipoprotein receptor-related protein 1 (LRP1) and has been used in systems for drug delivery to brain [2]. For this reason, ANG was chosen as reference in the experiment and the integrity of the barrier was checked with lucifer yellow (LY). It was observed that PEP1 peptide had almost the same percentage of crossing that ANG.

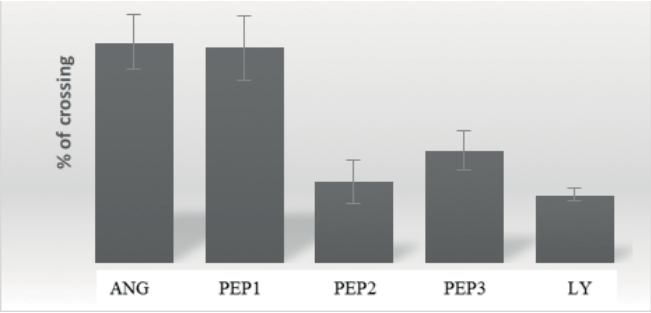

Fig 1. Fluorescent brain penetrating peptides transport experiments

An experiment of pass across the in vitro BBB model was done with peptide decorated and non-decorated nanoparticles (figure 2). The blank experiment was performed without cells in the insert and the concentration of nanoparticles was determined by nanoparticles tracking analysis (NTA). The amount of decorated nanoparticles in the luminal compartment (up) and in the abluminal compartment (down) was similar to the results obtained for the blank experiment. On the other hand, the non-decorated nanoparticles showed a very low pass across the barrier model.

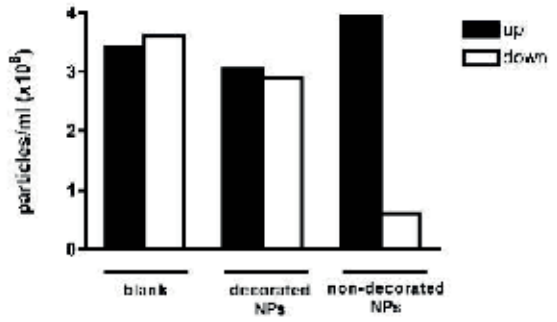

Fig 2. Decorated nanoparticles passage across the in vitro BBB model

Moreover, some experiments to study the mechanism of internalization of the decorated nanoparticles were performed. The expression of LRP-1 in BBB endothelial cells was detected by a specific antibody by optical microscope. After that, the internalization of nanoparticles decorated with fluorescent peptides was studied in bovine brain microvascular endothelial cells and U87 glioma cells at 4 ºC and 37 ºC. The internalization was observed in both cases only at 37 ªC that indicates an active transport of the nanoparticles through the cell membrane . Finally, an experiment of co-localization of nanoparticles decorated with fluorescent peptides and LRP-1 was done with U87 glioma cells at 37 ºC (figure 3). The images showed an overlap between fluorescent nanoparticles and LRP-1.

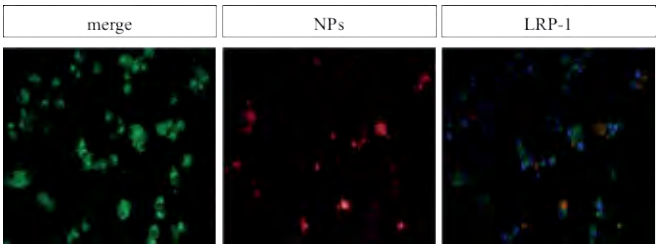

Fig 3. Nanoparticles on glioma cells: co-localization with LRP-1

CONCLUSIONS

The novel peptide linked nanoparticles of this study show to have a very interesting properties to be used for efficient drug delivery to the brain. The proprietary peptides can efficiently cross the BBB by receptor-mediated transcytosis via LRP-1. The in vitro determinations showed that the nanoparticles decorated with the peptide could successfully cross the BBB acting as a potential drug delivery carrier to the brain.

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BIOAVAILABILITY ENHANCEMENT OF ARTEMISININ THROUGH AMORPHIZATION BY NANO-CONFINEMENT VIA CO-SPRAY DRYING WITH ORDERED MESOPOROUS SILICA

KUMARAN LETCHMANAN, Shou-Cang Shen, Wai Kiong Ng, Reginald B.H. Tan

Bioavailability of poorly water soluble drug, Artemisinin (ART), was enhanced through amorphization by encapsulating the drug particles inside ordered mesoporous silica, SBA-15. Since ART is classified as a Biopharmaceutics Classification System (BCS) class II drug, the bioavailability of ART was enhanced by improving the dissolution rate and supersaturation solubility of the drug.
The amorphous solid dispersions of ART/SBA-15 at two different drug loadings (1:3 w/w and 1:1 w/w) were formulated via co-spray drying technique. The formulated solid dispersions were characterized using BET, PXRD, SEM, TEM and contact angle analyser. The drug release profiles of untreated and treated ART were investigated by using in vitro dissolution tester (USP-II). The storage stability of all the samples was examined for 6 months under 5 different storage conditions in open pan and Activ-Vial®, involving different relative humility and temperature. Finally, the cytotoxicity of ART and SBA-15 on human colon carcinoma (Caco-2) cells were analysed using flow cytometry after 24 hours incubation.
Characteristic studies Characterization studies (Table 1) demonstrated the outstanding features of SBA-15, including a high surface area, large pore volumes and uniform pore size distribution, which make it an excellent drug delivery vehicle for poorly water soluble drug. At the same time, the results of characterization also reveal the amorphization of ART once encapsulated inside the pore channels of SBA-15.

The in vitro drug release profiles (Figure 1) shows that within the first 15 min, crystalline ART, spray dried ART and physical mixtures of ART/SBA-15(1:1) and ART/SBA-15(1:3) achieved drug release of 15%, 47%, 27% and 14%, respectively. Under the same sampling time, the spray dried ART/SBA-15 showed an initial burst release, as 57% and 64% of ART was released from ART/SBA-15 (1:3) and ART/SBA-15 (1:1), correspondingly. The ART released from the mesoporous silica was approximately 4 folds higher than the corresponding value from untreated crystalline ART. In addition, the supersaturation of amorphous formulated ART was enhanced to two-fold higher (101 mg/L) than the thermodynamic solubility of crystalline ART (48 mg/L at 37 °C). The improved supersaturation of ART was sustained for 2 hours without any precipitation. The enhanced apparent solubility of ART would increase the availability of ART for absorption across the gastrointestinal epithelium, as well as improve the drug bioavailability.

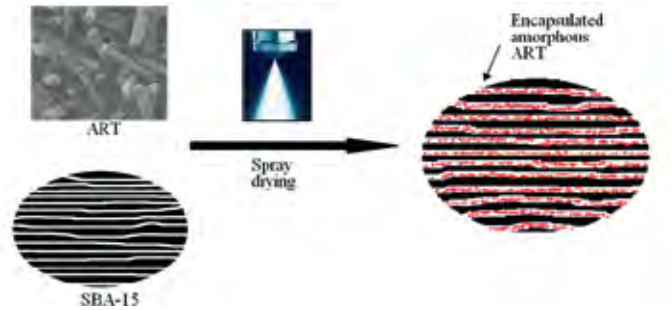
The ART/SBA-15 samples that were stored inside desiccators (25 °C/18% RH), Activ-Vial® at 25 °C and 40 °C displayed only a slight degradation of ART after 6 months of storage. However, the samples in open pans at 25 °C/75% RH and 40 °C/75% RH degraded significantly. This shows that hydrolytic degradation is predominant during the storage, whereby the relative humidity (% RH) plays an important role in chemical instability of amorphous ART compared to the storage temperature. All the samples that were stored at a higher relative humidity (75% RH), regardless of storage temperature, degraded easily compared to the samples that were stored inside Activ-vial and desiccators with low humidity. The presence of moisture at 75% RH acts as a medium for proton transfer to accelerate the rate of hydrolysis of ART.

The physical stability of ART/SBA-15 formulations upon storage was investigated under three different storage conditions involving low relative humidity for 6 months. These ART/SBA-15 samples still showed halo patterns and no evidence of PXRD peaks corresponding to crystalline ART. This indicates that the amorphous ART entrapped inside the pore channels of SBA-15 still remained physically unchanged as no crystal growth could be detected by PXRD. It is suggested that SBA-15 has a strong re-crystallization inhibition effect that is able to stabilize the ART amorphous forms from physical state modifications during long term storage periods. The size-constraint effect of SBA-15 on nucleation and crystal growth plays a major role in providing physical stability to amorphous ART. SBA-15 only has pore size in the range of 8-9 nm which is highly effective in restricting the ART molecules from nucleating and, thus re-arranging themselves in ordered structure.

Pure ART, SBA-15 and formulated ART/SBA-15 samples illustrate no harmful effect on the Caco-2 cells over a broad spectrum of tested concentrations. Even at the highest concentration of samples, the cell survival rate remained above 90%, confirming less particle interference on cell integrity, which causes low cytotoxicity effect. Importantly, none of the samples show IC50 value at any concentration after 24 h exposure. The morphologies of the treated cells were almost the same as the normal and untreated cells. Additionally, the proliferation of cells could be observed as the cells started to grow and adhered the bottom of the well plates. The unchanged shape and morphologies of Caco-2 cells clearly demonstrates the non-toxic nature and good biocompatibility of SBA-15 and ART/SBA-15 formulations.

In conclusion, the SBA-15 submicron particles with outstanding features were used as drug delivery vehicle for poorly soluble ART. Amorphization due to spatial confinement of ART inside the pore channels of SBA-15 mainly contributed to the remarkably enhanced dissolution kinetics compared to the crystalline ART. The amorphous ART/SBA-15 solid dispersions were able to achieve supersaturation more than two-fold of the equilibrium solubility of crystalline ART which can be sustained for 2 hours without addition of any precipitation inhibitors. The solid dispersions of ART/SBA-15 exhibited excellent physical stability under all storage conditions, whereas the chemical stability was affected by humidity regardless of storage temperatures. Thus, it is suggested to store the ART/SBA-15 samples under low humidity conditions to achieve long shelf life. Moreover, the results of cytotoxicity investigation indicated that SBA-15 and ART/SBA-15 formulations clearly demonstrate its good biocompatibility without obvious inhibition on the Caco-2 cell viability.

GRAPHICAL ABSTRACT



RESULTS

Table 1: Pore volume, surface area and average pore size of SBA-15 before and after spray drying with ART

Sample	Surface area (SBET) [m2/g]	Total pore volume (Vpore) [cc/g]	Average pore diameter (d) [nm]
SBA-15	809.0 ± 26.3	1.16 ± 0.04	8.70 ± 0.32
S.D SBA-15	778.9 ± 62.8	1.11 ± 0.13	8.19 ± 1.17
ART/SBA-15 (1:3)	370.7 ± 41.8	0.63 ± 0.04	7.34 ± 0.53
ART/SBA-15 (1:1)	112.8 ± 24.5	0.20 ± 0.04	6.40 ± 0.32

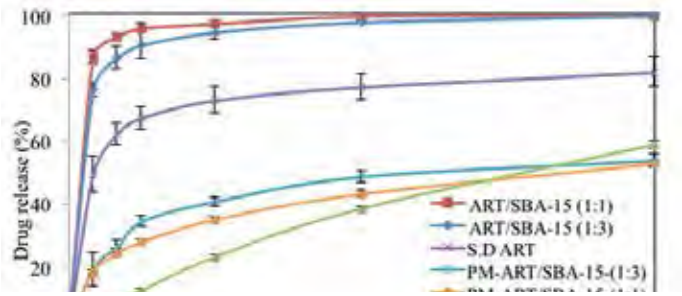


Figure 1: Dissolution profiles (sink condition) of pure ART crystal, physical mixtures and solid dispersions of ART/SBA-15

P-GLYCOPROTEIN (ABCB1) AND THE BREAST CANCER RESISTANCE PROTEIN (ABCG2) – SIMILARITIES AND DIFFERENCES

XIAOCHUN LI-BLATTER*, Estefanía Egido[§^], Rita Müller[+], Gracia Merino[§^] and Anna Seelig[+]

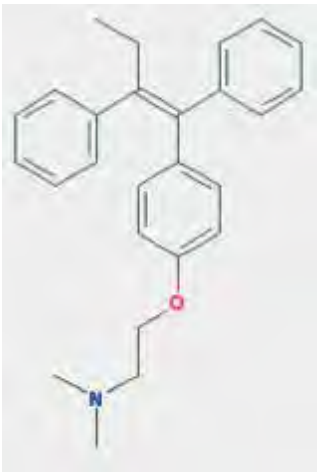
*Biophysics facility, Biozentrum, University of Basel, Switzerland;
[+]Biophysical Chemistry, Biozentrum, University of Basel, Switzerland;
[§]Departamento de Ciencias Biomédicas, Fisiología, Facultad de Veterinaria
^Instituto de Desarrollo Ganadero y Sanidad Animal (INDEGSAL), Universidad de León, Campus de Vegazana, 24071 León, Spain.

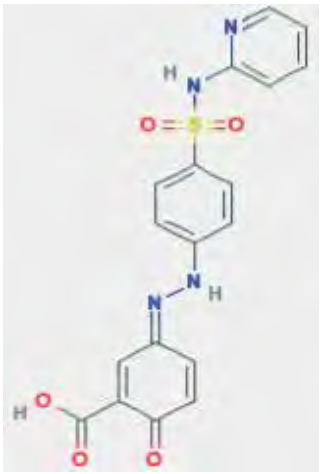
The breast cancer resistance protein, BCRP (ABCG2) and P-glycoprotein (ABCB1) are both members of the ABC-transporter family that use the energy from ATP hydrolysis to move toxins, including many drugs (collectively called allocrites) from the cytosolic membrane leaflet to the extracellular side of the membrane. Although, the two proteins exhibit very low sequence homology, they show partially overlapping allocrite specificity. The mechanism of substrate binding has been investigated in detail for Pgp. However, little is known on the interaction of allocrites with BCRP. Here, we report on the nature and the strength of the interaction of 36 compounds with P-glycoprotein and BCRP based on ATPase activity measurements and lipid-water partition coefficient measurements. Both transporters showed membrane mediated allocrite binding, based essentially on hydrogen bond formation. However, allocrite affinity to the transmembrane domains was generally higher in the case of BCRP than in the case of P-glycoprotein, suggesting a more hydrophobic binding cavity in BCRP. Out of the 36 compounds interacting with BCRP, 26 also interacted with P-glycoprotein. A clear differentiation between allocrites for BCRP and P-glycoprotein was possible using three molecular allocrite descriptors: electrical

charge, polar surface area, and amphiphilicity. The analysis shows that the two transporters perfectly cover the broad chemical space of membrane-intruding compounds.

Tamoxifen
cationic & amphiphilic

Sulfasalazine
zwitterionic & non-amphiphilic





P-glycoprotein substrate

BCRP substrate

Figure 1: Structures of Tamoxifen as P-glycoprotein substrate and Sulfasalazine as BCRP substrate.

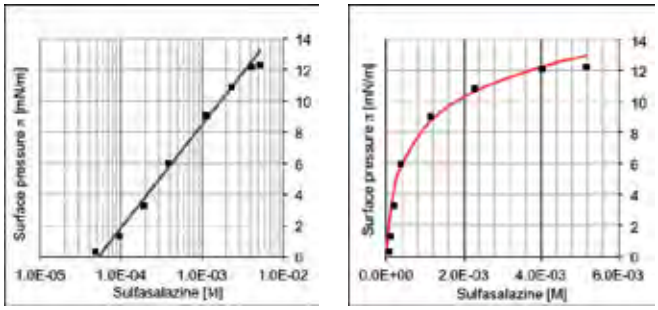


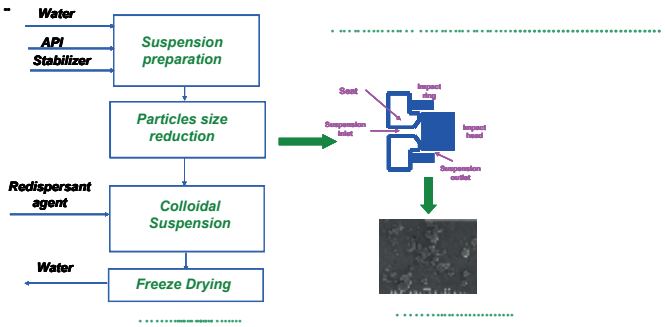
Figure 2: The surface pressure of Sulfasalazine as a function of concentration, measured in buffer solution at pH 7.4 (50 mM Tris/HCl, 114 mM NaCl).

NANOPARTICLES FOR PARENTERAL CLINICAL USE

PHILIPPE LIENARD, Jean-René Authelin, Ross Blundell & Mostafa Nakach

This poster will describe the technical and operational details of the SSNIV as Sterile Solid Nanoparticle for I.V. platform and provide process & finished product data to demonstrate its performances.
SANOFI decided to invest in injectable nanoparticle technologies in Paris. The facility is planned to be fully operational in 2015.
SSNIV is a European unique multi-purpose platform facility for scale-up and fabrication of nanocrystalline suspensions, emulsions and liposomes to supply products for human use.

Nanocristalline suspensions are currently one of the most promising "enabling technologies" for poorly soluble molecules. Nanoparticles are investigated for the parenteral administration route, when the classical ways to solubilize the active pharmaceutical ingredient (e.g. micellar solution, cyclodextrin, co-solvent…) do not work. The specific needs of parenteral route create very difficult technical challenges: these include the use of specific stabilizers that are compatible with i.v. administration, sterility of the nanocrystalline suspension and absence of large (micron-sized) particles.



The HPH as High Pressure Homogenizer was chosen, as the best compromise for the specific use. In this system, a crystal suspension is forced by a very high pressure (up to 1500 bars) to go through a very small orifice (few µm) at very high velocity (> 100 m/s). The process flow chart description and the schematic representation of the high pressure homogenization valve are pictured beside. The chosen HPH technology for the platform demonstrated its capacity to enhance the nanoparticle surface specific area. Milling results will be shown to highlight the performance of HPH vs Bead Milling, particularly regarding sharpness of particle size distribution, without any polymorphism transformation and no metal contamination observed.
Indeed, this cGMP pilot facility will be able to supply nanocrystalline suspension for parenteral route for highly active products up to the OEB5 internal level (HSE 1µg/M3). Terminal sterilization will be performed before vial fill and finish step.

Final product can be sterile nanocrystalline suspension or emulsion or liposome in vials ready for human use.
Typical batch size
- 0.2 to 6 kg API
- 10 to 60 liters

For human use, the final product is sterile
- I.V. or parenteral administration
- Nanocristalline suspensions, liposomes, emulsions in vials, when it is physically and chemically stable
- A freeze dried solid in vials for limited stability products.

TOXICOLOGICAL ASSESSMENT OF LOPINAVIR SOLID DRUG NANOPARTICLES

NEILL J. LIPTROTT[1], Christopher A.W. David[1], Marco Giardiello[2], Philip Martin[1], Steve Rannard[2] and Andrew Owen[1]
[1]Department of Molecular and Clinical Pharmacology, Institute of Translational Medicine, the University of Liverpool, Liverpool, UK
[2]The Department of Chemistry, the University of Liverpool, Liverpool, UK

Nanomedicines offer potential therapeutic benefits, such as increased bioavailability, without the cost of discovering new active pharmaceutical ingredients (APIs). However, the behaviour of some nanomaterials has been demonstrated to differ considerably from larger bulk material [1]. The production and use of engineered nanomaterials is constantly expanding, but there remain uncertainties surrounding the potential risks posed to human health and the environment. Nanoparticles (NPs) are believed to induce toxicity in cells, primarily through generation of reactive oxygen species (ROS) and caspase activation, the mechanism of ROS generation has also been found to vary considerably depending on the type of nanoparticle [2]. Through generation of oxidative stress it is possible that nanoparticles may then cause mitochondrial dysfunction, caspase activation and subsequent apoptosis [3-5]. We sought to assess reactive oxygen species generation, caspase activation, mitochondrial membrane polarisation and apoptosis in CEM (T-lymphocyte cell line) and THP1 (monocyte cell line) treated with an aqueous solution of the HIV antiretroviral lopinavir (LPV) and lopinavir solid drug

nanoparticles (LPV-SDN) formulated using a previously published approach [6]. CEM and THP1 cells were treated with either LPV (15µM) for 24 hours LPV-SDN (15µM based on API content). Camptothecin (10µM) and Menadione (10µM) were included as positive controls. CellROX reagent (ROS detection), polycaspase reagent (caspase activation), MitoProbe JC-1 (mitochondrial membrane polarisation detection) and Annexin V-488 conjugate (apoptosis detection) were used to assess various mechanisms of toxicity. Data were generated on a MACSQuant flow cytometer (Miltenyi Biotec), enabling appropriate gating for viable cells. Data are presented as mean ± Standard deviation of n = 4 experiments conducted in triplicate. Statistical analysis was conducted using unpaired t-test using Stats Direct software (version 2.7.9).

Menadione (MND) was used as a positive control to induce oxidative stress. The level of ROS in CEM when treated with MND was higher than untreated cells but not statistically significant (figure 1a). Neither LPV nor LPV-SDN elicited significantly higher levels of ROS in CEM cells. ROS levels in THP1 treated with MND were 3-fold higher than that in untreated THP1 cells (P=0.002; figure 1b). LPV incubation also resulted in higher ROS levels in THP1 (1.8-fold higher; P=0.0002) whereas incubation of THP1 cells with LPV-SDN only resulted in a 1.5-fold higher ROS accumulation (P=0.043).

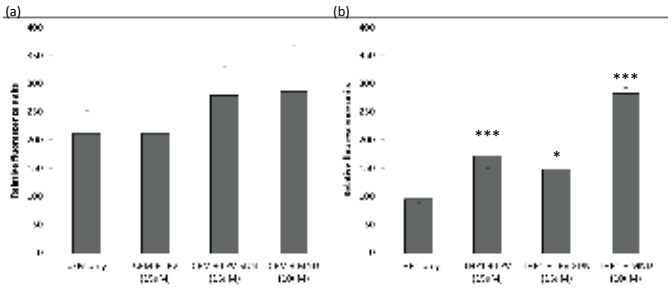


*Figure 1. Reactive oxygen species accumulation. CEM (a) and THP1 (b) were treated with either LPV or LPV-SDN for 24 hours. Menadione (MND) was included as a positive control. Data expressed as mean ± SD, N=4. *=P<0.05, **=P<0.01, ***=P<0.001*

Intracellular caspase activation was also assessed. Camptothecin was used as a positive control and resulted in a 2-fold (P=0.0004) and 1.5-fold (P=0.0002) higher level of caspase activity in CEM and THP1 compared to untreated cells (figure 2a & b), respectively. There was significant caspase activation in CEM cells treated with LPV (1.8-fold higher, P=0.005) compared to untreated cells whereas LPV-SDN did not significantly activate caspases in either CEM or THP1 cells (figure 2a & b).

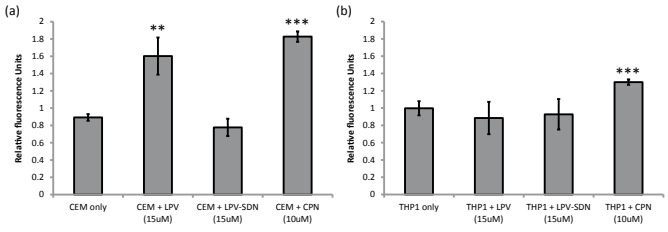


*Figure 2. Caspase activation. CEM (a) and THP1 (b) were treated with either LPV or LPV-SDN for 24 hours. Camptothecin (CPN) was included as a positive control. Data expressed as mean ± SD, N=4. *=P<0.05, **=P<0.01, ***=P<0.001*

Mitochondrial membrane potential was assessed using the JC-1 dye. JC-1 exhibits potential-dependent accumulation in mitochondria, indicated by a fluorescence emission shift from green (~529 nm) to red (~590 nm). Consequently, mitochondrial depolarization is indicated by a decrease in the red/green fluorescence intensity ratio. Camptothecin treatment resulted in significantly lower fluorescence ratios in CEM (3.3-fold lower; P=0.002) and THP1 cells (1.3-fold lower; P=0.006) (figure 3a & b). LPV treatment also resulted in significantly lower fluorescence ratios in CEM (2.5-fold lower; P=0.0002) and THP1 (1.2-fold lower; P=0.0004). However, LPV-SDN did not significantly affect mitochondrial membrane polarisation in either CEM or THP1 cells.

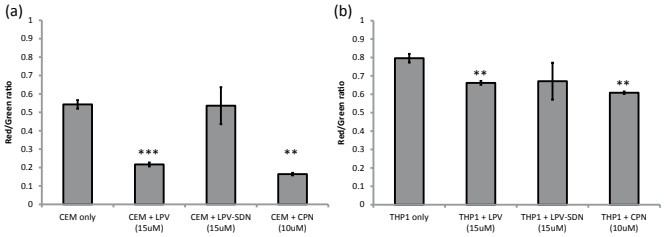


*Figure 3. Mitochondrial membrane polarisation. CEM (a) and THP1 (b) were treated with either LPV or LPV-SDN for 24 hours. Camptothecin (CPN) was included as a positive control. Data expressed as mean ± SD, N=4. *=P<0.05, **=P<0.01, ***=P<0.001*

Finally, the level of apoptosis was assessed in THP1 cells by measuring the amount of Annexin V bound cells. Camptothecin treatment resulted in significantly more apoptotic cells when compared to untreated cells (figure 4; 22% apoptotic cells; P=0.034). LPV (6.5%; P=0.04) and LPV-SDN (5.5%; P=0.046) treatment also resulted in significantly more apoptotic cells compared to control but there was no difference between LPV and LPV-SDN (18% difference; P=0.06).

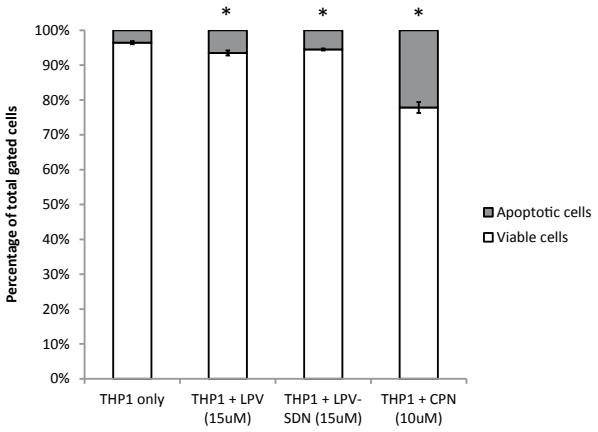


*Figure 4. Apoptotic cell determination. CEM (a) and THP1 (b) were treated with either LPV or LPV-SDN for 24 hours. Camptothecin (CPN) was included as a positive control. Data expressed as mean ± SD, N=4. *=P<0.05, **=P<0.01, ***=P<0.001*

These data illustrate the utility of specific methodology for medium-throughput detection of potential toxicity of SDNs (and potentially other nanomaterials) via flow cytometry. The data also show that the LPV-SDN formulation performed favourably in comparison to a LPV aqueous solution. This is possibly due to altered subcellular localisation of the LPV-SDN particles since we have previously shown a higher accumulation of LPV-SDN compared to LPV solution (data not shown). Work is now underway to further assess these properties in primary immune cells from healthy volunteers.

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SYNTHESIS OF MODULAR DELIVERY NANOSYSTEMS FOR TUMOUR IMAGING AND THERAPY

IRAIDA LOINAZ, Damien Dupin, Miren K. Aiertza, Germán Cabañero and Hans-Jurgen Grande
Biomaterials Unit, Materials Division, IK4-CIDETEC, Spain,
iloinaz@cidetec.es

INTRODUCTION

Currently chemotherapy treatment for solid tumours is based on highly efficient drugs that often do not improve the healing process. The reason is their heterogeneous distribution in the tumour due to irregular vascular perfusion and dense interstitial matrix.1 In order to develop more effiecient delivery system for anticancer agents several nanotherapies have already been approved for commercialization. The common mode of action relies on the passive accumulation of the carriers as a result of the enhanced permeation and retention (EPR) effect around leaky regions of the tumour vasculature. However, the centre of the tumour is unperfused and harbours the most aggressive cells with both low pH and PO2 conditions. As a consequence, the delivery efficiency of therapeutics to this region is low due to poor diffusion and the tumour can be regenerated after treatment. Moreover, the exposure of cancer cells to sublethal concentrations of drug facilitates the development of drug-resistance.2

To allow an effiecient delivery of the cancer drug into the tumour a modular delivery nanovehicle can be developed, changing size to facilitate the transport in each biological barrier. For instance, 150 nm size particles will favourably accumulate in the extracellular matrix of the tumour. Once there, they could be partially degraded by the over-expressed metaloprotenases (MMP) secreted by cancer cells to liberate smaller polymeric nanoparticles (NPs) of around 10-15 nm that will diffuse into the interstitial matrix. These final nanocarriers could be used for both detection and treatment of cancer tumours, mixing NPswith the required characteristics for the desired purposes.

In the last few years, researchers in the Biomaterials Unit of IK4-CIDETEC have been working on single-chain polymer NPs (SCPNs), especially for imaging and drug delivery applications.3 More recently, nano and microgel technology has been incorporated to offer a wider range of vehicles. As a combination of both processes, gelatin based nanogels that entrap SCPNs have been generated to be able to obtain a modular delivery nanosystem for solid tumour imaging and treatment.

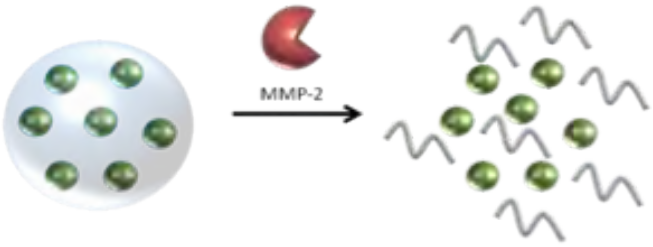

Figure 1. Gelatin based nanogels containing SCPNs (left); as soon as the MMPs degrade the gelatine SCPNs are released (right).

EXPERIMENTAL METHODS

Gelatin nanogel synthesis using nanoprecipitation. First, low molecular weight gelatin was eliminated. Then acetone was added under vigorous stirring to an aqueous solution of gelatin adjusted at acidic pH to obtain the nanogels, which were cross-linked by continuous addition of glutaraldehyde. For imaging purposes the nanogels were decorated with 2,2'-(7-(1-carboxy-4-((2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)amino)-4-oxobutyl)-1,4,7-triazonane-1,4-diyl)diacetic acid (maleimide-NODA-GA), which reacts directly with the primary amino groups from the gelatin. SCPNs were also incorporated into the gelatine nanovehicles using vinylbenzyl chloride (VBC) and Traut´s reagent.
Synthesis of SCPN. NPs based on poly(N,N-dimethylaminoethyl) methacrylate (PDMAEMA) were synthesised following the procedure published elsewhere.4 These NPs were decorated with NODA functionality, which was covalently incorporated on the surface of the NPs via thiol groups.
Dynamic Light Scattering (DLS) and Zeta potential studies were carried out using Malvern Zetasizer Nano ZS on particle dispersion of 0.01wt. % in PBS.
FE-SEM images were taken using Zeiss Ultra Plus. Samples were prepared on silicon wafer and sputtered with gold.

RESULTS AND DISCUSSION

Gelatin nanogels were produced using nanoprecipitation and the right conditions were established to obtain particles between 100-150 nm in size in solution (50-75 nm on a dry surface shown in Figure 2). FE-SEM characterization confirmed the nearly spherical and uniform morphology of the particles. The isoelectric point (IEP) of these nanogels was located between pH 5.5-6, depending on the quantity of primary amine used for the cross-linking reaction (Figure 2). A change in size could also be observed when the pH was around the IEP due to the protonation of the carboxylic acid resulting in the decrease of electrostatic attraction between carboxylate anions and cationic primary amine.

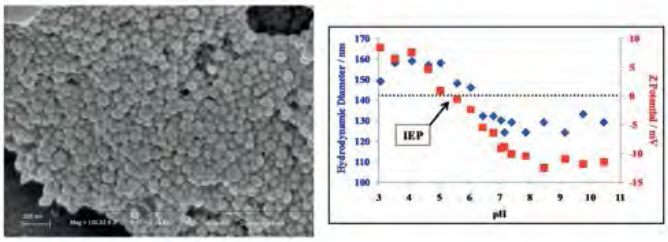

Figure 2. FE-SEM image of gelatin based nanogels (left); DLS (♦) and Zeta potential (■) gelatin nanogel particles in function of the solution pH in PBS buffer.

The functionalization of the nanogels with NODA was carried out following two different strategies: 1) decoration of the nanogel once it was formed or 2) functionalization prior to nanoprecipitation process. The former particles showed smaller sizes than the latter, which was more significant when the nanogels were functionalised with SCPNs compared to NODA following the two previously mentioned pathways. As can be seen in Figure 3, the final nanogels diameter varied from 150-200 nm if the SCPNs were decorating the surface of the particles to 350-450 nm if the SCPNs were embedded.

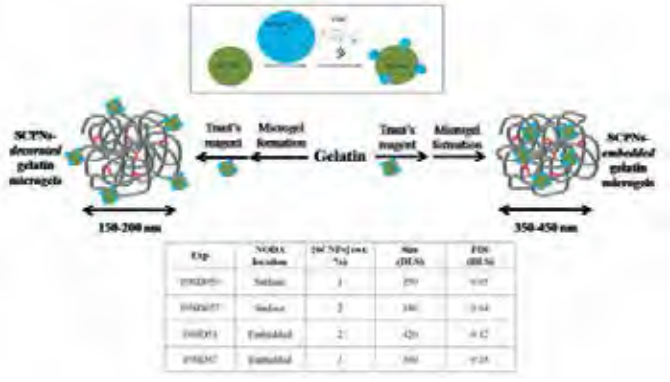

Figure 3. Schematic representation of the two methods to prepare gelatin nanogels containing SCPNs (top); summary of the DLS diameter of gelatin nanogels prepared following the two pathways (bottom).

CONCLUSION

Gelatin nanogels were successfully synthesised and characterized using glutaraldehyde as a reticulation agent. The functionalization of these particles with NODA was effectively achieved, starting from a NODA modified gelatin or decorating the nanogels with NODA once they were produced. Finally, SCPNs were also incorporated into or around the nanogels following the same strategy as for the NODA moiety, obtaining different sizes depending on the

process utilized. As soon as the amount of NODA is quantified, the functionalised nanogels will be tested in vivo in rats for biodistribution studies using Positron Emission Tomography (PET).

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ACKNOWLEDGMENTS

The authors would like to thank the ETORTEK program [[(BIOMAGUNE 11(E11-301)] for providing financial support to this project.

CHARACTERIZATION AND EVALUATION OF POLYACRYLIC ACID COATED NANOPARTICLES FOR BIOMEDICAL APPLICATIONS

JASNA LOJK[1], Vladimir B. Bregar[1], Katarina Miš[2], Mateja Erdani Kreft[3], Tomaž Marš[2], Peter Veranič[3], Mojca Pavlin[1]*
[1]Faculty of Electrical Engineering, University of Ljubljana, SI-1000 Ljubljana, Slovenia
[2]Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, SI-1000 Ljubljana, Slovenia
[3]Institute of Cell Biology, Faculty of Medicine, University of Ljubljana, SI-1000 Ljubljana, Slovenia

The small size of nanoparticles (NPs)1 and appropriate functionalization enabled the use of NPs in several biomedical and biotechnological applications as delivery vehicles, for cell labelling and tracking and other biotechnological applications2,3. One of the most prominent advantages of NPs is the possibility of targeted delivery, which facilitates NP accumulation mostly in the tissue of interest and thus reduces side effect of the treatment4. In general biodegradable NPs are preferred for in vivo use, but on the other hand, magnetic NPs give the possibility to manipulate them by a static or time-varying magnetic field. Currently magnetic NPs are used for magnetic separation, biosensors, tissue repair, drug delivery, hyperthermia treatments of tumors and as MRI contrast agents.3
The interactions between cells and NPs are highly influenced by NP properties such as size, shape5 and surface properties6biopharmaceutical molecules and imaging agents to target cells in disease sites. Such diagnostic and therapeutic nanomaterials, also termed \"nanomedicines\", often require site-specific cellular entry to deliver their payload to sub-cellular locations hidden beneath cell membranes. Nanomedicines can employ multiple pathways for cellular entry, which are currently insufficiently understood. This review, first, classifies various mechanisms of endocytosis available to nanomedicines including phagocytosis and pinocytosis through clathrin-dependent and clathrin-independent pathways. Second, it describes the current experimental tools to study endocytosis of nanomedicines. Third, it provides specific examples from recent literature and our own work on endocytosis of nanomedicines. Finally, these examples are used to ascertain 1. The internalization pathway and intracellular fate of the particles determine the rate and amount of uptake, nanoparticle retention and also influence the negative consequence of their presence, like cell stress or in worst cases even cell death. Besides NP properties, the NP-cell interactions are also highly dependent on the physiology of the specific cell type7. The biological properties of cell in terms of type and frequency of endocytosis, division rate and level of metabolism can affect the level of internalisation, intracellular fate and toxcity8. Determining the effects of NPs on different cell types is thus pivotal for proper evaluation of adequacy of the designed NPs for a certain application.
In this study, we analysed the cellular responses to cobalt ferrite (CoFe2O4) magnetic NPs, coated with poly acrylic acid (PAA) in three different cell types (CHO and B16-F10 cell lines and primary human myoblast cells - MYO). We determined the internalization pathway, intracellular trafficking and intracellular fate of our NPs using fluorescence and transmission electron microscopy (TEM), in parallel cell viability was assessed. This knowledge will help us evaluate the suitability of the designed NPs for certain applications and gain new invaluable knowledge for further design of NPs to target specific intracellular target organelles.
NPs were prepared and characterized as described previously9. We obtained a suspension of NPs approximately 33 nm in diameter and -50 mV surface charge, highly stable in water suspension and adequately stable in cell culture media supplemented with serum9,10. To determine the effects of NPs on the cells, all three cell types were incubated with different concentrations of NP for different time periods. Internalization pathways and intracellular fate of NPs were determined using TEM and fluorescence microscopy. After 1h incubation, NPs were found bound to the cell membrane and trapped in different membrane perturbations, typical of two endocytic pathways6biopharmaceutical molecules and imaging agents to target cells in disease sites. Such diagnostic and therapeutic nanomaterials, also termed \"nanomedicines\", often require site-specific cellular entry to deliver their payload to sub-cellular locations hidden beneath cell membranes. Nanomedicines can employ multiple pathways for cellular entry, which are currently insufficiently understood. This review, first, classifies various mechanisms of endocytosis available to nanomedicines including phagocytosis and pinocytosis through clathrin-dependent and clathrin-independent pathways. Second, it describes the current experimental tools to study endocytosis of nanomedicines. Third, it provides specific examples from recent literature and our own work on endocytosis of nanomedicines. Finally, these examples are used to ascertain 1; membrane ruffles characteristic of macropinocytosis, and small clathrin-coated pits (CCP) on the cell membrane as well as in already internalized clathrin-coated vesicles (CCV), confirming clathrin-mediated endocytosis (CME) as an internalization pathway. Once inside the cell, NPs were observed in different membrane enclosed vesicles. Immediately after endocytosis, NPs were located in early endosomes, which matured into late endosomes. Late endosomes fused with lysosomes to form hybrid organelles, where digestion of the endocytosed cargo occurs6biopharmaceutical molecules and imaging agents to target cells in disease sites. Such diagnostic and therapeutic nanomaterials, also termed \"nanomedicines\", often require site-specific cellular entry to deliver their payload to sub-cellular locations hidden beneath cell membranes. Nanomedicines can employ multiple pathways for cellular entry, which are currently insufficiently understood. This review, first, classifies various mechanisms of endocytosis available to nanomedicines including phagocytosis and pinocytosis through clathrin-dependent and clathrin-independent pathways. Second, it describes the current experimental tools to study endocytosis of nanomedicines. Third, it provides specific examples from recent literature and our own work on endocytosis of nanomedicines. Finally, these examples are used to ascertain 1. Additionally, NPs were also found in amphisomes, digestive vesicles formed by fusion of autophagosome and endosome. This indicates that the presence of NPs in the endosome does not interfere with the normal intertwining of the two intracellular digestion pathways.
To observe the intracellular fate of NPs on live cells, fluorescence microscopy was performed after 1 h and 24 h incubation with RITC-stained PAA NPs. Cells were additionally stained with LysoTracker® Blue dye to label acidic organelles. With longer incubation time, the number of internalized NPs increased, vesicles containing NPs increased in size and accumulated predominantly in the perinuclear region of the cell. At specific time intervals dependent on cell type, NP fluorescence colocalized with acidic organelles such as late endosomes, amphisomes and lysosome. Most colocalization was observed after 1 h in CHO cells, after 24 h in B16 cells while in MYO cells only a fraction of NPs colocalized at either time point. This shows that although the three observed cell types internalized NPs through the same endocytic pathways, the rate of intracellular trafficking is cell type specific and that for MYO cells colocalization was probably between the two analysed time points (1h and 24h).
Cells were also observed with TEM after 24 h of incubation to determine the intracellular fate. Larger quantities of NPs were found enclosed in vesicles that were mostly much larger than those observed after 1 h incubation. No exocytosis was observed, indicating

long-term accumulation of NPs.
To determine the effect of larger quantities of internalized PAA coated NPs on viability and proliferation of the chosen cell lines, cells were grown in the presence of four different NP concentrations (50, 100, 150 and 200 µg/ml) for 24 h and viability was analysed using PI viability test (Figure 1A). Based on these results, exposure to NPs had no effect on cell proliferation, as cell viability did not decrease with increasing NP concentration after 24 h incubation for all three cell lines.

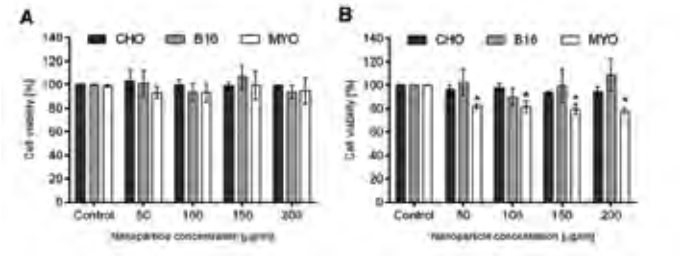

Figure 5: Cell viability after (A) 24 h or (B) 96 h exposure to increasing concentration of PAA coated Co-ferrite NPs for CHO cell line (CHO), B16 cell line (B16) and primary human myoblast cells (MYO). In both cases, viability was determined with PI viability assay. The results are presented as percentage of viable cells compared with the number of cells in the control sample for each cell line. Mean and standard error are shown for three independent experiments.

Due to considerable difference in division rate between the two stable cell lines (CHO and B16) and primary human myoblast cells (MYO), being 12-13 h for CHO and B16 and 3-5 days for myoblast cells, we wanted to verify whether the doubling time has an effect on long term cytotoxicity. Cells were incubated with NPs for 96 h, during which time most it not all cell population divided for all three cell lines. The same viability assay now enabled us to observe both the toxicity of NPs, by selective staining of dead cells with PI, and the effect of NPs on cell division rate. Based on our results (Figure 1B), increasing concentration of NPs again had no effect on cell viability in CHO and B16 cell lines. However, we observed a statistically significant decrease in cell number for slowly dividing MYO cells. The decrease in cell viability was not concentration dependent, dropping to approximately 80% at all four used NP concentrations. As well, the ration of dead cells did not increase with increasing concentration (results not shown).
Despite general similarities in NP uptake and their intracellular trafficking, we observed some morphological and physiological differences between the three selected cell lines. The most interesting observation, however, was the drop in cell viability in MYO cells after 96 h incubation. The seemingly non-toxic NPs, as observed after 24 h incubation, which is the standard incubation time in cytotoxicity studies, caused a concentration independent cell cycle arrest without causing additional cell death as assessed with PI staining. At the same time, there was no reduction in cell number in CHO and B16 cell lines. Although stable cell lines are known to be less susceptible to environmental stress compared to primary cells, the difference in response is most probably the result of the considerable difference in division rate. Due to shorter cell cycle, the internalized quantity of NPs in CHO in B16 cells did not reach a critical concentration before the cell divided, therefore dividing also the internalized NPs among daughter cells11. On the other hand, MYO cells presumably accumulated larger NP quantities, which interfered with the cell cycle progression.
In conclusion, here we showed that the three considerably different cell types used the same endocytic and intracellular trafficking routes, but still the dynamics of the processes were cell type specific. PAA coated NPs proved to be nontoxic, although we observed a drop in viability in MYO cells, most probably due to a cell cycle arrest. This could be due to known susceptibility of the primary cells, but also due to a longer generation time, resulting in higher NP uptake. This may lead to lysosomal dysfunction, but also results in prolonged intracellular NP persistence, as needed for certain applications.

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MAGNETIC PARTICLE INDUCED HYPERTHERMIA AFTER MAGNETIC DRUG TARGETING

STEFAN LYER, S. Dürr, C. Janko, R. Tietze and C. Alexiou
Department of Otorhinolaryngology, Head and Neck Surgery, Section of Experimental Oncology and Nanomedicine (SEON), University Hospital Erlangen, Waldstraße 1, 91054 Erlangen, Germany

Hyperthermia already is in clinical use as a pre-treatment before radio- or chemotherapy. In this context temperatures between 40-44°C are conducted. Temperatures above 60°C are applied as single treatment (thermal ablation) [1]. Additional to established heating methods the use of magnetic nanoparticles a magnetic AC fields have come into focus for the application of a hyperthermia that is localized to a very limited area of the body. Usually the magnetic NP's are injected directly into the tumour and the area is exposed to a alternating magnetic field between 100 kHz and 450 kHz. However, most of the respective trials are in preclinical status, although some studies already have been conducted in human glioblastoma patients [2].
Magnetic Drug Targeting (MDT) is another promising new cancer treatment based on magnetic nanoparticles. Superparamagnetic iron oxide nanoparticles (SPIONs) are coated with a biocompatible layer to produce a ferrofluid being stable in mammalian body fluids. Loaded with a chemotherapeutic, the SPIONs are injected near a solid tumour intraarterially into the tumour supporting vascular system. A the same time, an external magnetic field is directed onto the tumour and the nanoparticles as well as the chemotherapeutic are accumulated in the tumour area. In 2013 SEON could show in the to date worldwide largest animal study in the area of MDT, that by this approach an high portion of the applied chemotherapeutic agent mitoxantrone can be deposited in the tumour area, which lead to complete tumour remissions in 30% of the tumour bearing rabbits after one single application of MDT [3].

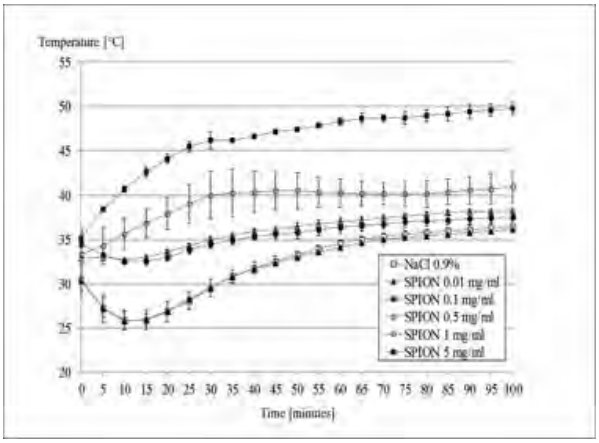

Figure 2: Heating curves of SPION-dilutions in NaCl 0,9%. concentrations under application of an AC magnetic field.

The idea of the work presented here was to combine these two promising therapeutic therapy options that are both based on magnetic nanoparticles. After the physicochemical characterization of the SPIONS phantom studies were done to study the heating properties of the ferrofluid before the in vivo application. It could be shown, that the particle suspension is capable of being heated by the utilized AC magnetic field generator. This alternating magnetic field system was developed to be able to treat animals from the size of mice to rabbits. Therefore a split-coil constructed from two flat pancake coils with variable distance was used (Siemens CT, Erlangen). A dilution series in physiolocical NaCl-solution showed, that the heating capacity was concentration dependent.
In a next step, a VX2-tumour was implanted subcutaneously at the left hind limb of a new zealand white rabbit. After approximately three weeks the tumour had reached a size so that it had to be treated. For application the particles were injected in the arteria femoralis while an tumour area was exposed to an external magnetic field. After particle accumulation the tumour area was placed in the middle of the two pancake coils and exposed to the AC magnetic field. Within this time a temperature rise (DT) of ca. 9°C could be observed in the tumour area, while temperature probes placed subcutaneously at the outer thigh and the abdominal wall did not show a DT of more than 2°C. After turning of the alternating magnetic field the temperature fell rapidly until the end of the monitoring.
This preliminary data show that the particles used in the experiments are capable of being heated by an AC magnetic field in vitro. The in vivo data shows, that using these particles as well as the used AC magnetic field generator it is possible to induce a significant DT after accumulating the particles in the tumour area via Magnetic Drug Targeting. Hence we could show, that a combination of the two strategies Magnetic Drug Targeting and Hyperthermia induced by AC magnetic fields is possible.

Acknowledgement: This work was supported by BMBF-Spitzencluster (01EX1012B), by the DFG (AL 552/3-3), and by the Else Kröner-Fresenius-Stiftung, Bad Homburg vor der Höhe, Germany.

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FTIR AS AN IN-PROCESS CONTROL METHOD TO EVALUATE LIPIDS CONTENT

ALEXANDER LYSKIN, Doron Friedman, Michael Raslin, Yechezkel Barenholz and Dima Shamrakov
Laboratory of Membrane and Liposome Research, The Hebrew University -Hadassah Medical School and LipoCure Ltd, both of Jerusalem Israel

LipoCure is developing a number of highly-potent therapeutics utilizing proprietary liposome-based nano-drugs with novel drug-loading methods and drug-release mechanisms. In the course of liposome-based nano-drugs manufacturing, in-process determination of total lipids content is a critical step during production.
Currently this analysis is performed by a lengthy HPLC procedure, which interrupts the production for many hours while waiting for the HPLC analysis results.
Therefore a rapid alternative procedure was introduced, where the "in-process" samples are assayed "as is" using an FTIR spectrophotometer equipped with multiple bounce Zn-Se ATR (attenuated total reflectance) sampling module. The measurement is based on asymmetric stretch vibrations of the phosphate band at ~1232cm-1.

In this work, we developed and validated a new rapid method to evaluate lipids content in-process by FTIR. The FTIR method is based on generation of a calibration curve with standard solutions. In the validation work, we found good linearity, accuracy and precision of the method. In addition, we verified this method by the HPLC/Evaporative Light Scattering Detection (ELSD) validated method. Therefore, measurements by the FTIR method can be performed for the lipids content determination in the manufacturing process. The FTIR method, which we developed and validated, meets all criteria for the in-process control method, allowing, if necessary, making adjustments in the production process.

ANAPHYLAXIS CAUSED BY ANTICANCER DRUG INFUSIONS: LABORATORY PREDICTION BASED ON COMPLEMENT FRAGMENT AND FACTOR-H ELISAS

TAMÁS MÉSZÁROS[1], Tamás Schneider[2], László Rosivall[3], András Rosta[2], Rudolf Urbanics[4], János Szebeni1,[4]
[1]Semmelweis University, Nanomedicine Research and Education Center, Budapest, Hungary
[2]National Cancer Institute, Budapest, Hungary
[3]Semmelweis University, Institute of Pathophysiology, Budapest, Hungary
[4]SeroScience Ltd, Budapest, Hungary

Anticancer drugs that contain nanoparticles (liposomes, micelles), as well as therapeutical monoclonal antibodies (mAbs) often cause hypersensitivity reactions (HSRs) as a consequence of their complement (C) activating effect. The phenomenon, called C activation-related pseudoallergy (CARPA) represents a risk for anaphylaxis, and may also contribute to the immunogenicity of certain nanoparticulate drugs and mAbs. In an effort to develop a laboratory test predicting CARPA, we quantified and correlated the HSRs caused by Taxol, Taxotere, Rituxan and Herceptin with the rises of C3a, C5a and SC5b-9 caused by these drugs in vivo and in vitro, following incubation with the sera of patients. The symptoms of HSRs (graded between 0-4) were correlated with C activation, using –among others- ROC analysis. HSRs occurred in 60% of patients despite premedication. There was significant correlation between the rise of SC5b-9 in the pretreatment sera of patients incubated with different reactogenic drugs and the grade of HSRs these drugs caused in vivo. In the case of Rituxan, our data suggest the use of whole blood for in vitro screening for C activation, since C activation by Rituxan occurs, at least in part, on (CD20+) lymphocytes. In the case of whole blood assay, hirudin anticoagulation is essential. Taken together, our data confirm the CARPA concept and show significant correlation between HSRs and SC5b-9 rises, suggesting the use of this C activation biomarker in HSR predictive tests. Among the patient factors that may influence sensitivity to CARPA, we measured the levels of factor H in reactive and non-reactive patients and found -according to preliminary data-, inverse correlation between proneness for C activation/CARPA and factor H levels. On the basis of the known role of C activation in the rise of specific immunity, the above in vitro C tests might also be used to predict the immunogenicity of Rituxan and other mAbs, which, just as the HSRs, presents a major barrier to the clinical use of these drugs.

COMPARISON OF POLYSACCHARIDE DAUNORUBICIN-LOADED NANOPARTICLES EFFICACY WITH THE DRUG ADMINISTERED IN PURE FORM ON MURINE MAMMARY CARCINOMA CELL LINE 4T1 AND HUMAN HEPATOCELLULAR CARCINOMA CELLS HEPG2

MAGDALENA MICHALAK[1], Iwona Cymerman[2], Tomasz Ciach[1]
[1]Faculty of Chemical and Process Engineering, Warsaw University of Technology, Warsaw, Poland.
[2]Laboratory of Molecular and Cellular Neurobiology, International Institute of Molecular and Cell Biology, Warsaw, Poland.

Due to its high cardiotoxicity, daunorubicin is currently used in anticancer therapy with extreme caution. Furthermore, administration of this cytostatic drug causes bothersome side effects, such

as bone marrow suppression, nausea and severe vomiting, skin changes and hair loss. This is the reason why unlike other drugs from the group of anthracyclines, it has been used only in treatment of acute leukemias and as a final step in the course of pediatric patients therapy.

To reduce the toxicity of daunorubicin during treatment and thus increase the extent of its operation in patients suffering from cancer, it has been loaded into polymeric dextran-nanoparticles. Usage of dextran as a carrier in this case has a number of positive aspects. Firstly, dextran is widely used in medicine as a blood substitute; as a polymer of glucose, it is not toxic inside the human body. In addition, as saccharide, dextran may be decisive in targeting tumor. This results from the assumption of the Warburg effect - the tumor cells show the need for increased absorption of saccharides. Such dextran-based nano-delivery system should result in no release of the drug in the patient's body until the nanoparticle is metabolized inside the tumor cell.

One of the characteristics of cancer cells, is their ability to carry out rapid and numerous divisions. As a result, they have an advantage over the immune system defenders that cannot keep up with the production of cells to fight the enemy. One of the criteria for selection of cell lines to this study, was fast propagation and performance during continuous divisions. The first choice was the cell line 4T1, which is a murine mammary carcinoma of the fourth degree. 4T1 spontaneously produces highly metastatic tumors and metastasizes in the lungs, liver, lymph nodes and brain, while the primary tumor is growing in situ. The second line selected was HepG2 of human hepatocellular carcinoma. In the case of these types of cancer, daunorubicin chemotherapy is not applicable. Therefore, demonstration of the efficacy of the contained drug might widen the choice of treatment options. Both lines are adherent and grow in a monolayer which allowed for a standardize testing scheme .
Cultures were grown in suitable media. For 4T1, American Type Culture Collection (ATCC®) approved high glucose RPMI-1640 medium (GIBCO) supplemented with 10% Fetal Bovine Serum (GIBCO). HepG2 cells were grown in Dulbecco's Modified Eagle Medium (Sigma-Aldrich) supplemented with 10% Fetal Bovine Serum (GIBCO), 5% Penicillin-Streptomycin (GIBCO) and 5% L-Glutamine (GIBCO). Conducted tests included verification of the effectiveness of the pure drug and of the daunorubicin-loaded nanoparticles with 50 nm diameter. The first stage of the study was to establish the most effective concentration of both tests systems – pure drug and nanoparticles. To compare the results we calculated how much of the drug is enclosed in the nanoparticles and we made a series of dilutions from 106ng / ml to 10-2ng/ml.

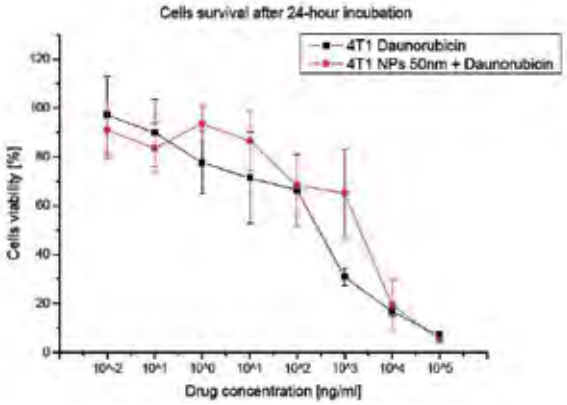
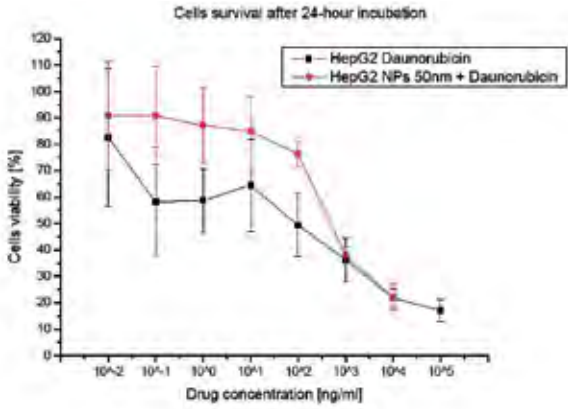


Fig. 1 Results of 4T1 cells viability based on the MTT assay after 24 hours of incubation with pure dauanorubicin and daunorubicin-loaded nanoparticles with 50nm diameter.

Fig. 2 Results of HepG2 cells viability based on the MTT assay after 24 hours of incubation with pure dauanorubicin and daunorubicin-loaded nanoparticles with 50nm diameter.



In case of 4T1 cells, the best result was reached for 105ng/ml concentration level. HepG2 cells reacted with smaller decrease in viability. There were some problems with reading of the results for concentration 105ng/ml. Probably they were caused by the intensive red color of samples. Thus, for HepG2 cells the most effective concentration level was 104ng/ml. The effectiveness of incubation time was examined in studies in the second stage.
In addition, to confirm the effectiveness of the drug-loaded nanoparticles and certainty of their entry into the tumor cells, images were taken with a confocal microscope. They showed the location of released drug in the 4T1 cell's nucleus, where anthracyclines intercalate with DNA.

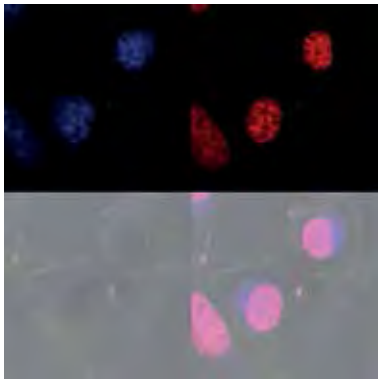


Fig. 3 Confocal microscope image of 4T1 cell line after one hour incubation with the pure drug at concentration level of 105ng/ml.

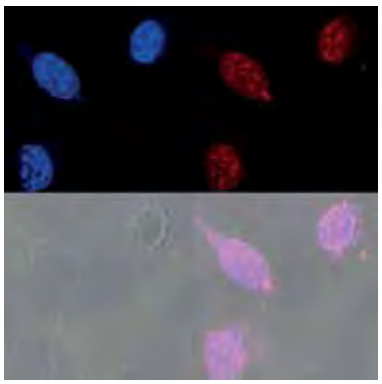


Fig. 4 Confocal microscope image of 4T1 cell line after one hour incubation with the daunorubicin-loaded nanoparticles with a diameter of 50nm at concentration level of 105ng/ml.

The obtained results confirm, that the synthesized nanoparticles cause a therapeutic effect on the 4T1 cell line. Particles with a diameter of 50 nm reduce the viability of cells after 24 hours to 19% at a drug concentration level of 104ng/ml. At this concentration level, there was also a significant decrease in survival of HepG2 cells. Viability fell to 20%, which would permit for the use of chemotherapy. For both lines, the use of nanoparticles resulted in a decrease of the amount of viable cells after 24 hours incubation. In both cases, it was similar to the result of the effectiveness of the pure drug which was about 20%. This allows to achieve a similar therapeutic effect for the pure drug and nanoparticles. Moreover, the images of 4T1 cell's confirm that both the drug released from the nanoparticles and the pure drug enter the nucleus were they intercalate with the DNA. Finally, the decrease of cell viability was observed in the images were the number of cells is significantly lower than in the control sample.

AGGREGATE FORMATION IN LIPOSOMES MEASURED BY FLOW CYTOMETRY: EFFECT OF PAYLOAD

GERGELY MILOSEVITS[1], János Milosevits[1], László Rosivall[2,3], János Szebeni[1,4]

[1]Nanomedicine Research and Education Center, Semmelweis University, Budapest, Hungary; [2]Institute of Pathophysiology, Faculty of Medicine, Semmelweis University, Budapest, Hungary; [3]Hungarian Academy of Sciences-Semmelweis University, Nephrology Research Group, Budapest, Hungary; [4]Department of Pathophysiology, Faculty of Health, Miskolc University, Miskolc, Hungary

SUMMARY

Vesicle aggregation is one of the stability issues that liposomal drug developers need to consider. Taking liposomal doxorubicin (Doxil/Caelyx) as an example, we developed earlier a flow cytometric method to quantitate the amounts of aggregates in a liposomal batch. In the present study, using this method, we attempt to assess the effect of payload (doxorubicin) on aggregation of drug-free liposomes whose structure exactly corresponds to Doxil/Caelyx (called Doxebo). Incubation of Doxebo with free doxorubicin at a concentration 5-10 % of the total encapsulated drug led to a 2-3-fold increase of particles ≥ 500 nm compared to control (Doxebo without added doxorubicin), suggesting that the payload drug may have a significant influence on vesicle aggregation above a certain threshold concentration. These findings may help to understand the factors underlying aggregation, an omnipresent potential problem with nanomedicines and protein-based biologicals.

INTRODUCTION

Supravesicular structure (SVS) formation, such as aggregation or fusion, is a known property of liposomes (Bailey et al.; Ahl et al.). It has been proposed previously that such larger than normal particles in pegylated liposomal formulations could induce complement activation which leads to an allergic reaction (CARPA) after i.v. liposome administration (Szebeni et al.). In a previous study we proposed the use of flow cytometry for quantifying SVS (Milosevits et al.), and here we focus on those experiments which were carried out to unveil the possible contribution of free doxorubicin, leaked out from Doxil, to aggregate formation. According to product label, minor fraction (up to 10%) of free, unencapsulated drug is allowed in commercial Doxil/Caelyx, guiding us to test the effects of 5 and 10% free doxorubicin (relative to the total, 2 mg/mL). For these studies we used Doxebo, an empty liposome structurally equivalent to Doxil/Caelyx, without doxorubicin. We incubated Doxebo with free doxorubicin and measured supravesicual structure (SVS) formation by flow cytometry.

MATERIALS AND METHODS

Materials

1. Doxorubicin HCl
2. Doxebo
- hydrogenated soy lecithin (HSPC, 9.58 mg/mL)
- cholesterol (Chol, 3.19 mg/mL)
- N-Carbamyl-poly(ethylene glycol methyl ether)-1,2-distearoyl-sn-glycerol- 3-phospho-ethanol-amine triethyl ammonium salt with a polyethylene glycol (PEG) moiety of 2000 Da (2K-PEG-DSPE), 3.19 mg/mL
- ammonium sulfate, ≈0.2 g/mL
- histidine, 10 mM (pH 6.5);
- sucrose, 10%

3. Fluoresbrite® carboxylated polystyrene latex-containing size ranging kit

Methods

1. Preparation of doxorubicin-free Caelyx-equivalent liposomes (Doxebo)
The (phospho)lipids found in Doxil/Caelyx, in corresponding amounts and ratios, were dissolved in an equivolume mixture of isopropanol and 96% ethanol at 70 ◦C.
The lipid solution was then admixed to pre-warmed Salsol A and the preparation was extruded 4 times via two superposed 80 nm polycarbonate filters, using a 10-mL extruder barrel from Northern Lipids (Vancouver, British Columbia, Canada).
The extruded liposomes were finally dialyzed against Salsol-A.

2. FACS measurements
FACS measurements were done using a Becton & Dickinson FACScan and were started by running of backgrounds (3 mL filtered PBS), followed by the measurement of liposomal solution with given free, unencapsulated drug content.
Calibration was done with Fluoresbrite® kit, containing fluorescence-labeled latex beads of known sizes (100, 200, 500, 750 and 1019 nm).
Particle detection continued for 1 min, or until a set number of events (e.g., 20,000) were reached. The counts and forward (FSC) vs. side scatter (SSC) plots (on log scales) were registered at room temperature.

RESULTS

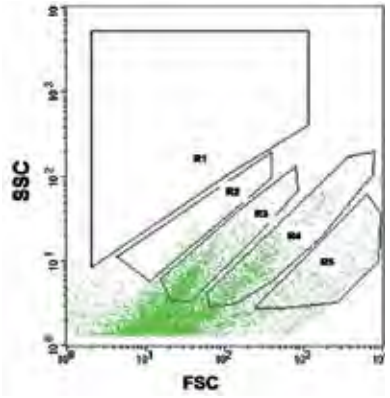


Figure 1. Gating of the SSC vs FSC scatterplot in SVS measurements conducted by flow cytometry analysis.

Free doxorubicin (mg/mL)	GATES				
	R1	R2	R3	R4	R5
0	0.9	27,8	0.8	4.8	0.1
0.1	0.9	24,7	2.2	8.8	0.2
0.2	0.9	24.1	1.8	7.1	0.2
0.4	1	25.9	1.1	5.8	0.1

Table 1. Effect of free doxorubicin on aggregate formation in doxorubicin-free liposomes.

Entries are % of events (dots) detected in the gated areas (R1–5) shown in Fig. 1.

Doxebo was incubated with doxorubicin for 30 min either at room temperature. Free doxorubicin values are final concentrations.
Light scattering patterns of particles in given solutions were analyzed by flow cytometry. According to size calibration conducted with latex beads, the size detection limit of particles registered as events in scattergams, such as shown in Figure 1., is 500nm, which implies that every event that was registered during the experiments signals structures larger than 500nm. Considering that Doxil/Caelyx contains 2 mg/mL doxorubicin and the fraction of free doxorubicin in it is within 10%, we incubated free doxorubicin with Doxil/Caelyx-mimicking, doxorubicin-free liposomes (Doxebo) in concentration ratios corresponding to that in Doxil/Caelyx, i.e., 0.1–0.4 mg/mL doxorubicin and 13 mM phospholipid. Incubations were carried out at 4 ◦C and at room temperature and the gating windows, upon FACS analysis, were the same as shown in Fig. 1. The data (Table 1) indicated 2–3 fold increases of events in the R3 and R4 regions following incubation with >0.1 mg/mL doxorubicin at room temperature, while the number of dots were not changed in the other regions, which are most easily rationalized by doxorubicin-induced aggregation of liposomes showing in R3 and R4.

CONCLUSION

These observations imply that free doxorubicin may have a significant impact on liposome structure, and, hence, in vitro stability of Doxil. The described FACS analysis can be utilized for qualitative and quantitative characterization of liposomes for the presence of aggregates,homogenicity and stability.

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THE INFLUENCE OF POLYVINYLPYRROLIDONE AND MERCAPTO POLYETHYLENE GLYCOL COATED GOLD NANOPARTICLES ON CELLULAR AND VASCULAR FUNCTION

TEBA MOHAMED[1], Sabine MATOU-NASRI[3], Glen FERRIS[1], Debra WHITEHEAD[2], May AZZAWI[1]*

[1]School of Healthcare Science and the [2]School of Science and the environment, Faculty of Science and Engineering, Manchester Metropolitan University, Manchester, M1 5GD, United Kingdom; [3]King Abdullah International Medical Research Center, Riyadh, Saudi Arabia

Corresponding authors:

*Dr. May Azzawi, The School of Healthcare Science, Faculty of Science and Engineering, Manchester Metropolitan University, Manchester, M1 5GD, UK.

Tel +44 0161 247 3332, Email m.azzawi@mmu.ac.uk

† Dr. Debra Whitehead, The School of Biology Chemistry & Environmental science, Faculty of Science and Engineering, Manchester Metropolitan University, Manchester, M1 5GD, UK.

Tel +44 161 247 3341, Email d.whitehead@mmu.ac.uk

BACKGROUND

Gold nanoparticles (AuNPs) demonstrate potential for cell tracking and imaging diagnostics, however, their impact on cellular and vascular function remains uncertain. The AuNPs emission characteristics can vary when they are suspended in different physiological fluids due to their aggregation; hence, to overcome this effect, organic polymer composite coatings have been applied. The aim of the present study is to investigate the effects of polyvinylpyrrolidone (PVP) and mercapto-polyethylene glycol - (mPEG) coated AuNPs on endothelial cell viability and vascular function of murine aortic vessels, ex vivo.

METHODS

AuNPs (12±3nm) were synthesised according to the Turkervich method. They were then stabilised with the polymers PVP and mPEG and characterised by transmission electron microscopy (TEM), energy dispersive x-ray spectroscopy (EDAX) and UV-Vis spectroscopy (Figure 1). Cellular uptake of AuNPs by cultured bovine aortic endothelial cells (BAECs) was visualized using transmission electron microscopy (TEM). AuNP effects on BAEC proliferation, cell viability and apoptosis were determined using the automated cell counter and flow cytometry for cell number counting, exclusion dye propidium iodide (PI) and Annexin V/PI detection, respectively. Aortic vessel rings from male Wistar rats were mounted between two fine steel wires in an organ bath system and constantly superfused in oxygenated physiological salt solution (PSS) at 37oC degrees. Cumulative doses of the endothelial dependent agonist acetylcholine (Ach; 0.01-100μM) and endothelial independent sodium nitroprusside (SNP; 0.1nm-10μM) were added to KCl preconstricted vessels, before and 30 minutes after incubation with stabilised and non-stabilised AuNPs.

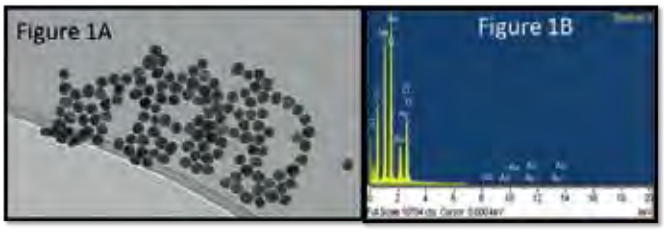

Figure 1A: TEM showing the distribution of monodispersed non-modified AuNPs (12±3nm); 1B: EDAX of AuNPs identifying the elements present in the nanoparticles.

RESULTS

Maximum cellular uptake of AuNPs was observed 24 hours and 48 hours, after incubation in non-stabilised and stabilised AuNPs, respectively (Figure 2). Both stabilised and non-stabilised AuNPs significantly decreased cell viability and proliferation and increased apoptosis up to 24 hours after incubation, whereas no inhibitory effect was observed after 48 hours incubation. PVP coated AuNPs (AuPVP), but not mPEG coated AuNPs (AumPEG) lead to a significant attenuation in endothelial dependent Ach dilator responses (Ach concentration 0.01-100μM; $p<0.05$). mPEG coated AuNPs at 2.9μg/mL had no overall significant effect on either ACh or SNP responses; however, at higher concentration (5.8μg/mL) AumPEG NPs led to a significant reduction in ACh dilator response at most ACh concentrations.

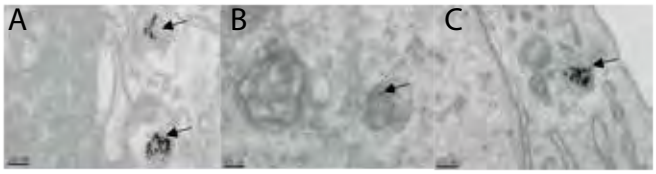

Figure 2: TEM demonstrating uptake of gold nanoparticles into endosomal structures within the cytoplasm of bovine aortic endothelial cells (arrows), after 24h incubation. A) uncoated AuNPs, B) mPEG coated AuNPs, and C) PVP coated AuNPs.

CONCLUSIONS

We demonstrate that polymer coating of gold nanoparticles (AuNPs) enhances their stability in physiological solutions and culture media, and also reduces cellular uptake into BAECs. Furthermore, we show that cellular and vascular effects of AuNPs depends on the type and concentration of polymer coating used. mPEG coated AuNPs show greater biocompatibility, than PVP coated AuNPs, thus showing greater potential use as agents for diagnostic imaging and therapeutics.

ACKNOWLEDGEMENTS: We thank Dave Maskew, MMU, for technical support; Dr. Aleksander Mironov, EM facility, Faculty of Life Sciences, University of Manchester, for his assistance in TEM.

A COMPLEMENTARY ANALYSIS OF NANOPARTICLE-PROTEIN INTERACTION AS A POTENTIAL "PRE-IN-VIVO-SCREENING"

KRISTIN MOHR[1,3], Maria Sommer[3], Grit Baier[1], Svenja Winzen[1], Laura K. Müller[1], Ralf G. Meyer[3], Volker Mailänder[1], Manfred Schmidt[2] and Katharina Landfester[1]

[1]Max-Planck-Institute for Polymer Research, Ackermannweg 10, D-55128 Mainz

[2]Institute of Physical Chemistry, Johannes Gutenberg-University Mainz, Welder Weg 11,D-55021 Mainz

[3]Dept. of Hematology, Oncology, and Pneumology, University Medical Center of the Johannes Gutenberg-University Mainz, Langenbeckstr. 1, D-55131 Mainz

Nanoparticles have reached clinical reality beyond experimental trials. This development has stimulated the hope to deliver therapeutic as well as diagnostic substances specifically to well-defined compartments, tissues or even single cells within a patient's body. Still, the bio-distribution of nanocarriers in vivo depends on innumerable possible interactions within the body that can hardly be predicted by cell-culture experiments. Consequently, assays need to be defined that help to better predict in vivo performance of these NPs.

Our group is investigating protein-nanoparticle interactions with multiple complementary physicochemical analyses to include dynamic light scattering (DLS), isothermal titration calorimetry (ITC), high pressure liquid chromatography (HPLC) and asymmetric field flow fractionation (AF-FFF). For identification of aggregation inducing serum components, different serum fractionation techniques were successfully applied. Albumin, IgG and lipoprotein solutions, as well as low abundant serum protein mixtures were isolated and utilized for further interaction studies via DLS, ITC and AF-FFF.

We have recently investigated low dispersity polystyrene nanoparticles (PS-NPs) in vivo in mice and in situ with dynamic light scattering (DLS) in blood serum.[1]

Miniemulsion polymerization was used to produce the PS-NPs of ~90 nm with various surface functionalities to include carboxyl and amino functionalization. The PS-NPs were injected into mice and their bodily distribution measured and subsequently compared with nanoparticle aggregation patterns identified by DLS (Figure 1).

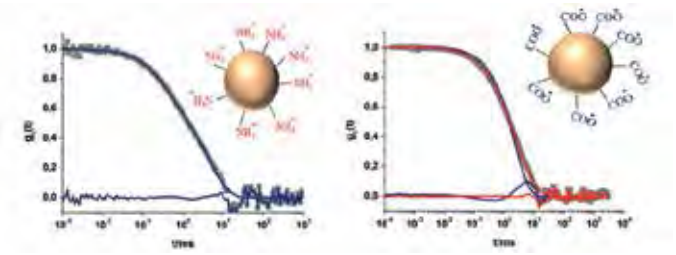

Figure 1: Dynamic light scattering analysis of particles in human serum: squares show the data points of the mixtures of amino functionalized (left) and carboxyl functionalized (right) polystyrene particles and human serum, force fit by the given parameters of particles and serum (blue line) and fit with an additional function for the aggregate caused by serum particle interactions (red line).

We found that particles showing no aggregation in DLS experiments were well distributed in mice while particles that showed aggregation in the size range of 200 nm to 2 µm in DLS were either not distributed in the body or only up taken by the liver.

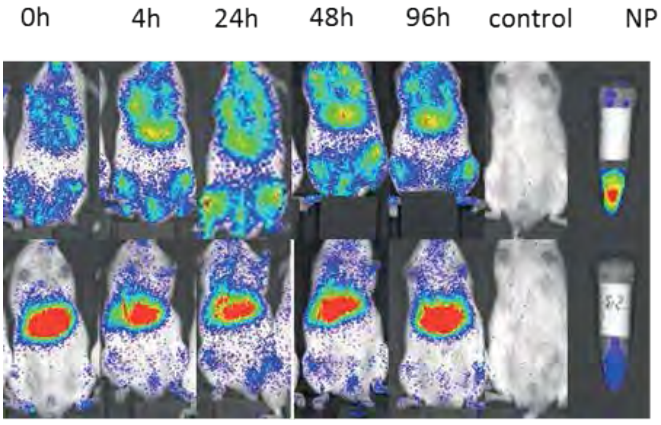

Figure 2: In vivo distribution of amino (top) and carboxyl functionalized (bottom) polystyrene particles in mice at different time points.

As shown in Figure 2 (top) the amino functionalized particle is well distributed in mice and shows no aggregation in human blood serum (Figure 1, left). In contrast the carboxyl functionalized particle is mainly taken up by the liver and shows aggregation in DLS (Figure 1, right). We perceive using DLS of nanoparticles in blood serum as a beneficial "pre-in-vivo-screening" technique.

A Combination of DLS with further analytical methods allows us to address multiple aspects of protein-polymer interactions and provides the potential possibility to pre-select nanoparticles for the final in vivo approach.

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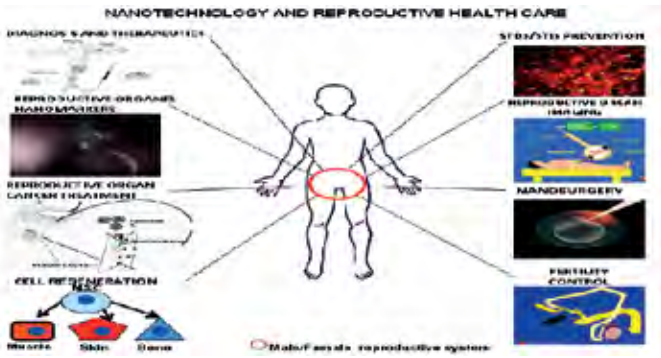
NANOMEDICINE AND REPRODUCTIVE HEALTH INCLUDING HIV/AIDS

SITARAMAIAH MOKKAPATI

President, Indian Nanomedicine Society,

Director, Kamineni Health Group, Hyderabad, India

Nanomedicine has a great impact on reproductive health care and has a promise for improving the performance of devices. Nano-based drugs such as nanocapsules, nanoparticles, nanomers, nanoformations, nanobiomaterials for control release of drug and lower drug loading for bioefficacy while keeping side effects to the minimum. These result in a new delivery-like forms for enhanced acceptability of such composite tablets, forms for quick disperse and long-acting drugs. Such nanoformulations improves compatibility and acceptance avoiding invasive procedures like injections. The protocols are likely to be cost-effective but can reach large population. They could be more stable providing larger shelf life however environmental issues need to be considered while targetting these technologies. Among the possible candidates under the present study are nanoformulations for contraceptive pills, microbicidal agents etc. Also, the biomedical devices in reproductive health care combining contraception with microbicide is the focus.



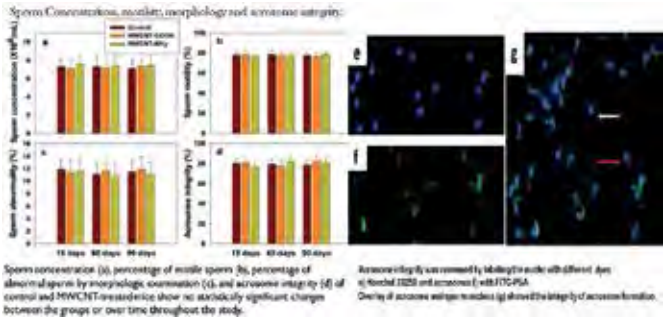
Reproductive Health Care devices such as condoms both male and female, IUDs and pleasure enhancing products are targets in our

industry. This kind of health care technologies need to bring together reproductive biologists, material scientists, nanotechnologists. The possible problems to be solved in this study include the following but are not limited to them:

- Long acting microbicides in tablets
- Once a month acting contraceptive pills
- Failure resistant stronger condom using nanocomposite of latex.

Improving contraception through thermal property, surface structures, electrical charging vibratory action and other mechanisms, for management of male factor infertility.

Nanobiotechnological applications in Reproductive Biology, especially addressing the concerns of Male infertility has seen few advancements in recent times. Use of nanomaterials as experimental tools in a highly delicate system of male reproductive tissues and gametes still remains a potentially controversial approach in clinical setting. However, available evidences on nanomedicine as a research tool to investigate, diagnose and treat male reproductive pathologies are a hope.



"Fertility chip" has been developed using nanotechnology to count spermatozoa in sperm, this can act as a reliable pre-scanning method of male fertility. Several of nanomaterials, such as soluble carbon nanotubes which have a potential applications as in vivo delivery and imaging tools were tested for their nano-reproductive toxicity for male fertility. And the pilot study indicated minor adverse effects on mice male reproductive system. Mesoporous silica nanoparticles (MSNPs) have been characterized as a powerful and safe delivery tool, rendering them to be used upon mammalian sperm for investigative, diagnostic and/or therapeutic purposes. Recently, scientists has developed techniques to utilize MSNPs, as investigative tools in mystery cases of infertility. Nanomedicine is being looked at as an promising new approach in andrological investigations, especially considering the semen parameters to enhance the predictive value of fertility potential. The current 'Lab on chip' nanomedicine appear to have more immediate potential for analysis of living sperm, however these methods and their utility awaits clinical verification and validation.

Schematic of the microfluidic semen analysis system showing the chip connected to an impedance analyser, which is in turn connected to a PC and an oscilloscope. Visualisation of the sample using a microscope shows a sperm approaching an electrode. (Segerink LI, Sprenkels AJ, Oosterhuis GJE, Vermes I & van den Berg A 2012Lab-on-a-chip technology for clinical diagnostics: the furtility chip. Ned Tijdschr Klin Chem Labgeneesk 37 61-63.)

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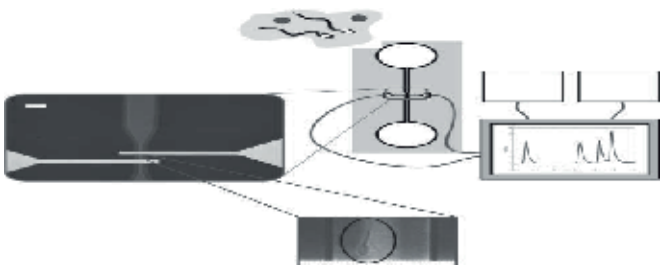

SOLID LIPID NANOPARTICLES-EMBEDDED MICROPARTICLES FOR INHALATION THERAPY

NOHA NAFEE[a],*, Ayman Husari[a], Claus-Michael Lehr[b], Marc Schneider[a]

[a]Pharmaceutical Technology and Biopharmacy, Philipps-University Marburg, Marburg, Germany

[b]Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS), Department of Drug Delivery (DDEL), Saarland University, Saarbrücken, Germany

*Department of Pharmaceutics, Faculty of Pharmacy, Alexandria University, Alexandria, Egypt

INTRODUCTION

Nowadays, nanoparticles have gained wide interest in pulmonary local and systemic delivery of drugs. The deposition of dry powders for inhalation in the lungs is a function of the aerodynamic properties (mean median aerodynamic diameter, MMAD) of inhaled particles. As previously reported, particles with MMAD between 5 - 10 µm mainly deposit in the upper airways, while those having MMAD of 1 - 5 µm are subject to sedimentation by gravitational force in smaller airways and respiratory bronchioles.1 Pulmonary application of nanoparticles (< 1 µm) as such is expected to end up in the alveolar region or more often exhaled due to their tiny size. Therefore, to ensure better nanoparticle deposition in the bronchial area, the aerodynamic diameter needs to be increased. Attempts to increase the MMAD of nanoparticles include the nano-in-micro approach that implies spray drying of nanoparticles in presence of a carbohydrate/polymeric carrier to form microparticles.

However, applying such strategy to solid lipid nanoparticles (SLNs) raise many challenges. While polymeric nanoparticles are highly robust to elevated temperature during spray drying, SLNs are more prone to aggregation, particle fusion and polymorphic changes of the lipidic components, which is the reason why spray drying of SLNs was seldom reported.2 Our objective is, thus, to prepare SLNs-embedded microparticles for inhalation. Optimization procedure implies variables such as SLNs and/or carrier composition, solvent used as well as proper selection of the spray drying parameters.

EXPERIMENTAL METHODS

SLNs prepared by hot homogenization technique using precirol (P), compritol (C) and dynasan (D) as solid lipids, in presence of tween 80 (T), poloxamer 407 (F) or PVA (P) as emulsifiers. Mannitol was chosen as carrier for SLNs being a widely used excipient for dry powder inhalers, approved by FDA for inhalation purposes.3 Mannitol solution in deionized water or water/ethanol mixture was mixed with SLNs dispersion prior to spray drying. For better aerodynamic characters, leucine was added to mannitol spraying solution. The effect of mannitol:leucine weight ratio, water:ethanol volume ratio, and spraying parameters was investigated. SLNs-embedded microspheres were characterized for their actual size distribution by the Mastersizer 3000 (Malvern Co., UK) and morphology by SEM. In addition, the colloidal stability of SLNs in mannitol solution and after spray drying was verified.

The distribution of Nile Red-labeled SLNs in fluorescein-labeled microspheres was visualized by confocal laser scanning microscopy (CLSM). In addition, particle deposition experiments using the next generation impactor (NGI) were performed to predict the deposition pattern of SLNs-embedded microspheres in the respiratory tract and to determine the aerodynamic properties; MMAD, fine particle fraction (FPF) and geometric standard deviation (GSD).

RESULTS AND DISCUSSION

SLNs produced were in the size range (50-150 nm) with negative zeta potential of -15 to -30 mV according to lipid-emulsifier combination. For SLNs-embedded microparticles, the size of all formulations was in the respirable fraction (1.5 - 11 µm) with a mean D50 of 4 - 6 µm. SPAN values were calculated as a measure of width of distribution relative to the mean diameter. In this study, small SPAN values ranging from 1.4 to 2 were obtained, indicating narrow distribution. The size of the particles was verified after mixing with mannitol solution and after disintegrating the spray dried micro-

spheres. In comparison to SLNs dispersion in water, SLNs retained their size in the nano-range in the spraying solution and after spray drying with the exception of C-PVA SLNs that were found to form huge aggregates (> 5 µm) after spray drying. Compared to mannitol microparticles, inclusion of SLNs increased surface roughness of the particles formed. The emulsifier used was found to play a key role in particle morphology leading to formation of hollow or continuous matrix microparticles, Fig. 1.

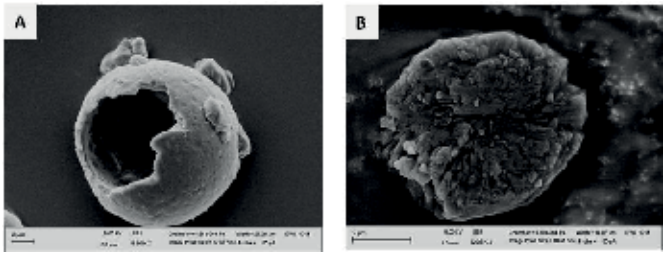

Fig. 1: SEM micrograph of SLNs-embedded microparticles: (A) hollow, (B) matrix particles While the effect of ethanol (at the percentage tested) on particle size and shape was minor, leucine proved to significantly modify particle morphology in a concentration dependent manner. Interestingly, SEM and CLSM images clearly demonstrated that SLNs were homogeneously distributed in the carbohydrate matrix with no signs of particle fusion or aggregation, Fig. 2.

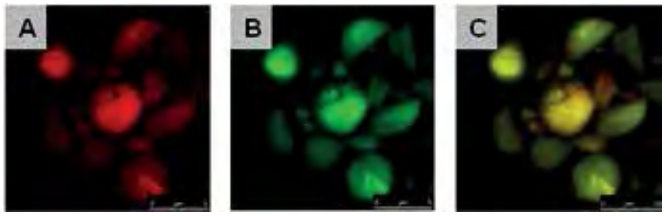

Fig. 2: CLSM image of SLNs-embedded microparticles: (A) Nile Red-labeled SLNs, (B) Fluorescein-stained mannitol, (C) Overlay of red and green channels

The deposition pattern demonstrated that only approx. 10% of the inhaled microparticles reached the alveoli (< 1.66 µm), whereas the majority is deposited onto stages 1-4 representing the upper airways. All formulations showed peak deposition at stage 2 (4.46 µm), Fig. 3. The MMAD values ranged between 4.3 and 5.3 µm with GSD in the range between 1.5 and 2.0. The fraction of all particles with an aerodynamic size smaller than 5 µm represented by the inhalable fine particle fraction (FPF<5 µm) varied between 48 – 58 %.

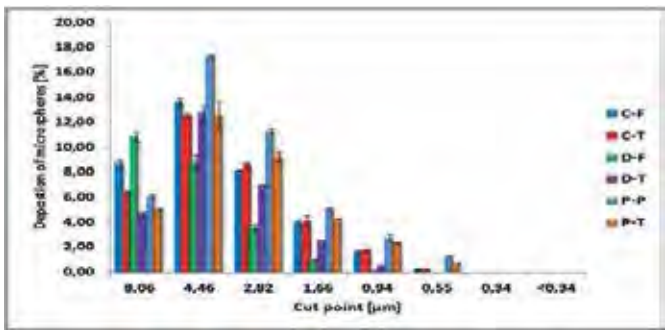

Fig. 3: Deposition of different SLNs-embedded microparticles at different stages of the NGI

In conclusion, SLNs could be efficiently spray dried in presence of sugar carriers. Spray drying did not negatively affect SLNs integrity or colloidal characteristics. The size of spray dried SLNs-embedded microparticles was indeed in the respirable fraction. SEM images demonstrated the surface roughness of the microparticles, with the possibility of producing hollow and continuous matrix particles. In addition, homogeneous distribution of SLNs in the carbohydrate matrix was confirmed by CLSM. Deposition studies with the NGI revealed a MMAD of 4.3 – 5.3 µm and FPF of 48 – 58 %.

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DEMAND QUANTIFICATION OF BACTERIOLOGICAL CULTURE MEDIA IN PUBLIC HOSPITALS IN KENYA

NGONGO DORIS NIGHT[1], Kimotho JH[1], Ochwoto M[1] , Basiye FL, Wandera CM, Kaiguri PM.
[1]Kenya Medical Research Institute (KEMRI), P.O. Box 54840 00200 City Sq. Nairobi.
Ngongo Doris Night. dnight@kemri.org Tel. +254-722-736368

Declaration: This survey was wholly sponsored by CDC-Foundation, with technical support by the KEMRI Production Team and the Marketing Team under the guidance and supervision of the Head-Production.

INTRODUCTION

Microbial Culture and Sensitivity is still the gold standard procedure for diagnosis of infectious diseases and, in certain cases, the only method that can be used reliably for diagnosis of these diseases. Only in exceptional cases can an organism be identified on the basis of its morphological characteristics alone1. Although there is a gradual shift towards molecular diagnosis in developed countries, it will take longer for this to happen in the developing countries. Studies have shown that some microbial diseases are best diagnosed through Microbial Culture & Sensitivity2. Widal testing, for example, was banned in Kenya in 2011 and bacterial culture is now the expected mode of typhoid diagnosis3. Unfortunately, Widal test kits are still found in use in Kenya4, mainly because of unavailability of culture media2. While culture is the gold standard for tuberculosis diagnosis globally, most high burden countries like Kenya rely on microscopy (sensitivity <65% c.f. culture). In 2009, WHO called for universal access to TB culture by 2015 and the STOP TB partnership set up the Global Laboratory Initiative (GLI) to help facilitate this5. Vibrio cholerae is confirmed through culture from stool or rectal swabs by use of selective media like thiosulfate-citrate-bile salts agar. Confirmation is serologically done by O1- or O139-specific antisera. Rapid test kits of these are commercially available but do not yield an isolate for antimicrobial susceptibility testing or sub typing. In other countries, rapid test kits are not used for routine diagnosis6. The role of suitable quality CM cannot be over emphasized, as the success of isolation of aetiological agents depends on it. Due to increasing quality standards, regulatory scrutiny and efficiency pressure in the world market, the demand for CM is shifting quickly to ready-to-use culture media7. Globally, there are companies specialized in Media production. Such companies include Eliava Media Production (EMP), a member of Eliava Institute of Georgia (the oldest Scientific research Institute in the former Soviet Union) 8. EMP employs modern technologies and equipment, experienced scientists, standardized production process and strict quality control, which make it the unique media production facility in the region. Other companies that manufacture ready-to-use Culture Media include Merk Chemicals9, LIP Diagnostics of UK10, Hardy Diagnostics (3rd largest producer in USA)11 and Cherwell Laboratories12, among others.

Kenya Snapshot: Kenya has an estimated population of 40 million, majority of who live in rural and sub-urban areas. Traditionally, donor funding has gone to the development budget of the Ministry of Health, which for many years has amounted to 60–90% of budget support13. HIV/AIDS and malaria pose the greatest disease burden on the healthcare system13, 14. There is currently unmet demand for quality culture media in Kenya and the region. Most public facilities in Kenya, laboratories included, suffer from limited quality

infrastructure, equipment/supplies and human resources. Constant supply of CM is hampered due to high cost and, sometimes, expertise to prepare them. Quality control of the prepared media is a challenge, compromising the integrity of the results obtained using the prepared media. There is thus need to enhance the capacity of CM productivity in, say, KEMRI Production facility which has basic infrastructure and expertise, to produce cost-effective culture media to serve the region and help Kenya achieve her Vision 2030 health goals15 and MDGs16. It was envisaged that the KEMRI Ready-to-use Culture Media Project, supported by partners like Bienmoyo Foundation17, could serve not only Kenya but also the larger Common Market of Eastern and Southern Africa (COMESA).

Weak Healthcare System: There are over 5,000 health facilities across the country 41% of which are run by the government, 15% by non-governmental organizations (NGOs) and 43% are for profit private businesses. The Kenyan healthcare sector faces four major challenges that augment the need for local capacity to develop and manufacture culture media: First, population and economic growth that has outpaced the service capacity of the existing healthcare infrastructure. Secondly, there is the increasing incidence of non-communicable diseases. Thirdly, a "vicious circle" of poor services in remote and rural areas due to insufficient supply of qualified health professionals and the limited number and inconsistent quality of facilities and equipment13, 18. Lastly there is the growing headache of antimicrobial resistance (AMR) and microbial threats that is beginning to retard healthcare growth, and even take us back to the pre-penicillin era19. Besides, the cost of various reagents for use in the medical laboratories in Kenya and neighboring countries are high due to their high costs of shipment that results from their bulk. Generally, ready-to-use culture media have short shelf lives (e.g. Nutrient Agar Plates have a shelf life of less than 7 - 90 days20 so that by the time the shipments arrive in the country, substantial parts of their shelf lives are normally gone. Many of them tend to expire on the shelf, heightening their costs.

Aim /Objective: The objective of the study was to quantify the demand for Bacteriological Culture Media in Kenya with a view to developing local capacity to Sustainably Produce Quality-assured and cost effective culture media.

METHODOLOGY

A marketing research schedule and questionnaire were developed to ensure relevant data was collected from target health facilities from around the country by field visits. The first field visits targeted the KEMRI Centers in Nairobi and environs. The second circuit of visits covered randomly selected Regional hospitals, reference laboratories and other health facilities in Central and Eastern Kenya. Thereafter, Coastal Region followed by the Western and Nyanza circuit was visited hence finalizing the survey. The questionnaires were filled on the spot by respondents and some information gathered by observation. The collated primary data was hence entered in Ms Excel and analyzed by SPSS. A total of 61 participants representing the various health facilities in Kenya were engaged on the use of culture media with the aim of identifying and quantifying the target market. The 61 participants were selected randomly from the eight regions in Kenya, with ten health facilities targeted for each.

RESULTS AND DISCUSSION

Majority of the participants in this study were Laboratory Technicians in Charge, 21(34.4%), followed by Laboratory Technicians, 20(32.0%). The least were the Research Scientists. Out of all the personnel interviewed, 51 (83.6%) stated that they prepared bacteriological culture media for their facility, 7 (11.5%) did not know if bacteriological culture media was prepared in their facility and only 3(4.9%) stated that culture media was not prepared in their facility. The laboratory personnel interviewed prepared several types of media which indicated the type of experiments they performed and the types of bacterial pathogens they handled. The media ranged from the general media (non-specific) to specific bacteriological media. The media also varied from enriched to non-enriched. In general, majority of the laboratories used Mac-Conkey Agar (65.6%), followed by Blood Agar and CLED media with 59.0% and 57.3% respectively. The lowest proportion was that of Chocolate Blood Agar (CBA) and Muller Hinton (MH) media, with 19.7% and 37.7% respectively. The other types of CM prepared included; XLD, DCA, Urea broth and Lowenstein Jansen. All these media had a proportion of less than 5% out of the total laboratories. Eastern and Central regions had a higher proportion of participants who prepared Blood Agar, Mac-Conkey and CLED media for their routine work (33.3%, 31.4% and 27.5% respectively). They were closely followed by Nyanza and Western regions. Majority in Coast and Nairobi regions did not prepare Blood Agar, Mac-Conkey and CLED media; however, CLED media was prepared by all ten participants at the coast which represented 25.0% of the 40 participants who used CLED. In all the regions, Nairobi had highest proportion of participants who did not prepare the media: Blood agar (44.4%), Mac-Conkey (42.3%) and CLED (57.1).The trend was different for the three other media; majority did not prepare Chocolate Blood Agar (CBA) and Muller Hinton Agar and there was no big difference observed in proportions of those who prepared other media and those who were using the media discussed.

Majority of the laboratory facilities (82.3%) performed 1 to 50 cultures per day and among these the District hospitals perfomed the highest number of tests (38.1%). They were followed by the Medical Research Institute with 19.0% and Private hospitals (16.7%). Least were the Referral Hospitals and Religion based hospitals. Very few facilities performed more than 50 cultures per day. The few that performed more than 50 tests included Medical Research Institute that performed between 51-100 tests and also performed all ranges of tests up to above 500 tests per day. The culture media purchased locally were 66.7% while in-house produced media stood at (11.8%). However, other participants imported the culture media (7.8%) while some were given as a donation (3.9%) from their project donors. Depending on the nature of the test, some participants combined two or more sources of obtaining the culture media. In this combination there were an equal proportion of those who combined locally purchased and in house and those who locally purchased and imported (both at 3.9%). Only one laboratory combined all the three (2.0%). On the other hand, frequency of making orders varied from one laboratory to the other, majority of the laboratories ordered their culture media quarterly (66.7%) and bi-annually (19.6%). Only 4/51 ordered monthly. (5.9%) participants did not know how the culture media was ordered. There was a direct relationship between the number of tests performed per day and the capacity ordered. The District hospitals that performed higher number of tests ordered above 2kg of the media. The highest capacity cited was 5kg of each medium. On average, the District hospitals ordered 500grams to 1kg of each media. Once the culture media was ordered, 18/47 (38.3%) took between two months to three months to be delivered, while in 13/47 (27.7%) took less than a week. Other media took 1-3months (23.4%). Most of those that took longer period to be delivered were those that were imported.

There were various factors considered when choosing culture media for use in the laboratory, but top in the list of most respondents was "High Quality Media" 44/52 (86.6%). This factor was closely followed by "Best Performance" 82.7% (43/52) and lastly in the top three factors was "Affordability" 51.9% (27/52). Other factors included the Value of the media (26.9%), Broad product selection (26.9%) and Recognized brand name (13.5%). Least amongst the factors was Good marketing or Sales representatives 1/52 (1.9%) and being given a Discount when purchasing the media 2/52 (3.8%). These factors were evenly distributed in all types of health facilities selected in all regions and among the target professional groups. Majority of those who mentioned Value and Easy/fast service as factors to consider were from District hospitals 7/14 (50.0%) and 3/11 (27.3%) respectively and from Medical Research Institute 5/14 (35.7%) and 5/11 (45.5%) respectively. Discount provision was mentioned only at the district hospital and Good marketing and Sales representative was only mentioned at Regional hospital. A higher proportion (53.1%) of the respondents agreed and 39.7% strongly agreed that locally produced microbial CM would save them on time and cost, only 2/49 (4.1%) were of the contrary opinion. On the other hand, majority (63.2%) of the respondents strongly agreed and another 28.6% agreed that standardized media would improve disease diagnosis in their facility. On RTU media, a higher proportion strongly agreed (49.0%) while 42.9% agreed that these

would save them time for their core activity i.e. diagnosis. However, 18.4% of them were of the contrary opinion on whether RTU packed culture media would improve on short shelf life of the product. In general, a higher proportion felt that locally produced media would save them on cost and time and that standardized media would improve disease diagnosis in their facility.

From the data analysis done the culture media project are viable. From the 61 facilities that were visited 83.6% of the respondents agreed that they prepared bacteriological culture media in their facilities although Quality Control remained the main challenge. Majority of the facilities utilized Mac-Conkey Agar (65.6%) followed by Blood Agar (59.0%) and CLED Media (57.0%). Other media categorised as 'Other' including XLD, DCA, Urea Broth and Lowenstein Jansen must be equally considered since a lot of such media is utilized by research and learning institutions. It is important to note that priority must be given to the commonly utilized media but this will depend on the niche KPD chooses to focus on. Referral hospitals were in all four circuits visited while the research institute centres were also geographically located in Nairobi, Coast, Nyanza and Western Region in Busia. These may be chosen as areas to start with incrementally spreading to other key areas of interest. Majority of the respondents consider 'High Quality Media' as the most important factor when choosing culture media. Best Performance came second followed by 'Affordability 'of the media. KPD is at an advantage considering that it is in the process of acquiring ISO 9001-2008, ISO 17043 and ISO 13847. These standards will ensure that all products emanating from the facility including the culture media are of the highest international standards. Additionally, having a local base will ensure that the prices of the culture media are priced competitively compared to the imported products hence giving the customers value for their money. This is further supported by the fact that, generally, a higher proportion of the respondents felt that locally produced media will save them on cost and time which means that the lead time between imported supplies shall be drastically reduced. They also believed that standardized media would improve disease diagnosis in their facility thus making them focus more on their core activity which is diagnosis rather than media preparation. From the statistics, KPD should mainly target the Medical Research Institute, Referral Hospitals and District Hospitals as a niche market then afterwards consider the other laboratories. This is due to the numerous cases they handle everyday for either research purpose, first-hand patients or due to referrals. Frequency of ordering culture media has a major impact on which market segment KPD should focus on. There was a direct relationship between the number of tests performed per day and the capacity ordered. Those District Hospital laboratories that performed higher number of tests ordered above 2kg of the media. The highest capacity cited was 5kg of each medium. On average the laboratories ordered a capacity of 500grams to 1kg of each media. Current shelf life of ready- to- use media is 7 to 14 days thus translating to either daily, weekly or after every two weeks deliveries by KPD (a much shorter time than quarterly deliveries). With a high capacity commercial autoclave installed, KPD would be able to meet high demand of ready-to-use culture media. In fact the higher number of orders the more economic it would be for the commercial autoclave. Based on the findings, a lot of awareness must therefore be carried out by the marketing team to ensure the target market get to know of the RTU media that will be offered by KPD. Relevant promotional strategies such as product brochures and catalogues must be distributed to target markets. It is evident that were KPD to engage in the production of RTU media, the greatest competition would be from the imported media; Quality products would be necessary given the short shelf life of ready-made media. KPD would have to manufacture media in strict conformity with the ISO 9001:2003 specifications then deliver under strict cold-chain. Since most imported CM come in powder for reconstitution just before use, KPD would have to consider the best selling proposition to counter this challenge.

CONCLUSION AND RECOMMENDATIONS

For production of affordable and sustainable culture media products the following interventions should be in place: Establishment of viable, affordable and sustainable products that will serve our target markets. Formulation of an integrated working committee to facilitate the effective planning and implementation of this project. Allocation of both human and financial resources including capacity building of key players in both production and marketing. The Marketing Team be fully supported so as to create adequate awareness of KPD products to target markets to solicit positive purchase response from them. Encouraging local (in-house) procurement by linking the KEMRI centers and projects to consume the RTU media products. Strengthen collaboration between the Ministries of Health, NGOs and other relevant stakeholders for prosperity and posterity. Strengthen and make regular the sensitization programs for medical personnel and other prospective users of the KPD products and services. Lastly, conduct regular needs assessment to identify key issues impacting on implementation of this project.

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EVALUATION OF TREATMENT EFFICIENCY OF PATIENTS SUFFERING ISCHEMIC HEART DISEASE COMPLICATED WITH CHRONIC HEART FAILURE BY USING NONINVASIVE INSTRUMENTAL METHOD OF FUNCTIONAL NEAR INFRA-RED SPECTROSCOPY

[1]**PAIZIEV A.A.**, [2]Gadaev A.G. and [2]Sayfiyev N.Ya.
[1]Institute of Ion-Plasma and Laser Technologies Uzbek Academy of Science
[2]Tashkent Medical Academy Ministry of Public Health Republic of Uzbekistan

INTRODUCTION

Nowadays, the skeletal muscle oxygenation is one of the principal endpoints in all CHF patients, but it remains one of the least monitored organs, despite the fact that would allow the assessment of the patients' detailed hemodynamic status, the severity of the organism's ischemia and the treatment efficacy in all patients suffering from CHF. At present all the techniques utilized to measure skeletal muscle hemodynamic status have some limitations that diminish their preciseness and common clinical implementation. Moreover, existing methods are quite complicated in their manipulation and expensive in cost . On the contrary, offered in present work new technology Near Infra-Red Spectroscopy (NIRS) is non-invasive, all-round and low-cost technique that enables the muscle oxygenation measurement both at rest and during the exercise, thus supporting the sufficient information about the muscle's oxidative metabolism.

It has shown that nearly all morphological and biochemical changes of Cr depletion were completely reversible upon normalization of myocardial Cr levels. Therefore, Cr metabolism is expected to be a significant target for future pharmacological therapy to increase myocardial efficiency and maintain structural integrity of the failing heart. NIRS technology will reveal the dynamic changes in peripheral hemodynamics muscle in ischemic heart disease complicated with CHF before and after PCr therapy.

MATERIALS AND METHODS

The study population consisted of 14 patients with ischemic heart disease complicated with CHF. In our study we are using NIRS technique for the evaluation of peripheral muscle oxygenation before and after conventional treatment along with exogenous PCr therapy (1 gr/day) in ischemic heart disease with CHF patients in the course of 10 days. Skeletal muscle oxygenated hemoglobin concentration [HbO2], deoxygenated hemoglobin concentration [HHb] and total hemoglobin concentration [tHb] measured with the "Oxy-Prem" Near Infrared Spectrometer (BORL, Zurich, Switzerland).

RESULTS AND DISCUSSIONS

Using simple physiological intervention (occlusion) in order to control the circulation and to calculate various quantitative parameters in arm muscle both at rest as well as during venous (VO) and arterial (AO) occlusion. In course of VO when blocks venous outflow, but does not impede arterial inflow we can observe increasing of O2Hb, HHb and tHb but after release of VO (during rest time) all signals rapidly return to baseline level. But difference behavior we can observe in course of and after AO. Here observed increasing of O2Hb and accordingly decreasing of HHb during AO and sharp hyperaemic response after release of AO. Blood volume increases rapidly. Blood flow (BF) in muscle measure during venous occlusion (VO) by evaluation the linear increase in tHb within first seconds of VO. This possibility is connected with blocking of venous outflow and increasing of tHb what is directly related to arterial inflow.

CONCLUSION

This study demonstrated that peripheral muscle oxygenation measured after 10 day standard treatment along with PCr therapy by NIRS test shown improvement of patients with ischemic heart disease complicated with CHF. NIRS technique enable not only to detect and assess the changes in skeletal muscle oxygenation after pharmacological interventions, but also to create diagnostic and prognostic as well as therapeutic criteria for ischemic heart disease patients with CHF. The correlation among different NIRS indicators of skeletal muscle oxidative metabolism with the hemodynamic indexes of patients let us to propose that delivery or/and utilization of the O2 is the main cause of the impairments observed in skeletal muscle oxidative metabolism, because it is principal in the further management of patients. NIRS could be a potentially useful clinical tool for the evaluation of the effect of treatment on the skeletal muscle oxygenation in CHF patients.

MUCUS A BARRIER TO OVERCOME: ARE VIRUS-LIKE NANOPARTICLES A POSSIBLE SOLUTION?

PEREIRA DE SOUSA I.[1]; Steiner C[1], Pearson J.P.[2], Veldhuis G.J.[3], Salvenmoser W.[4], Bernkop-Schnürch A.[1]
[1]Department of Pharmaceutical Technology, Institute of Pharmacy, Leopold-Franzens-University of Innsbruck, Innrain 80/82, 6020 Innsbruck, Austria
[2]Newcastle University, Institute for Cell and Molecular Biosciences, Framlington Place, Newcastle upon Tyne, NE2 4HH, United Kingdom
[3]Nanomi B.V., Zutphenstraat 51, 7575 EJ Oldenzaal, The Netherlands
[4]Institute of Zoology, Leopold-Franzens-University of Innsbruck, Innrain 80/82, 6020 Innsbruck, Austria

When a pharmaceutical carrier, exploiting the oral route, is designed the major obstacle that must be kept in mind is its absorption in the gastro-intestinal track. In fact, the surface of this anatomical region is covered by a mucus gel layer, a barrier to overcome for improving the bioavailability of pharmaceutical compounds. The aim of this study was to design a nanoparticle (NP) delivery system able to overtake the mucus barrier. To achieve this purpose a lesson from nature was taken. In fact, viruses can permeate through the mucus as fast as in water [1, 2]. Accordingly, NPs with highly densely charged surface, mimicking the nature of viruses, were formulated. NPs were produced by ionic gelation method, combining cationic chitosan (CS) with anionic chondroitin sulfate (ChS). The NPs were characterized by particle size, zeta potential and hydrophobicity. The interactions occurring between NPs and diluted porcine intestinal mucus were investigated according to a new method set up in our research group. Furthermore, the NPs permeation ability was investigated in fresh undiluted porcine intestinal mucus. As reference 50/50 DL-lactide/glycolide copolymer NPs (PDLG 5002 NPs) were used.

Two types of particles were designed with a particle size in the rage of 400-500 nm. One type (ChS/CS 1 NPs) showed a slightly positive zeta potential (4.02 mV) and a hydrophobic shell. The other type (ChS/CS 2 NPs) presented a slightly negative zeta potential (-3.55 mV) and a hydrophilic character. When incubated with diluted intestinal mucus, ChS/CS 1 NPs drastically change in size and zeta potential, while ChS/CS 2 NPs did not significantly vary in dimension and surface charge. However, both type of NPs showed higher permeation ability in fresh porcine intestinal mucus compared to the reference PDLG 5002 NPs.

According to the results, when compared to particles that do not present a highly densely charged surface (PDLG 5002 NPs), ChS/CS 1 and 2 NPs showed improved mucus permeating properties. Thereby, exploit the strategy of viruses gives great potential for the formulation of mucus-permeating NPs. This research provide valuable information for the production of nanoparticulate delivery systems able to overcome the mucus, opening the door for the future design of carrier capable of increase the bioavailability of incorporated pharmaceutical compound.

Acknowledgments: The research leading to these results has received funding from the European Community's Seventh Framework programme [FP7/2007-2013] for ALEXANDER under grant agreement n° NMP-2011-1.2-2-280761.

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LIPOSOME SIZE DISTRIBUTION AND MORPHOLOGY ANALYSIS BY CRYOGENIC TRANSMISSION ELECTRON MICROSCOPY

SIVAN PERETZ DAMARI[a], Dima Shamrakov[c] , Yechezchel Barenholz[d], and Oren Regev[a,b]
[a]Department of Chemical Engineering, [b]The Ilse Katz Institute for Nanoscale Science and Technology, Ben-Gurion University of the Negev, Beer Sheva, Israel; cLipoCure Ltd; d Institute of Medical Research Israel-Canada (IMRIC), The Hebrew University-Hadassah Medical School, Jerusalem, Israel.

Liposomes play an important role in medical science as nanoscale drug carriers. Two of their most important structural parameters are their morphology and size distribution, influencing their biodistribution, including passive targeting efficiency and, as a result, their performance. Currently there is no fast, accurate and easy way to perform quantitative structural analysis that combines size distribution and morphology at nanoscale resolution. Dynamic light scattering (DLS) is the most commonly used technique to determine size distributions, but DLS does not determine liposome morphology. In addition DLS measurement of size distribution may be misleading since it assumes an isotropic spherical particle shape, from which, using the Stock-Einstein equation, the hydrodynamic radii distribution is calculated, this calculation requires a complex deconvolution of the multi exponential autocorrelations functions which introduce uncertainty into the size distribution analysis. Direct imaging via cryogenic transmission electron microscopy (cryo-TEM) provides a more reliable approach for obtaining structural data in liquid dispersions with no artifacts. However, the consequent structural analysis is performed manually in most cases or by commercial software which is not specific for liposomes thus not accurate enough. Therefore we developed an automated, MATLAB-based analysis of cryo-TEM micrographs. We show that our approach enables determining liposomes' major morphology parameters such as shape, aspect ratio, size and volume distributions. It can be applied to a large number of micrographs and thereby enables statistical analyses. As a case study we investigated morphologies of commercial Lipodox® (FDA approved generic pegylated liposomal doxorubicin) and liposomes of identical lipid composition but without drug (Figure 1) and compared it to DLS analyses. Our study demonstrates the superb utility of the quantitative cryo-TEM analysis in determining liposome size distribution, shape, and the physical state of the drug in the intraliposome aqueous phase.

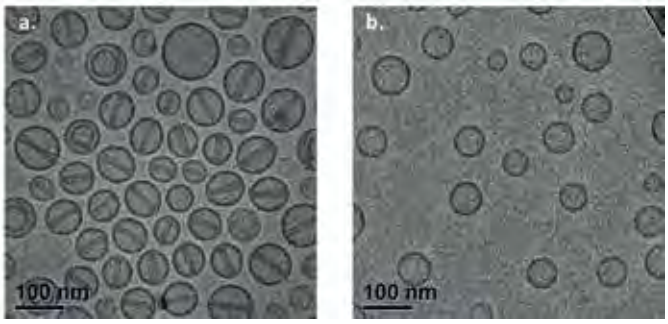

Figure 1: Cryo-TEM micrographs of: a. Lipodox® liposomes b. empty liposomes (DOX-free).

DENSE HYDROXYAPATITE NANOCERAMIC FOR HARD TISSUE IMPLANTS

E. PIETRZYKOWSKA[1], S. Kuśnieruk[1], T. Chudoba[1], J. Wojnarowicz[1], J. Rogowska-Tylman[1] , B. Woźniak[1] , J. Ločs[2] , W. Łojkowski[1]
[1]Institute of High Pressure Physics, Polish Academy of Sciences, Warsaw, Poland
[2]Riga Technical University, Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre, Riga, Latvia

Hard tissue implants are still a challenge for regenerative medicine. In the Institute of High Pressure Physics of the Polish Academy of Sciences (IHPP) two technologies have been developed with high potential to fulfill technical, societal and market demands.
The first, technology is the Microwave Solvothermal Synthesis (MSS) technology to produce phase pure nanopowders with uniform morphology, and high specific surface area. MSS technology permits doping of powders with various additives. Recently the MSS technology was successfully applied to produce hydroxyapatite nanopowders with average grain size 9 nm, specific surface area 240 m2/g, shaped in the form of plates mimicking natural bone particles (1).
The second technology is High Pressure Consolidation Technology. This method allows to produce dense ceramic by applying extreme high pressure and low temperature. The process conditions are high pressure in the range from 1 to 8 GPa, and temperature from 24 to 600ºC. These conditions cause the acceleration of the driving force for sintering and reduce pores in the material. Also thanks to this method the nanopowder structure is maintained. The nanopowder structure is significant for material's bioactivity and resorbability (2).
Implants produced with these technologies are characterized by grain size in the range from 20–40 nm; mechanical properties (nanohardness) 8.3 GPa; Young's Modulus 124 GPa.

Acknowledgments: GoIMPLANT M-era Net project
1. Patent application P-369906, Lojkowski et al, The method of nanoplates and method of nanopowder with nanoplates obtaining from synthetic hydroxyapatite.
2. Patent application P-399701, Lojkowski et al, The method of bone implants fabrication and the bone implant.

"CLINICAL APPLICATION OF NANOMOLECULAR CONJUGATED ALLERGEN / IMMUNOGEN DIAGNOSTIC TOOL FOR INDIVIDUALIZED ASSESSMENT, AS HIGHLY SPECIFIC, SUPER - SENSITIVE DEVICE IN DAILY PRACTICES OF ALLERGY AND CLINICAL IMMUNOLOGY"

WOJCIECH PODLESKI,Nowak Stanislaw H., Ph.D.,IfG - Institute for Scientific Instruments GmbH, Berlin, Germany, Podleski Wojciech Konstanty, M.D., Ph.D. The Podleski Foundation Art & Humanity & Medicine (U.S.A.), Marly - Fribourg, Switzeland

INTRODUCTION

In 2012, during The European Academy of Allergy and Clinical Immunology Congress in Geneva - Switzerland, our original document entitled " Grazing incidence x-ray technique for assessment of immune system " was acclaimed and endorsed, see abstract no 598.
The purpose of present poster is to overcome the transient intellectual gap in enduring, when we expect fundings, of such novel concept with enormous practical potential in precise diagnostic and then therapeutic principles associated with unimaginable high quality of personalized medicine in constantly threatening circumstances of our today's environment.

METHODOLOGY

Via non - invasive molecular profiling, the specific new skin diagnostic delivery nanomedicine system, will be formulated. All diagnostic entities are by definition supramolecular assembles of nano-particulate-based conjugates. They superiority are expressed by very high allergen / immunogen discriminative specificity at a

very low concentration, in order to minimize impending toxicity. The common standardized background, of valid internationally comparative methodology, will be established in order to optimize pathways of individualized medicine.

RESULTS

Finding the comprehensive and evolutive balance between innovation and its translation to practical clinical personalized nanomedicine by analyses of accumulated data will secure and master the existing uncertainty related to environmental diseases, not only. Targeted diagnostics emerges as fountains of hidden, provoking QUANTUM LEAPS promote our today health management sophisticated expertise. Of particular importance is application of novel nano - particles diagnostic design technique in developmental field work, with combined socio - ecologic diseases identifications.

CONCLUSIONS

Mastering the uncertain adaptive, evolutionary disturbances of our environment by its proper eco - protective surveillance identification is the hallmark of our 21st century medicine. Using precise nano - diagnostic technique interpretations of imminently hazardous aberrant components in preventive health management, is the universal principal strategy for the future. Extreme diagnostic distinction of applied personal nanomedicine, deployed ahead of time to particular geographical location in danger, is the key indicator for relevant local specific eco - protection measurements.

ENHANCING SELECTIVITY IN DRUG THERAPIES: MAGNETIC LIPOSOMES AS EFFECTIVE MULTI-FUNCTIONAL NANOCARRIERS

EUGENIO REDOLFI RIVA[†,§], Agostina Francesca Grillone[†,§], Yang Zehui|, Tianshu Li[¥], Alessio Mondini[†], Shinji Takeoka[|,¥], Gianni Ciofani[†] and Virgilio Mattoli[†]

[†]Center for Micro-BioRobotics @SSSA, Istituto Italiano di Tecnologia, Viale Rinaldo Piaggio 34 Pontedera, Pisa, 56025, Italy

[§]The BioRobotics Institute, Polo Sant'Anna Valdera, Scuola Superiore Sant'Anna, Viale Rinaldo Piaggio 34 Pontedera, Pisa, 56025, Itlay

[|]Department of Life Science and Medical Bioscience, Graduate School of Advanced Science and Engineering, Waseda University, Tokyo, Japan

[¥]Institute for Nanoscience & Nanotechnology, Waseda University, Tokyo, Japan

Unspecific biodistribution and lack of selectivity towards the disease site are among the major issues in modern systemic pharmacological therapies, which restrict the possibility of treatment of highly localized pathologies.[1],[2] Encapsulation in unilamellar lipid vescicles (liposomes) can improve drug biodistribution and enhance its selectivity towards specific cells upon proper surface functionalization, thus increasing therapeutical efficacy.[3]

Herein, we report on the fabrication and characterization of magnetic liposomes as biocompatible carriers to locally deliver a drug and to enhance its cellular uptake in a well defined target through an external magnetic field.

Liposomes have been prepared through a lipid thin-film evaporation method, re-hydrated in the presence of an aqueous solution containing dextran-stabilized magnetic nanoparticles, and thus extruded to obtain a stable dispersion of unilamellar magnetic vesicles. Gel chromatography has been further performed in order to separate magnetic liposomes from non-encapsulated magnetic nanoparticles. With this method has been possible to obtain nanometric lipidic vescicles with average diameter of 176.3 ± 1.8 nm and a magnetic nanoparticles concentration of 1.057 mg/mL. Dipalmitoylphosphatidylcholine (DPPC), cholesterol, and DHSG (1,5-O-dihexadecyl-N-succinyl-L-glutamate) have been used as main components for the lipid bilayer, whereas DSPE-PEG has been chosen to stabilize the dispersion by steric hinderance and a Glu2C18-PEG-N3 lipid with an azide moiety for further ligand functionalization. In order to allow the in vitro detectability of the liposomes, they have been stained through a lipophilic dye (DiI). Average diameter and morphology of liposomes were studied by dynamic light scattering, atomic force microscopy and scanning electron microscopy, respectively.

Liposomes targeting under different flow rates was investigated using a microfluidic channel, in order to assess the possibility of their accumulation thanks to an external permanent magnet (Br = 1.32 T) placed close to the region of interest (1.18 mm under the channel). This system has been linked to a perfusion set capable to produce a constant flow cell culture medium doped with the liposomes. Software interface allowed for a fine control of the pressure inside the microfluidic channel. Human umbilical vein endothelial cells (HUVECs) have been seeded on the bottom of a chamber connected to the microfluidic channel and grown at confluence. Moreover, liposome biocompatibility, tracking and internalization were assessed through evaluation of cell metabolic activity and confocal microscopy, respectively.

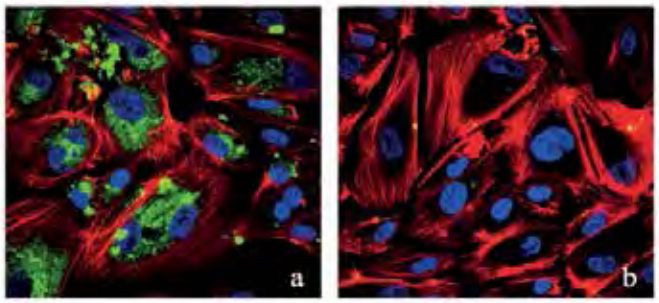

Figure 1. Magnetic liposomes targeting inside the microfluidic circuit. Liposomes are stained in green, cytoskeleton of cells in red. HUVECs seeded in the magnet-side channel (a) show enhanced liposomes internalization with respect to cells seeded in the control channel (b).

Figure 1 shows enhanced HUVECs internalization of green-stained magnetic liposomes inside the magnet-side channel (a), with respect to the control channel (b). These experiments have been carried out by setting a flow rate of 3.25 mL/min for 6 h and putting the microfluidic channel inside an incubator at 37°C. HUVECs have been stained at the end of the experiments for f-actin detection though TRITC-phalloidin, and imaged at confocal microscope.

Moreover, cytotoxicity of magnetic liposomes has bees assessed with WST-1 assay after 24 hours of incubation at 37°C.

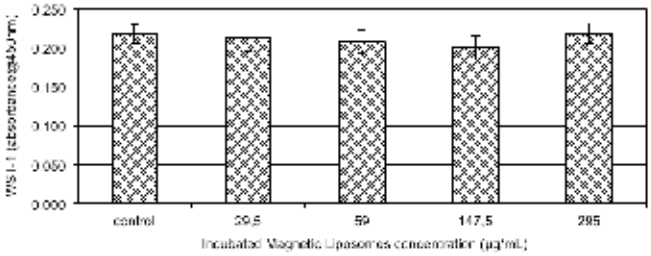

Figure 2. WST-1 assay results on HUVECs incubated with magnetic liposomes.

WST-1 assay shows absence of cytotoxic effects on HUVECs with respect to the control in terms of metabolic activity, confirming high biocompatibility of the magnetic carriers.

Obtained results confirm the possibility to prepare biocompatible magnetic-lipidic carriers, and to control their accumulation by means of an external magnetic force.

By exploiting their magnetic properties, moreover, future applications exploiting hyperthermia-assisted drug delivery can also be envisaged.

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OSTEOGENESIS OF MESENCHYMAL STEM CELLS: COMBINED EFFECTS OF HYPERGRAVITY AND BARIUM TITANATE NANOPARTICLES

ANTONELLA ROCCA[a-b], Attilio Marino[a-b], Veronica Rocca[c], Stefania Moscato[d], Giuseppe de Vito[e-f], Vincenzo Piazza[e], Barbara Mazzolaia, Virgilio Mattoli[a], Gianni Ciofani[a]

[a]Istituto Italiano di Tecnologia, Center for Micro-BioRobotics @ SSSA, Viale Rinaldo Piaggio 34, 56025 Pontedera (Pisa), Italy

[b]Scuola Superiore Sant'Anna, The BioRobotics Institute, Viale Rinaldo Piaggio 34, 56025 Pontedera (Pisa), Italy

[c]Università di Pisa, Dipartimento di Ingegneria dell'Informazione, Largo Lucio Lazzarino 2, 56122 Pisa, Italy

[d]Università di Pisa, Dipartimento di Medicina Clinica e Sperimentale, Via Roma 55, 56126 Pisa, Italy

[e]Istituto Italiano di Tecnologia, Center for Nanotechnology Innovation @NEST, Piazza San Silvestro 12, 56127 Pisa, Italy

[f]Scuola Normale Superiore, NEST, Piazza San Silvestro 12, 56127 Pisa, Italy

INTRODUCTION

Physical cues can regulate cellular physiology through several signaling pathways1. In recent years, particular attention has been focused on the effects of different stimuli on differentiation of mesenchymal stem cells (MSCs). As an example, it has been found that ceramic nanoparticles and hypergravity independently promote the differentiation of MSCs towards osteoblasts2.

In the proposed study, we investigate the combined effects of 20 g hypergravity and barium titanate nanoparticles (BTNPs) on the proliferation and the osteogenic differentiation of MSCs. To induce a 20 g acceleration, we performed our experiments in the framework of the "Spin Your Thesis!" 2013 campaign, by using the Large diameter centrifuge (LDC) of the European Space Research and Technology Centre (ESTEC, Noordwijk, The Netherlands). Moreover, to take advantage of nanotechnology in bone regeneration, we tested the simultaneous administration to the cultures of BTNPs, a class of piezoelectric nanoparticles investigated for their ability to enhance bone cells differentiation3. Hence, we report on the effects of increased gravity combined with BTNPs on the MSC behavior, in particular focusing on the osteogenic marker expression both at gene and at protein level.

EXPERIMENTAL METHODS

We performed analyses on proliferating and differentiating rat mesenchymal stem cells (SCR027 Millipore) that underwent 3 hours treatment at 20 g in the LDC system, incubated with and without 20 µg/ml of BTNPs. Results were compared with their pertinent controls at 1 g. Both treatments and controls were at least in biological triplicate and the whole experiment was replicated twice.

Cytoskeleton/focal adhesion staining Kit (FAK100 Millipore) was used to obtain qualitative data about spatial positioning and structural distribution of vinculin that was revealed by incubation with a primary antibody (1:200 diluted in 10% goat serum) followed by a FITC-secondary antibody (AP124F Millipore, 1:400 diluted in 10% goat serum), 100 µM TRITC-phalloidin, and 1 µM DAPI.

The transcription of the genes involved in the osteogenesis was evaluated through quantitative real-time RT-PCR. Briefly, the RNA was extracted through the High Pure RNA Isolation Kit (Roche) following the manufacturer's protocol and then 100 µg of RNA was retrotrascribed into cDNA with iScriptTM Reverse Transcription Supermix (Bio-Rad). Finally, SsoAdvancedTM SYBRGreen® Supermix (Bio-Rad) was used for the amplification.

Western blotting was used to identify proteins separated by polyacrylamide gel electrophoresis. Twenty µg of protein were incubated overnight at 4°C with specific antibodies: ALPL (1:8000 Abcam Biotechnology), or COL1 (1:1000, Abcam Biotechnology) or GAPDH (1:2000 Abcam Biotechnology). Chemiluminescence (ECL clarity, BioRad) was performed to detect the complexes between primary antibodies and the secondary horseradish peroxidase (HRP)-conjugated (KPL, Gaithersburg, final concentration 0.2 µg/ml).

Alizarin red S assay (Millipore) was performed to evaluate calcium-rich deposits and the inspection of fine structures by phase contrast microscopy.

RESULTS AND DISCUSSION

The immunofluorescent staining of cytoskeleton of proliferating mesenchymal stem cells qualitatively demonstrated that cells are affected by the hypergravity treatment (Fig. 1). In particular, cells undergone 20 g stimulation appear more stretched and with actin fibers organized in parallel with respect to the 1 g gravity; they are more spread and irregular both with and without BTNPs administration. These changes in the cell morphology are significant since it is widely recognized as spreader cell morphologies are indicative of a more pronounced commitment towards osteogenic differentiation4.

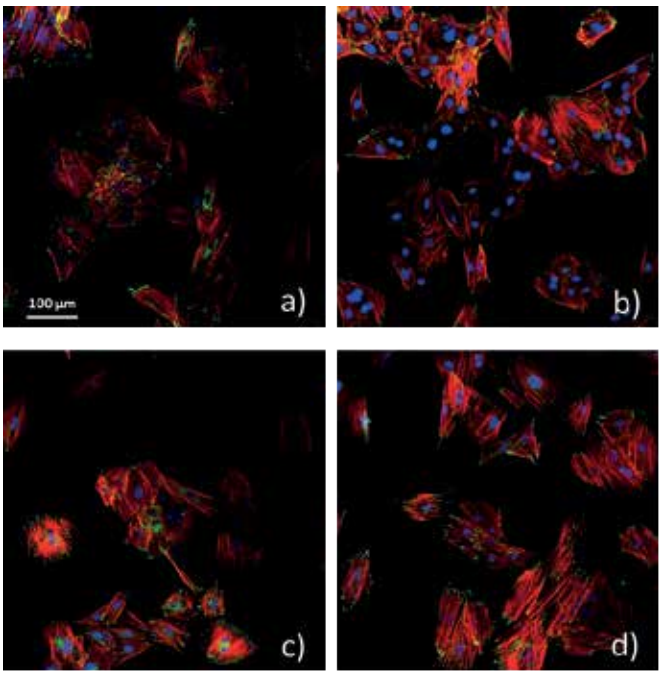

Figure 1. Immunofluorescence staining of vinculin (green) and f-actin (red) in proliferating mesenchymal stem cells after 3 hours of treatment: 1 g (a); 1 g + BTNPs (b); 20 g (c); 20 g + BTNPs (d). Nuclei counterstained in blue.

Thereafter, we analyzed differentiation markers through quantitative RT-PCR, and the results demonstrated a significant increment of transcription of Runx2, alkaline phosphatase and collagen type I genes when the cells internalizing BTNPs are stimulated by hypergravity, with respect to the 1 g controls.

The analysis of protein expression through Western blotting highlighted that the combined stimuli are able to foster an enhancement of collagen type I synthesis, that is a protein typical of osteoblasts.

Calcium deposits, finally, were significantly increased in samples stimulated with hypergravity and BTNPs respect both to 20 g and 1 g (Figure 2).

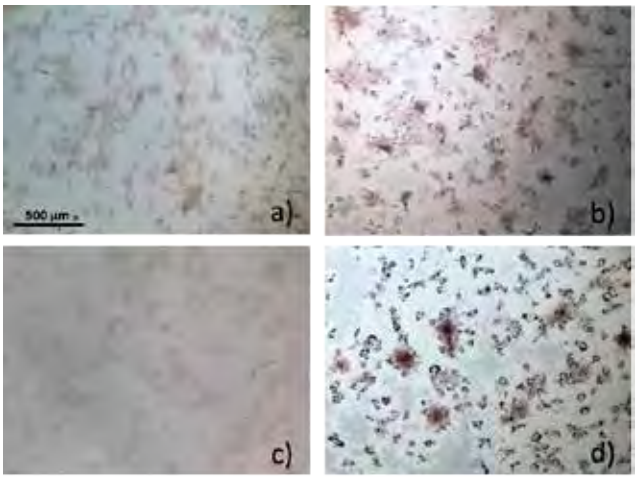

Figure 2. Alizarin red assay: 1 g (a); 1 g + BTNPs (b); 20 g (c); 20 g + BTNPs (d).

Our hypothesis is that MSCs accelerated to 20 g internalize nanoparticles at a higher extent, and therefore this could contribute in the promotion of the osteogenesis both in terms of gene transcription and of phenotype development. Moreover, our results demonstrated that hypergravity alone is able to enhance osteogenic differentiation. These promising results grow the interest on gravitational effects of force-sensitive cells.

CONCLUSIONS

Overall, our results demonstrated that hypergravity conditions and BTNPs can synergistically improve the osteoblastic maturation of MSCs. These findings, even if preliminary, could contribute to improve knowledge of the stimuli involved in osteogenic differentiation, as well as to the design of new approaches for nanoparticle-mediated drug delivery and regenerative medicine.

Acknowledgements: The experiments reported in this work were performed in the framework of the Spin Your Thesis! 2013 program organized by the ESA Education Office. Authors are particularly grateful to the staffs of ESA Education Office, to the Technical Directorate of ESA and to ELGRA for their constant support before, during and after the campaign.

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IN VIVO SCREENING OF A CELL PENETRATING PEPTOID LIBRARY TO ISOLATE BRAIN SPECIFIC TRANSPORTER MOLECULES

FRANZISKA ROENICKE

The aim of an organ specific drug delivery is to increase the efficiency of a drug and simultaneously minimize its toxicity (Grietje Molema 2001). A promising approach to improve this technology is the development of carrier molecules that guide the therapeutically active drug to its point of destination. Attractive candidates are monoclonal antibodies, liposomes, polymers, nanoparticles, proteins and many more. But all entail their distinct problems, ranging from synthesis problems to unpredictable biological difficulties or toxicity. Hence these criteria need to be considered during an early stage of drug development. For an in vivo application a compound needs to possess the physicochemical properties of being water soluble and lipophilic at the same time in order to pass important biological membrane barriers.

Present strategies thus focus on cell penetrating peptides (CPPs). CPPs are small cationic peptides, which generally consist of arginine and lysine side chains. They are characterized for their rapid penetration through tissue, but the underlying mechanism how they translocate a membrane is not yet completely unraveled. After all, while passing the cell membrane, these CPPs are able to function as carriers, transporting cargoes even multiple times larger than their size. CPPs are not known to be organ specific and their use in biological systems is limited due to their short blood-stream circulation.

A new class of CPPs mimetics are N-substituted oligoglycines, called peptoids (CPPos). In comparison to CPPs, their side chains are shifted from the α-carbon to the nitrogen of the peptide backbone. Benefits of this structural transformation are among others the high resistance against denaturing solvents, temperature or chemicals, the enhanced stability relating proteolytic enzymes, and a major advantage in drug development, the ability to introduce diverse non-natural side chains during the synthesis. This enables the generation of highly variable CPPo libraries with the intention to make them more organ specific.

For high throughput screenings in biological systems, large amounts of peptoids are necessary and a high variety is desired. These requirements can be achieved with the IRORI technology with an automated split and mix synthesis and a directed sorting technology based on glass coated radiofrequency tags (Dominik K. Kölmel 2012).

Based on this new technology, first small libraries have been generated and tested on cell. Lipophilic peptoids showed an accumulation in mitochondria, whereas cationic candidates concentrated in endosomes and the cytosol. Experiments in mouse models already detected peptoids specific to the organs: heart, lung and liver, there in particular in the lipid droplets.

A completely permutated peptoids library should now elucidate structure function correlations. Therefore screening approaches on cells, zebrafish embryos and adult zebrafish will be established on a high throughput scale. With these methods we also hope to identify organ specific transporter molecules.

As a proof of the targeting principle, a screening procedure is essential in the early phase of drug development as this property represents a criterion for exclusion. Searching through an entire library for a potential carrier with the requested bioviability, an adequate high throughput whole animal screening has to be established. A compromise between complexity, concerning the organ system and comparability to the mammalian system, and the high throughput availability should be given. These demands strengthen the zebrafish danio rerio as an ideal model to study the organ specificity of the CPPo library. Despite the usual advantages of the zebrafish as there should be noted the high generation rate, high reproductive rate, external development, transparency of the embryo and the comparably unproblematic and inexpensive animal handling, the zebrafish represents a vertebrate model, whose organ system is comparable with the human one (Stoletov and Klemke 2008). It also possesses a tight junction-based blood brain barrier, similar to that of higher vertebrates (Jeong et al. 2008). Hence, the zebrafish offers a simple and useful model to screen CPPos for their organ targeting, including the brain which plays a strategically significant role in drug delivery research.

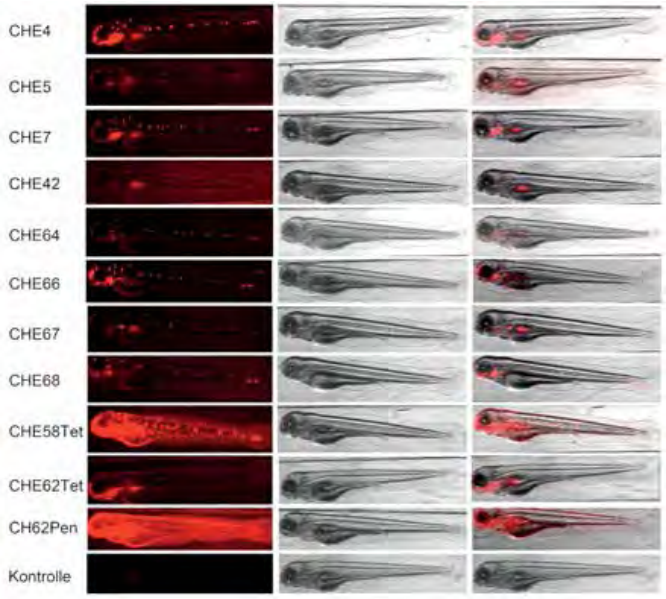

Figure 1: 96 hpf old embryos, incubated for 2 hours with the peptoids. Peptoid are coupled with the fluorophore Rhodamin B. Micorscope: Leica TCS SP5 DMI6000, Objective:63x/1.4

First experiments in the zebrafish embryo displayed peptoid carrier, which accumulate amongst others in neuromast cells (see Fig.1, CHE 4/5/7/42/64/66/67) These initial findings should be transferred to the adult zebrafish mode because its organ system and also the brain development are completed. For a better imaging analysis, a transparent zebrafish has been established (White et al. 2008). A combination between the nacre mutant and the roy mutante, which possess a complete lack of melanophores respectively iridophores, results in the double mutant casper, which demonstrates almost an entire transparency throughout the adulthood. This transparency makes it in ideal tool to study the fluorophor-tagged peptoids. Already developed strategies for high-throughput

imaging of zebrafish, as the self-made LED fluorescence macroscope or the magnetic resonance microimaging don't combine the must haves to be reproducible for everyone (Blackburn et al. 2011). Important facts are the financial feasibility, speed, easy handling, commercial availability or application to high throughput. These requirements taken into account, the PearlTM Imager (LI-CPR, Biosciences) is a promising technology to scan zebrafish for organ-directed peptoids. Although it is a freely available system, it is so far only available in one academic facility in Germany, namely the KIT. Until now it was used for mouse scans but is adaptive to the zebrafish model. A notable advantage is the easy handling of the PearlTM Imager and the minimal burden for the animals.
Our goal is to develop a high-throughput screening technique via a laser-based imaging system to systematically monitor the organ-directed concentration of peptoids in a new adult zebrafish model (see Figure 1). In particular brain-specific carriers should be focused and eventually chemically modified to enhance their blood-brain barrier mobility. In parallel the bioavailability, the way of degradation and the degradation rate will be observed. Finally, the isolated candidates will be tested in mouse models, to confirm the transferability to mammalian systems.

COMPARABILITY OVER TIME AND SPACE DUE TO EFFECTIVE IN-TERLABORATORY COMPARISONS

MATTHIAS RÖSSLEIN[1], John T. Elliott[2], Nam Woong Song[3], Agnieszka Kinsner-Ovaskainen[4], Cordula Hirsch[1], Patrick Hole[5], Katherine Sillence[5], François Rossi[4], Peter Wick[1]
[1]Materials – Biology Interactions Laboratory, Swiss Federal Laboratories for Materials Sci-ence and Technology (Empa), St. Gallen, Switzerland
[2]Biosystems and Biomaterials Division, National Institute of Standards and Technology (NIST), Gaithersburg - MD, USA
[3]Center for Nanobio Convergence, Korea Research Institute of Standards and Science (KRISS), Daejeon, Rep. of Korea
[4]Institute for Health and Consumer Protection, Joint Research Center (JRC) of the European Commission, Ispra, Italy
[5]NanoSight Ltd., Minton Park, London Road, Amesbury, Wiltshire SP4 7RT, UK

Engineered nanomaterials (ENM) that should go once into clinical trials and application have to pass beforehand a rigorous characterization cascade. Such tiers of testing have to guarantee comparability over time and space to ensure a constant level of biological effectiveness. This cascade includes among others physical chemical characterization and cytotoxicity evalua-tions. There have been a number of successful interlaboratory comparisons determining the hydrodynamic diameter of ENMs using dynamic light scattering (DLS) [1] or nanoparticle tracking analysis (NTA) [2]. The later have demonstrated that a detailed description of the experimental procedure is a precondition for achieving a sufficient level of comparability (Figure 1). Beside that standard reference materials support the measurement infrastructure and quality quite well. Such tools and infrastructures are still lacking for in vitro assays, such as cytotoxicity. Therefore it is important to develop highly reliable assays that provide a strategy to ensure confidence in the measurement results and their uncertainty estimates. Such an assay will include an appropriate experimental design with a number of suitable controls and a detailed experimental procedure [3]. By performing an interlaboratory comparison we obtained an idea of the comparability of the results and an estimate of the measurement uncertainty. In addition we are able to identify the size and relevance of influence quantities. Furthermore it has verified transferability of the procedure to different laboratories. This allows validating the chosen approach.

A common assay for cell viability and therefore also usable for cytotoxicity is the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay. It is an assay with just a few operator interactions and hence an ideal starting point for such prove of principles. Based on a cause and effect analysis Empa and NIST engineered an assay plate design with a number of controls. They provide measurements related to each of the steps in the measurement procedure (e.g. pipetting, cell ceding, cell growth etc.). If these measurement results are within given specifications, then they provide confidence that the test results of unknown samples are valid and can be compared with other test results that have been gained with the same assay. This approach is illustrated with the results of a recent in-terlaboratory comparison between 5 laboratories (Empa, KRISS, JRC, Nanotech and NIST) [4]. Our results substantiate that control measurements are critical for achieving confidence in the measurement results and that comparability of nano-cytotoxicity assays results can be achieved in this way. After all these process improvements MTS-assay is now "fit for pur-pose" and ready for the introduction to routine measurements under strict regulatory compliance. The beauty of this is that the entire approach can be transferred directly to other 96 well plates and plate readers based assays without substantial changes of the experimental design.

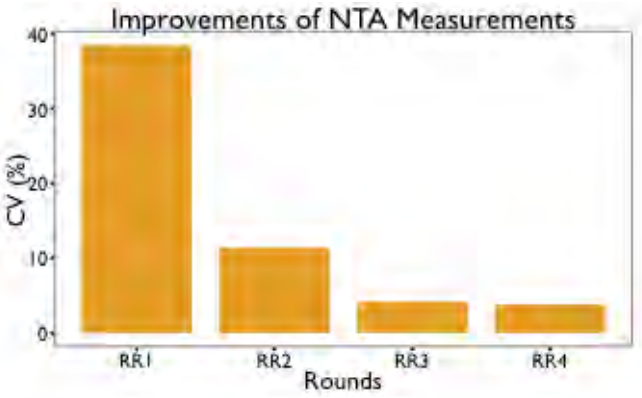

Figure 1: NTA Interlaboratory comparisons rounds 1 to 4 (RR1 – RR4) with considerable improvement of the coefficient of variation (CV) of all contributing labs

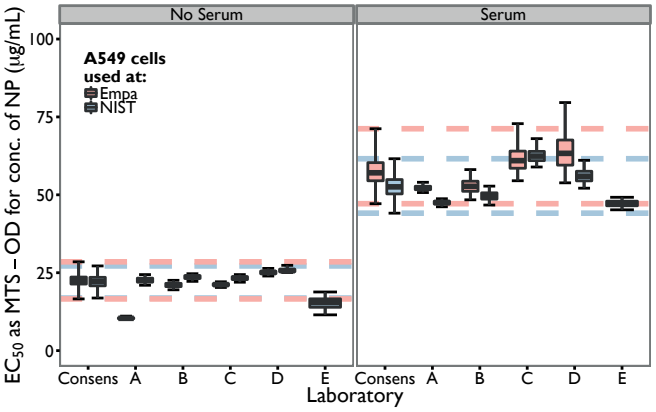

Figure 2: Interlaboratory comparison of the MTS assay using PS-NH2 NP

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SYNTHESIS AND PROPERTIES OF GOHAP - HIGHLY BIOCOMPATIBLE, NANOCRYSTALLINE HYDROXYAPATITE MATERIAL FOR MEDICAL APPLICATIONS

J. ROGOWSKA-TYLMAN, B. Woźniak, S.Kuśnieruk, D.Smoleń, J.Wojnarowicz, E. Pietrzykowska, T. Chudoba, W. Łojkowski
Laboratory of Nanostructures for Photonics and Nanomedicine, Institute of High Pressure Physics, Polish Academy of Sciences, Sokołowska 29/37, 01-142 Warsaw, Poland
e-mail: rogowskatylmanjulia@gmail.com

The area of bone regrowing materials has dynamically developed in recent years, yet the most popular approach to bone regeneration is still the use of autografts and allografts. Though, alternative methods are gaining momentum as researchers have embarked on a quest for more biocompatible, cell-friendly materials that could replace autografts. One of them is hydroxyapatite (HAp), a bioactive ceramics that is used in the form of paste and granules to fill small bone defects. For large bone defects, a regrowing process is still a challenge. The key issue is mainly low regrowth rate, poor mechanical properties, high inflammatory risk and low resorption rate or implanted material. Therefore the main objective of the current regeneration medicine projects is to develop the technology for bioactive scaffold with improved shape control, better mechanical properties, bioactivity and resorbability. Aforementioned goal can be achieved through the production of nonstoichiometric nanoparticles of hydroxyapatite with the grain size smaller than 10nm and the shape close to the natural HAp, which will be used as a material for bioactive, mechanically strong scaffolds. Such nanoparticles due to their calcium deficiency and high surface to volume ratio may achieve necessary solubility level and increased osteoblasts adhesion.
The Institute of High Pressure Physics of the Polish Academy of Science (IHPP) is an expert in the synthesis of doped nanoparticles with a 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 reaction time, temperature and pressure.
By leveraging unique MSS technology for nanoparticles synthesis, IHPP is able to synthesize innovative HAp nanoparticles using the standard reaction between calcium hydroxide and phosphoric acid. The reaction is carried out in water solution in time shorter than 5 minutes. The specific surface area is almost 270m2/g the average grain size smaller than 10nm with shape in the form of platelets mimicking the natural bone particles. 28 days of degradation test conducted according to norm ISO 10993-14 indicated material solubility equal 20mg/dm3.

IN VITRO DEGRADATION STUDY OF HYBRID PLA NANOFIBERS MODIFIED WITH BIOACTIVE GLASS NANOPARTICLES INTENDED FOR BONE TISSUE ENGINEERING APPLICATIONS

AGATA ROGUSKA[2], Małgorzata Lewandowska[1], Anna Matras[1], Przemysław Suchecki[1], Oscar Castano[3]
[1]Faculty of Materials Science and Engineering, Warsaw University of Technology (WUT), Warsaw, Poland
[2]Institute of Physical Chemistry, Polish Academy of Sciences (PAS), Warsaw, Poland
[3]Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain

INTRODUCTION

The growing demand of effective therapies in regenerative medicine including orthopaedics imposes the need to develop new types of tailored materials stimulating and orchestrating the regeneration process. The main interest consists in developing materials that masterly combine bioactivity and biodegradability. Bioactivity is required in order to attract cells and to stimulate their migration and growth for the regeneration of each specific tissue. Biodegradability is required to provide the initial chemical cues to activate the regeneration process, and to biodegrade gradually while releasing the signals required to guide the tissue repair. These bioactive and biodegradable materials should end up totally replaced by the natural regenerated tissue.
A variety of scaffold materials have been used in tissue engineering as support structures for cell and tissue growth, including synthetic polymers and ceramics, decellularized tissue, and proteins, with varying degrees of success. To date, hybrid materials appear to be one of the most promising candidates for the development of novel scaffolds for bone regeneration due to their good bioactivity and mechanical properties. Thus composite materials combining a polymeric matrix with glass particles are a suitable option for developing flexible and versatile scaffolds. Polylactic acid (PLA), a biodegradable polymer approved in many different devices by the FDA, has been widely studied and used in novel scaffolds for tissue repair. Biodegradable calcium phosphate (CaP) glass nanoparticles offer interesting solutions as reinforcing phase for composite materials. In addition, osteoconductive biodegradable CaP glasses have the ability to trigger vessels formation. This phenomenon is mainly due to the release of Ca2+ ions which have shown to elicit an angiogenic effect.
The aim of this study is to analyze the biodegradation of the hybrid PLA-bioactive glass nanofibrous scaffolds in the in vitro degradation tests where physiological conditions are simulated.

MATERIALS AND METHODS

Hybrid materials used in this study involved glass nanoparticles embedded in PLA electrospun fibers. Precursor solution was hydrolyzed in the presence of an excess of water and using CTAB and ethylenglycol as pores and shape modification agents, in order to obtain particles with a controlled, homogeneous and narrow size distribution. The nanoparticles obtained were centrifuged and thermally treated bellow their crystallization temperature to obtain nanoparticles. Finally, nanoparticles were ultrasonically dispersed in 2,2,2-trifluoroethanol and mixed with a PLA solution to obtain a electrospinnable slurry with the suitable viscosity. Subsequently, aligned and random hybrid PLA/glass nanofibers were successfully produced by electrospinning.
The obtained materials are supposed to degrade during the implantation. Therefore, it is of prime importance to have a comprehensive view of their behaviour along the degradation period in order to know both, the evolution of their structural properties as well as the release of degradation by-products. In order to evaluate the degradation of the as-obtained materials, nanofibers were immersed in SBF at 37ºC in static conditions for various periods of time. The SBF solution was replenished every two days. The degradation kinetics and mechanisms were evaluated by means of scanning electron microscopy (SEM) observations and chemical analysis: Fourier-transform infrared spectroscopy (FTIR) and photoelectron spectroscopy (XPS). In addition, changes in the pH values were monitored during the degradation test.

RESULTS AND DISCUSSION

Hybrid PLA/CaP glass nanofibers of three different compositions varying in CaP glass content were investigated, namely containing 10, 20 and 30 wt.% of glass nanoparticles. In all cases, microscopic observations revealed the aligned or random orientation of the fibers depending on the processing conditions. XPS analysis proved that the hybrid nanofibers are enriched in Ca, P, Ti and Na. All those elements are the components of the glass nanoparticles used in the fabrication process of the scaffolds.
Fig. 1 shows SEM images of randomly oriented pure PLA and hybrid nanofibers with 10, 20 and 30% of CaP glass nanoparticles after 14 days incubation in SBF. Samples containing 10 and 30% of nanoparticles exhibited high level of porosity. In addition, nanofibers with 30% of nanoparticles possessed high number of transverse cracks and contractions. For nanofibers containing 20% of nanoparticles, biomineralization process after 14 days incubation in SBF was observed. Characteristic precipitation was revealed for this sample under SEM, see Fig. 2c.

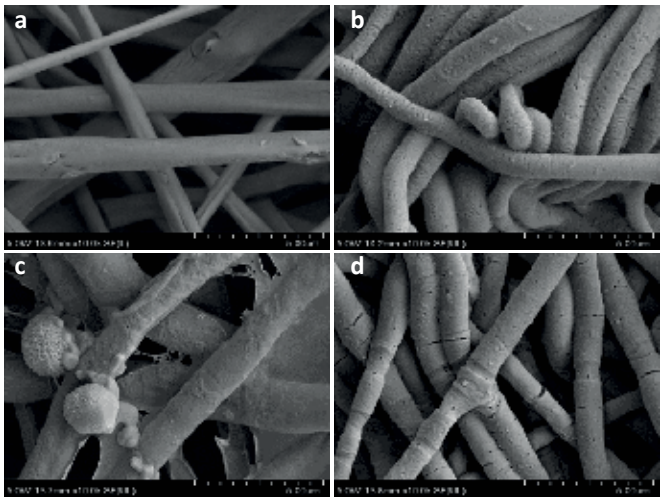

Fig. 1. SEM images of (a) randomly oriented pure PLA and hybrid nanofibers with (b) 10, (c) 20 and (d) 30% of CaP glass nanoparticles after 14 days incubation in SBF.

The chemical analysis of the hybrid nanofibers after the degradation test revealed that there is a significant decrease of glass content in the hybrid nanofibers in the first day of the degradation test. Between 1st and 21st day of degradation test, the glass content in the hybrid fibers is more stable. FTIR investigations of hybrid nanofibers containing 20% of nanoparticles after 14 days of incubation in SBF confirmed the SEM observation. A new broad band at about 560 cm-1 was observed in the spectrum, which can be attributed to phosphates bonds, see Fig 2.

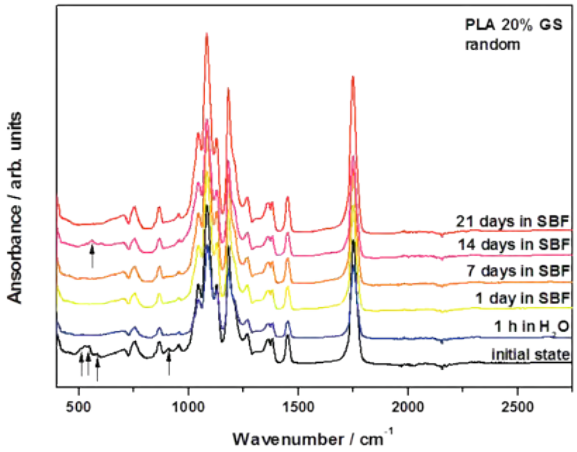

Fig. 2. FTIR spectra of as-obtained hybrid nanofibers with 20% of CaP glass nanoparticles and after incubation in SBF for various periods of time.

CONCLUSIONS

One of the most useful methods to analyze the biodegradation behaviour of a material is to perform in vitro degradation tests where physiological conditions are simulated. In this sense, simulated body fluid (SBF) is a well-known acellular and aproteic medium widely used to evaluate biomaterials degradation and their ability to mineralize or to form a CaP apatite layer that precipitates on their surfaces.

Physico-chemical characterization of hybrid PLA/CaP glass nanofibers after degradation test in SBF for various periods of time has shown that there is a significant decrease of glass content in all nanofibers (independently on amount of glass) in the first day of the test. Between 1st and 21st day of degradation, the glass content in the hybrid fibers remains stable. In addition, for sample with 20% of CaP glass nanoparticles, biomineralization process is observed after 14 days of incubation. This may suggest that this sample exhibits the highest bioactivity.

Acknowledgements: We thank the European Commission (European ERANET project no. PI11/03030, NANGIOFRAC) and the National Center for Research and Development (Project no. 04/EuroNanoMed/2012) for financial support.

IS BLOOD VESSEL WALL THE BARRIER FOR QUANTUM DOTS?

ROTOMSKIS R[1,2], Kulvietis V[1]
[1]Biomedical Physics Laboratory, Vilnius University Institute of Oncology, Vilnius, Lithuania
[2]Biophotonics group of Laser Research Center, Vilnius University, Vilnius, Lithuania

Quantum dots (QD) are semiconductor nanopartcles (NP) which exhibit exceptionally stable and bright photoluminescence (PL) and size tuneable spectroscopic properties. The surface of QD can be chemically modified with various organic ligands to obtain specific biological functionality. Due to their size QD exhibit new spectroscopic and physicochemical properties which are important for biomedical applications.

After intravenous injection one of the first steps of QD passage to the organism is the penetration through the wall of blood capillaries. Praetner et al.,2010 showed that neutrally charged QD (PEG terminated) have the lowest adhesion to the vessel wall and therefore exhibit longest circulation lifetime [Praetner et al., 2010]. It was also shown that QD might be endocytized by the leukocyte and transmigrates through the vessel wall within the cells [Rehberg et al., 2012].The ability of QD to penetrate through the blood vessels is one of the major factors determining their distribution in the organism and it plays important role in fluorescence angiography, selective tumours imaging and therapy, magnetic resonance imaging, etc. It is known that inflammation, cancer and other pathological conditions alter vessel permeability for QD [He et al., 2013; Rehberg et al., 2012]. The studies [Smith et al., 2010, Praetner et al.,2010, Rehberg et al., 2010, Kim et al., 2012] don't answer what is the mechanism of QD transport across the capillary wall (transcellular, intercellular or both). More to add, QD penetration through the blood vessels with additional wall layers (e.g., veins, arteries) are not explored.

Therefore we aimed to investigate the penetration of QD through the blood vessels in apical-basolateral direction and vice versa. The distribution of the QD in tissues of rat in vivo was investigated.

The handling and experiments with animals were performed using protocols approved by the State Food and Veterinary Service. QD were injected intravenously to the Balb/c mice under ketamine narcosis. QTracker-655 QD (Life Technologies) composed of CdSe/ZnS core/shell, coated with mPEG-5000 polymer at the dose of 200 µl and 0.4 µM were used. QD localization in the organism was investigated 1 h and 24 h after injection. After QD administration the animals were euthanized by cerebrospinal dislocation. Fluorescence spectroscopy of the specimens of tissues was performed using Cary Eclipse spectrometer (Agilent Technologies, USA) with fiber optics module (Smeasured≈20 mm2) in order to determine QD accumulation in the tissues. The organs (skin, lungs, thymus, brain, liver, muscle, kidney, spleen) were excised, washed with saline and dried before measurements. The excitation wavelength of 450 nm was used. Brain, skin, cardiac and thigh muscle were excised and cut in ~0,5 mm slices for imaging under confocal fluorescence microscope. Additional preparation of tissues was performed by fixating samples in 4% formaldehyde solution for 24 h and embedding in paraffin. Sections of 4 µm thickness were made with Leica RM2145 microtome. The slices were prepared without deparaffinising and staining the sections in order to avoid QD elution or photoluminescence (PL) quenching. After fluorescence analysis tissue slices were deparaffinised and stained with hematoxylin-eosin (HE) to visualize tissue structure.

The fluorescence microscopy was performed using Nikon C1si confocal laser scanning fluorescence microscope. The standard RGB detector was coupled with 500-590 nm band-pass filter for green and 620-750 nm band-pass filter for red channel. QD localization in the tissues of experimental animals was evaluated by analyzing strong red QD PL with the green tissue autofluorescence background of the unstained tissue specimens.

Spectroscopic analysis reflects that QD concentration in the blood reaches its maximum at 2.5 h after injection, which is followed by

an exponential decrease and complete clearance after 24 h.
Spectroscopic analysis shows general accumulation of QD in the organs, however, it lacks information about spatial distribution of QD in the tissues. Therefore confocal fluorescence microscopy was used to assess QD localization in the tissue specimens. The analysis of the frozen tissue sections revealed that QD (red) mainly accumulated in the blood vessels of skeletal muscles, cerebral hemispheres, uterus, heart, skin and other tissues. The network of blood vessels could be clearly seen in the background of green tissue autofluorescence. There was no exatravasation of QD in the extracellular space (Fig. 1).

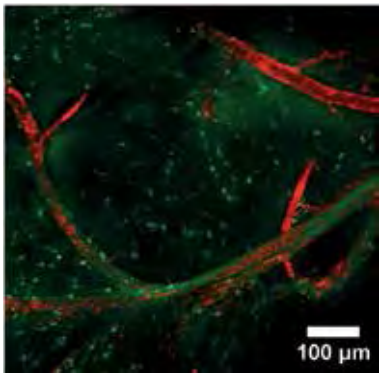

Figure 1. Confocal fluorescence microscopy image of a frozen mice cerebral tissue section 1 h after intravenous injection of CdSe/ZnS-mPEG QD (red). Green colour corresponds to the tissue autofluorescence (x20/0,5 obj., λexcitation=488 nm).

Optical sectioning of single blood vessels showed that QD localize in the lumen of the vessels but they are not found in the vessel wall. Confocal microscopy images clearly showed that QD accumulated in the outer layer of the wall tunica adventitia (t. adventitia), but they could not cross the border of the middle layer of the wall (t. media) and didn't accumulate in this layer. QD could also be detected in the lumen of the vessels due to QD presence in the blood. QD adhered to the endothelium, but they couldn't penetrate through it and pass to the t. media. It shows that QD cannot pass to the t. media from both sides of this layer.

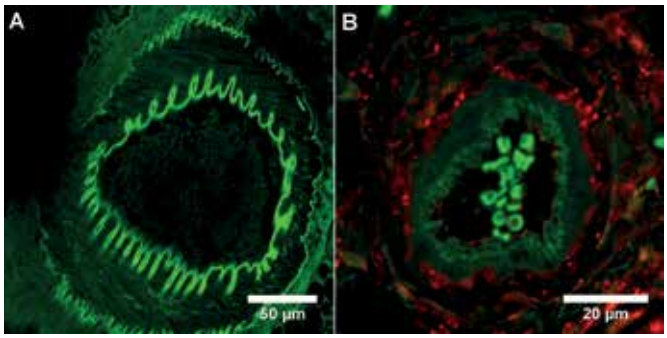

Figure 2. Confocal fluorescence microscopy images of paraffin embedded tissue sections of dermal arteries from the injection site 1 h after intravenous injection of saline (A) and of CdSe/ZnS-mPEG QD (red) (B). QD accumulation in the tunica adventitia, but not in tunica media is visible. Green color corresponds to the tissue autofluorescence. (x20/0,5 obj., λexcitation=488 nm).

QD outlined even small blood vessels: arterioles, venules and capillaries without accumulation in the wall of the vessels. It shows that the barrier properties of the vessel wall don't depend on the presence of the muscle fibers. The barrier of the capillaries probably is formed by the basement membrane of the endothelium and the neighbouring fibers of the surrounding connective tissue. The results of confocal microscopy show that intravenously injected CdSe/ZnS-mPEG QD localize within the blood vessels and the extravasation of QD to the extracellular space in most organs is negligible.
The results of confocal microscopy show that intravascularly located QD mainly resided in the blood plasma. QD adhered to the endothelium of blood vessels, but there was no QD PL in the deeper layers of the vessel walls. It indicates that QD are not transferred through the endothelium to the middle layer (t. media) of the wall. It was shown that endothelial cells might internalize QD and this process depends on the surface coating of the particle: QD terminated with carboxyl or amine groups were observed in the endosomes, but PEG coated QD could not be detected in the endothelial cells [Rehberg et al., 2012, Praetner et al., 2010]. However, it remained unclear if QD can be translocated to the basal side of the endothelium. Our results show, that QD do not penetrate t. intima and don't accumulate in the deeper layer of the vessel wall. We assume that the basement membrane (BM) could play important role in the barrier properties of the endothelium. BM is a special element of all epithelial tissues and vascular endothelium, which is formed by densely organized protein sheets (IV collagen, laminin, proteoglycans, etc.) [Kalluri , 2003]. Few studies show that this structure may act like a passive filter and restrict the diffusion of NP [Bennett et al., 2008, Kulvietis et al., 2013]. However, the role of BM as a biological barrier for NP is poorly explored. Internal elastic lamina separating the t. intima and t. media is another important extracellular structure which could impede QD diffusion. It contains fenestrations allowing for passage of diffusible vasoactive substances [Kirby et al., 2013]. However, these fenestrations could be too narrow for QD passage. The intercellular junctions between endothelial and smooth muscle cells [Gairhe et al., 2011] may also contribute to the restriction for QD penetration.
The results also show that QD which were located outside the blood vessels penetrated into the external layer of the vessels (t. adventitia) and they outlined the border of the t. media, but didn't cross it. QD penetration through the t. media and t. adventitia was not investigated before.
We relate the biodistribution of QD in the vessel wall with the extracellular tissue structure. T. adventitia constitutes of loose collagen and elastin fibers and sparse cells with negligible intercellular junctions. This tissue structure allows QD diffusion. Meanwhile t. media is composed of tightly organized smooth muscle fibers which are interconnected with gap junctions [Gairhe et al., 2011]. They also share a common BM which contributes to the barrier properties of t. media [Hedin et al., 1999]. Larger vessels additionally contain external elastic lamina. These extracellular features could explain the QD retention in the t. adventitia without penetration to the t. media.
This study shows that QD may adhere to the endothelium and diffuse in the areolar connective tissue of tunica adventitia, but they are unable to pass to the tunica media from outer as well as inner surface of this layer. The limited penetration through the vascular wall determines the retention of QD within the blood vessels without remarkable extravasation to the extracellular tissue fluids in most organs. Intravascular retention is a major factor affecting the biodistribution of QD in the organism and pharmacokinetic parameters. The specificity of QD diffusion in different tissue could be used in the diagnostics and treatment of certain diseases which involve vascular wall damage.

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DEVELOPMENT OF SOLID LIPID NANOPARTICLES FOR ANTIMICROBIAL DRUG DELIVERY

GIULIO SANCINI[1], Rosario Musumeci[2], Roberta Dal Magro[1], Silvia Beretta[1], Alessandra Bulbarelli[1], Elena Lonati[1], Paola Galletti[3], Daria Giacomini[3], Clementina E. Cocuzza[2], Paolo Gasco[4]
[1]Department of Health Sciences, University of Milano-Bicocca, via Cadore 48, 20900 Monza, Italy;
[2]Department of Surgery and Translational Medicine, University of Milano-Bicocca, via Cadore 48, 20900 Monza, Italy
[3]Department of Chemistry ''G. Ciamician'', University of Bologna, via Selmi 2, 40126 Bologna, Italy;
[4]Nanovector S.r.l., via Livorno, 60 - 10144 Torino, TO, Italy

INTRODUCTION

Our previous Solid Lipid Nanoparticles (SLN) pharmacokinetics and biodistribution studies raise the possibility that interstitial lung macrophages, in closer contact with the circulation than alveolar macrophages, significantly contributed to SLN uptake [1, 2]. Prolonging plasma circulation time, lowering renal drug exposure, and markedly increasing lung tissue deposition would be important attributes for an ideal antimicrobial compound in treating methicillin-resistant Staphylococcus aureus (MRSA) pneumonia [3]. Furthermore, preferential deposition of the new antimicrobials into liver and spleen tissue may add benefits to the patient. Indeed the natural history of MRSA pneumonia involves lung necrosis and bloodstream invasion. Kupffer cells within the liver may represent the last line of host defense against S. Aureus bacteremia resulting in sepsis, multi-organ failure, and death [4]. A hypothesis can be drawn that SLN-loaded antimicrobial targeting liver tissue macrophages provide an additional benefit that could translate into improved outcomes in patients with MRSA pneumonia complicated by bacteremia.

GOAL OF THE WORK

With the ongoing efforts in the nanomedicine field, here we explore the possibilities that nanoparticle-based drug delivery systems will improve treatment to bacterial infections, especially in life-threatening diseases such as staph infections.

METHODS

Preparation of SLN has been previously explored. Here, novel SLN has been prepared by Nanovector s.r.l. Starting from Nanovector's expertise we focused on characterization and control by DLS/PCS technique for process step of microemulsion formation by applying a developed knowledge for the on-line process micro-control. SLNs has been specifically designed to encapsulate antimicrobial agents. Their preparation is based on an original warm microemulsion technique, allowing the production of nanocarriers in an aqueous solution with a size ranging from approximately 20 nm to 200 nm. The new lead antimicrobial compounds FF357 loaded by SLN has been administered by pulmonary route by means of intratracheal aerosolization. By applying intratracheal aerosol technique [5], more uniform distribution can be obtained with greater penetration into the peripheral or the alveolar region of the lung, thus limiting the difficulty in measuring the exact dose inside the lungs which is affected by standard aerosol technique.

RESULTS AND DISCUSSION

Our preliminary results confirmed that [3H]-SLN developed by Nanovector represents a suitable drug delivery system to improve drug bioavailability within the lungs.

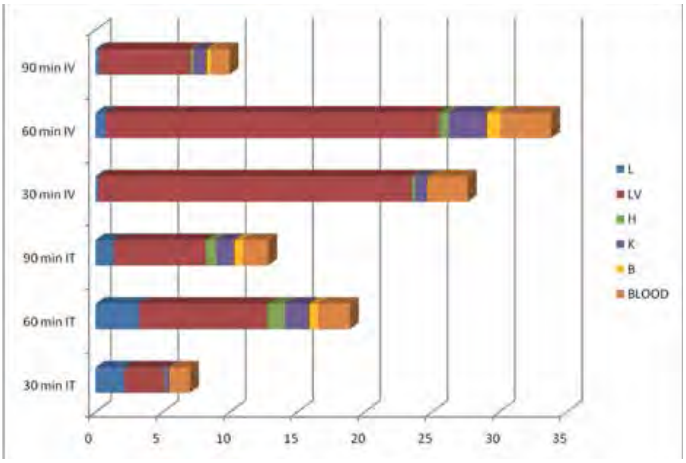

Fig. 1. SLN biodistribution after intravenous and intratracheal administration in rats, (L=lung; LV=liver; H=heart; K=kidney; B=brain).

Results reported in Fig. 1 indicate that inhaled [3H]-SLN, ensures greater localization in the lung and lowered their retention in the liver with a reduced first pass effect. Moreover, in order to assess the SLN biocompatibility we evaluated in the bronchoalveolar lavage fluid (BALF) of SLN IT treated mice, total and differential cell counts, inflammatory marker (TNFa) and cells damage markers (total proteins, LDH, ALP). No evidence of lung inflammation and lung injuries have been found. We have shown that the SLN, namely the vector itself, are distributed within the lungs being retained for hours after IT aerosolization. It has therefore been identified a phase that determines a fairly prolonged permanence of SLN in the lung without a significant accumulation in extrapulmonary districts such as the liver, contributing to significantly reduce the first pass effect and possible drug related systemic side effects incurred by drugs with a critical toxicity profile.
We recently developed a new SLN formulation provided by Nanovector that demonstrated longer retention time after pulmonary administration in mice. The biodistribution profile of indocarbocyanine iodide(DiR)-labeled SLN was evaluated in adult mice after single intratracheal instillation (50 µL, 9-18 mg/mL as total lipids). As shown in Fig. 2 DiR-SLN (DiR, near-infrared fluorescent cyanine dye) were clearly detectable within lungs as longer as 24h after IT administration (3) thus indicating that this new SLN formulation is a promising tool to deliver antimicrobial agent to the lungs.

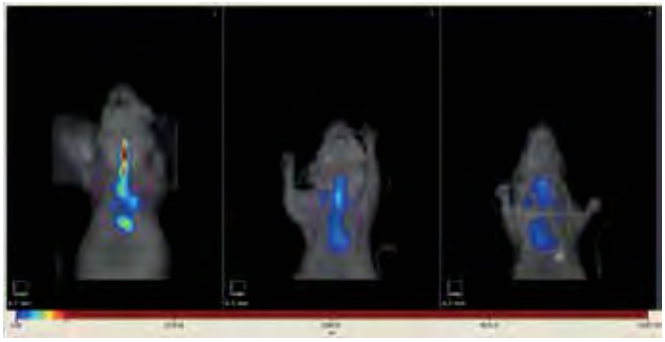

Fig. 2 : In vivo DiR-SLN biodistribution studies: FMT scans after 15 min (1), after 1h (2) and after 24h (3) from intratracheal aerosolization (IT) in mice.

Total protein concentration in the BALF was evaluated as marker of alteration of the alveolar-capillary barrier permeability, but no significative increase was detected in SLN-treated-mice. Differential cell count confirmed no significative differences in the percentage of alveolar macrophages and polymorphonuclear leukocytes (99% neutrophils) between sham and SLN-treated mice, 24 hours after IT instillation. Finally the persistence of SLN in the lung did not induce any enhancement in the BALF level of IL-1β.
Biodistribution studies of the FF357 antimicrobial compound are currently running. FF357 loaded by this new SLN formulation IT administered in 4 mice up to the final dose 0.129 mg/mice did not exert any signs of acute toxicity being preserved respiratory functionalities and behavior.

CONCLUSIONS

Many antimicrobial drugs are difficult to administer because of their low water-solubility, cytotoxicity to healthy tissues, and rapid degradation and clearance in the blood stream. Their antimicrobial activities against intracellular microbes are also severely limited by poor membrane transport ability. Extensive studies have demonstrated that nanoparticles such as liposomes and solid lipid nanoparticles (SLN) are able to overcome these issues and facilitate antimicrobial delivery to microbial infection sites.

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DEVELOPMENT OF A MULTIMODAL DRUG DELIVERY SYSTEM FOR RADIOCHEMOTHERAPY OF TUMOR TISSUE

CARMEN SEIDL[1], Christin Bednarek[1,2], Götz Hofhaus4, Rasmus Schröder[4], Marlon Veldwijk[5], Frederik Wenz[5], Claus Feldmann[3], Ute Schepers[1]

[1]Institute of Toxicology and Genetics; [2]Institute of Organic Chemistry; [3]Institute of Inorganic Chemistry, Karlsruhe Institute of Technology (KIT); [4]Bioquant Cell Networks, University of Heidelberg; [5]Department of Radiation Oncology, Medical Faculty Mannheim

To date, radiotherapy is playing an essential role in modern multimodal treatment of cancer as the chemotherapy alone often leads to therapy resistance[1]. However, the side effects due to irradiation induced destruction of healthy tissue are often the dose limiting step[2]. Therefore, there is a great demand for novel multimodal therapeutics that increase the therapeutic range. Herewith we report the development of a nanosized multimodal drug delivery system for improved treatment of tumors. The system is based upon a liposomal structure and combines two conventional types of tumor therapy, chemotherapy and radiotherapy. Therefore, it employs doxorubicin (DXR) as a cytostatic and stearylamine coated gold nanoparticles (sAu-NPs) as radiosensitizers. In addition, tumor-specific accumulation of the system should be given due to the preparation of a defined size (150 nm) and exploitation of the EPR-effect. Furthermore, tumor cell specificity and liberation of the drug should be achieved by using a polyamine transporter, which is covalently coupled to the cytostatic via an acid-labile linker (doxorubicin-transporter). Simultaneous incorporation of doxorubicin-transporter and sAu-NPs into the liposomal membrane forms the multimodal drug delivery system (Figure 1). Cell uptake as well as accumulation of the components and liberation of doxorubicin inside the cells was shown.

Figure 1: Multimodal drug delivery system PC egg based scaffold (blue) Targeting peptoid (black)Doxorubicin (red) Stearylamine-coated gold nanoparticles (yellow)

Similar approaches we address to combine chemo- and radiotherapy are based on the incorporation of doxorubicin into metallic hollow spheres. Moreover, we focus on the so called photodynamic therapy (PDT). The nanoparticulate photosensitizer β-SnWO4 is suggested for therapy of near-surface tumors via blue-LED illumination.

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TUMOUR PENETRATING PH-SENSITIVE POLYMERSOMES FOR CANCER THERANOSTICS

LORENA SIMÓN GRACIA[a], Jeppe Madsen[b], Giuseppe Battaglia[b], Tambet Teesalu[a]

[a]Lab of Cancer Biology. Institute of Biomedicine and Translational Medicine. University of Tartu (Estonia).

[b]Department of Chemistry and the Centre for Materials Discovery. University College London

Main challenges in delivery of therapeutic agents to tumours are: rapid clearance before reaching the cancerous tissue, insufficient extravasation and penetration of tumor tissue, and low specificity towards tumour cells. This results in poor response to therapy, adverse side effects, and, over time, in development of drug resistance. Conjugation of tumour-specific targeting ligands can increase selectivity of anticancer drugs. We have discovered a new class of tumour penetrating peptides (TPP) that not only home to tumour vessels, but also extravasate and spread through tumour parenchyma and internalize into cells. This new class of homing peptides follows the C-end Rule: the consensus R/KXXR/K CendR motif must be exposed at the C-terminus of the polypeptide chain to elicit cell and tissue penetration. The CendR receptor, NRP-1, is a pleiotrophic cell surface receptor with essential roles in angiogenesis and regulation of vascular permeability development of the nervous system [1,2]. Natural ligands possess a C-terminal R/KXXR/K sequence motif that interacts with NRP-1, and causes cellular internalization and vascular leakage [3]. Short synthetic CendR peptides (such as prototypic active CendR peptide RPARPAR) have similar effects, especially when made multivalent by coupling to a molecular scaffold or a particle. NRP-1 is widely expressed in normal tissues and strongly overexpressed in a variety of malignant cells in vitro and in vivo, and in tumor stromal cells (immune cells, fibroblasts) [4]. Prototypic tumour penetrating CendR peptide, iRGD (sequence: CRGDKGPDC), contains RGD motif (bold), a cryptic CendR sequence (underlined), and a CendR convertase cleavage site. iRGD enhances tumour penetration of coupled and co-administered compounds through a multistep process. First, the RGD homing motif directs the peptide to αv integrins on angiogenic tumour endothelium, where the peptide is proteolytically processed to expose the CendR motif at the C-terminus. The activated CendR motif then acquires the ability to bind to NRP-1, triggering extravasation, tumour penetration, and cell entry of the C-terminally exposed CendR motif.

Compared to free drugs, nanoparticle (NP) encapsulated drugs have improved biodistribution, pharmacokinetics, and toxicological parameters. Polymersomes (PS) are liposome-like, yet very stable, nanosize structures that can be loaded with hydrophilic and hydrophobic drugs and imaging agents. pH-sensitive PS disintegrate rapidly at mildly acidic conditions in endosomes and release the contents intracellularly.

Our work combines tumour penetrating peptide and polymer technologies to develop cancer theranostics. We have developed TPP-functionalized PS loaded with paclitaxel (PTX) and assessed cellular internalization and cytotoxicity. TPP-functionalized PS provide a platform to deliver drugs and imaging agents deep into tumour tissue, and to release the drug payloads into the target cells using

the pH switch. This will result in better efficacy of the drugs, and decreased side effects. Our platform will establish a new paradigm in cancer diagnosis and therapy: enhanced tumour penetration.

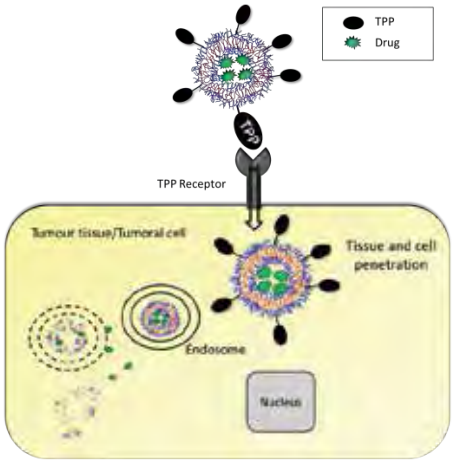

Figure 1. Overview of the TPP-PS internalization mechanism. TPP-functionalized pH-sensitive polymersomes binds to TPP receptor in tumoral cells and tissue. The drug-loaded polymersomes extravasate, spread over the tumour stroma and enter into tumour cells. Inside the cell, polymersomes dissolve in acidic endosomal compartment releasing the drug to the cytosol due to the "proton sponge" effect.

The pH-sensitive PS composed of the diblock co-polymer containing poly(2-(diisopropylamino)ethyl methacrylate (PDPA) were developed in the laboratory of our collaborator, Prof. G. Battaglia at the University College of London [5,6].
For pilot studies on CendR-mediated targeting, we conjugated the RPARPAR peptide to the co-polymer poly(oligo(ethylene glycol) monomethyl ether methacrylate)- poly(2-(diisopropylamino)ethyl methacrylate) (POEGMA20-PDPA90) label with rhodamine dye.
To study the specific binding and internalization of RPARPAR-PS, PPC-1 prostate cancer cells (that overexpress CendR receptor NRP-1), were incubated with RPARPAR-PS or with non-targeted PS. Human melanoma M21 cells, not expressing NRP-1, were used as negative binding control. The incubation of PPC-1 cells with RPARPAR-PS resulted in higher internalization after 1 hour of incubation compared with non-targeted PS or compared with M21 cells (Fig. 2).

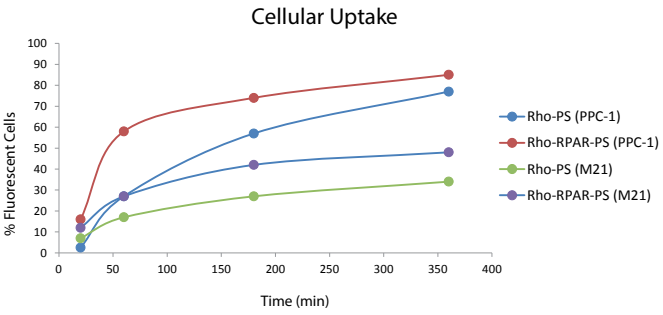

Figure 2: Cellular uptake of Rho-label PS by PPC-1 and M21 cells. The cells were incubated with 1mg co-polymer/mL at different time points. Rho-PS: PS label with 10% of rhodamine. Rho-RPAR-PS: PS label with 10% rhodamine and containing 20% of RPARPAR peptide.

The in vitro cytotoxicity of PTX-loaded PS was also tested in PPC-1 and M21 cells.
After 30 minutes and 1 hour of incubation the PS-RPAR loaded with PTX showed significant higher cytotoxicity in PPC-1, at 10 nM of PTX, compared with non-targeted PS and free drug. The same experiment performed in M21 cells did not show significant differences regarding cytotoxicity when using PS-RPAR or non-targeted PS. The empty PS (PS without PTX) did not show cytotoxicity in any of the cell lines. These results, together with the internalization experiments demonstrate a specific and receptor-dependent binding and internalization of the pH-sensitive PS.

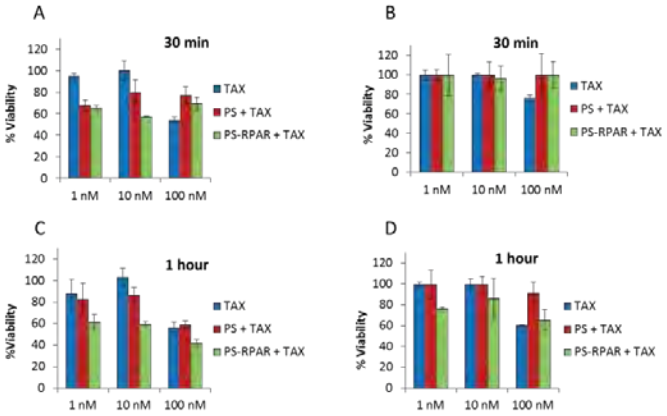

Figure 3: Cytotoxicity assays in PPC-1 and M21 cells. A: PPC-1 cells incubated with free paclitaxel (TAX), PTX-loaded PS (PS + TAX), or PS-RPAR loaded with PTX (RS-RPAR + TAX) for 30 min. B: M21 cells incubated with the samples for 30 min. C: PPC-1 cells incubated with the samples for 1 hour. D: M21 cells incubated with the samples for 1 hour.

Our in vitro results demonstrate the suitability of the pH-senstive polymersomes for CendR-mediated drug delivery to malignant cells. We will next explore the in vivo and ex vivo performance of polymersomes functionalized with TPP that are more complex that RPARPAR (iRGD, LyP-1, uCendR). For testing the antitumor activity of our nanosystems, we will use orthotopic tumor xenografts of glioblastoma (005, P3) breast (BT474, MDA-MB-231), prostate (PC3, PPC1, Du145, LnCAP), and pancreatic (MiaPACA) cancer in immunodeficient mice, as well as syngeneic 4T1 breast tumors in immunocompetent Balb/c mice. For ex vivo evaluation of the ability of the drug-loaded TPP-PS to bind and penetrate into glioblastoma tissue we will use freshly isolated explants of resected clinical glioblastoma (obtained from our clinical collaborators at the Tartu Univeristy Clinics). These studies will provide the first proof of translational potential of the pH-sensitive TPP-PS platform.

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PRELIMINARY COMPARATIVE STUDY OF A TOPICAL FORMULATION OF HEXADECYLPHOSPHOCHOLINE (HPC) WITH THE GOLD STANDARD INTRAMUSCULAR MEGLUMINE ANTIMONIATE IN A MURINE MODEL OF CUTANEOUS LEISHMANIASIS

ALEJANDRO SOSNIK[3], Ángela María Gómez-Galindo[1], Romina Glisoni[2], Diana Granados-Falla[1], Gabriela Delgado[1]
[1]Grupo de Investigación en Inmunotoxicología, Departamento de Farmacia, Universidad Nacional de Colombia, Bogotá, Colombia; [2]Departament of Pharmaceutical Technology, Faculty of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina; [3]Department of Materials Science and Engineering, Technion-Israel Institute of Technology, Haifa, Israel.

INTRODUCTION

Gold standard treatments against leishmaniasis are parenteral and associated with adverse effects that compromise the adherence to the therapeutic regimens and favor the development of antimicrobial resistance. For these reasons, efforts directed to reduce the use of traditional drugs or to obtain a better-tolerated and effective treatment are a first priority of the World Health Organization.

In this work, we evaluated for the first time the antileishmanial efficacy of free hexadecylphosphocholine (HPC) and its inclusion complex with hydroxypropyl-b-cyclodextrin (HPb-CD), dispersed in a water-in-oil (W/O) cream, on a murine model of the cutaneous infection and compared it to a conventional intramuscular formulation containing meglumine antimoniate (AIM).

Materials and Methods: 10 BALB/c female mice (one mouse for Group 1 and three mice Groups 2, 3 and 4) four weeks of age from the central biotery of the Faculty of Veterinary Medicine and Zootechny (Universidad Nacional de Colombia) were housed in the animal facility of the Department of Pharmacy (Faculty of Sciences, Universidad Nacional de Colombia). Mice had a quarantine period of two weeks before being infected. Animals were kept in individual transparent polycarbonate boxes (One Cage 2100TM AllerZoneTM Micro-IsolatorTM) with bedding and ad libitum access to fresh water and LabDiet®. The assays were performed in accordance to the provisions provided in the Colombian law 84 of 1989 (National Statute for the Protection of Animals) and under Title V of Resolution 008430 of 1993 emitted by the Ministry of Health about biomedical research involving animals. Mice were infected with 1 x 107 promastigotes of Leishmania panamensis (MHOM/CO/87/UA140), provided by Dr. Sara Robledo (Universidad de Antioquia, Colombia) by intradermal inoculation at the lumbosacral area using a 1 mL syringe. After the primo-infection, animals were maintained for six weeks to allow the formation of cutaneous ulcers with a mean size of 6 mm. This was the final point to start the treatment employing the different samples (Table 1).

Table 1. Treatments applied to the different mice groups.

Group	Treatment	Doses	Administration way	Period of treatment
1	AIM	120 mg/kg	Intramuscular, once a day	20 days
2	HPC W/O cream	4.5 mg HPC per g W/O cream	Topic, once a day	30 days
3	CHPC W/O cream	4.5 mg HPC complexed per g W/O cream	Topic, once a day	30 days
4	INT W/O cream	Drug-free W/O cream	Topic, once a day	30 days

AIM: Meglumine antimoniate; HPC W/O cream: Hexadecylphosphocholine dispersed in Water-in-Oil cream
CHPC W/O cream: Hexadecylphosphocholine/Hydroxypropyl-b-cyclodextrin inclusion complex dispersed in Water-in-Oil cream; INT W/O cream: Mice infected and treated with Water-in-Oil cream without active pharmaceutical ingredients.

One hundred mg of each formulation (W/O cream Groups 2-4, Table 1) was properly applied to each lesion once-a-day until its disappearance. Then, lesions were covered with bandages. The clinical follow up was done twice-a-week (registering data about food/water consumption, general status and weight). In addition, the size of the lesion was measured with a Vernier Calliper, which allowed determining the area of each lesion (transverse diameter of the lesion X sagittal diameter of the lesion) at each time point. Once a week, the size of the lesion was recorded and at day 32, the animals were sacrificed according to the Guidelines on Euthanasia of the American Veterinary Medical Association. Samples of lesion skin, inguinal lymphonodes, liver and spleen were collected in order to conduct histopathology and immunohistochemistry analysis (including CD4+ and CD8+ cell populations). With lesion diameters, we established the index of evolution of the lesion (IE) as the ratio between the initial area of the lesion and area of the lesion at a given time point.

RESULTS

In animals treated with HPC W/O cream, the IE was similar or slightly greater than INT (animals treated with drug-free W/O cream) during the first 3 weeks (Figure 1). Then, a gradual decline of the efficacy was observed. Complexation with the cyclodextrin increased the efficacy over the whole the treatment, IE being similar or greater even than that of the standard AIM.

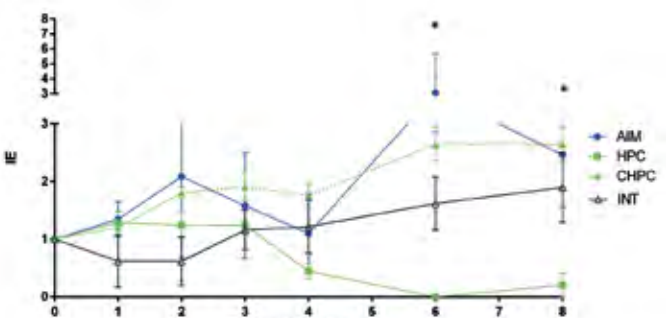

Figure 1. Progression lesions (expressed as IE) in BALB/c mice inoculated with amastigotes of Leishmania panamesis treated with formulations containing HPC and AIM. The values are expressed as SEM were calculated using ANOVA, p ≤0.05. INT: Mice infected and treated with with Water-in-Oil cream without active pharmaceutical ingredients; AIM: Meglumine antimoniate administrated by the intramuscular route.

Histopathology results of skin specimens showed in general, a severe mix infiltrated with neutrophils, macrophages and epithelioid cells and, in less proportion, lymphocytes and plasmatic cells that indicated immunomodulation. However, this kind of infiltrate was not found in two mice, one of them treated with HPC W/O cream (Group 2; Table 1) and the other only with the drug-free cream (INT, Group 4; Table 1). In addition, amastigotes were observed in free form (severe and infective form) or in macrophages with the exception of two mice of the Group 2 (treated with HPC W/O cream); these animals did not present macro and microscopic ulceration, panniculitis and myositis. Finally, the immunohistochemical analysis of the skin showed the absence of CD4+ and CD8+ cell markers in mice treated with HPC and that had appropriate ulcer evolution over the treatment.

CONCLUSIONS

Our results showed an antileishmanial activity of HPC against Leishmania panamensis (responsible of cutaneous leishmaniasis) when formulated as a HP-b-cyclodextrin inclusion complex and dispersed in a W/O cream with respect to the non-complexed drug. Moreover, the activity was comparable or slightly better than that of AIM. Remarkably, the proposed strategy would enable the replacement of the gold standard parenteral and painful treatment that requires trained personnel for the administration by a topical one that is more compliant.

Acknowledgements: AS thanks the European Union's - Seventh Framework Programme under grant agreement #612675-MC-NA-NOTAR. GD thanks to Universidad Nacional de Colombia, Dirección de Investigación Sede-Bogotá (Project #16015).

NONPHASE TRANSITION MAGNETIC-RESPONSIVE LIPOSOMES CONTROLLED BY LOW INTENSITY MAGNETIC STIMULI

R. SPERA[a], C. Merla[b], R. Pinto[b], M. Liberti[c], F. Apollonio[c], S. Petralito[a]
[a]Dep. of Drug Chemistry and Technology, Sapienza University of Rome, Italy
[b]Technical Unit of Radiation Biology and Human Health, RC Casaccia, Enea, Italy
[c]Dep. of Information Engineering, Electronics and Telecommunications, Sapienza University of Rome, Italy

In drug delivery, nanomedicine is a recently developed term to describe nanometer sized (1-1000 nm) multicomponent drug, or drug delivery systems for disease treatment [1-2].
Recent developments in drug therapy are focused on minimizing side effects and improving treatment efficacy. This can be achieved by using a nanocarrier that releases the drug in response to a stimulus. In particular, the ability to control the timing, dosage and release profile of the agent would greatly increase the efficacy of the therapeutic agents. The short and long-term damage to healthy

tissue will also be minimized. Spurred by recent progress in materials chemistry and drug delivery, stimuli-responsive devices that deliver a drug in spatial-, temporal- and dosage-controlled fashions have become possible.

Release of drugs from a nanocarrier can be facilitated by either a local or remote stimulus. A local stimulus is one that relies on change in condition within the body, often taking advantage of physical and/or chemical characteristics that are unique to diseased tissue to release the dug from the carrier. A remote stimulus, instead, is one that is applied outside the body, therefore it is non-invasive, but still able to penetrate the body for finely localize drug release. Remote stimuli are of particular interest because they can provide both temporal and spatial control over drug release.

The use of an alternating magnetic field as remote stimulus for controlled drug release, in what is known as magnetic hyperthermia induced controllable drug delivery, has gained much interest recently. In magnetic hyperthermia induced release, the drug carrier should consist of a magnetic nanomaterial that interacts specifically with an oscillating magnetic field. Typically this interaction results in the generation of heat within the nanocarrier, which in turn initiates release of the drug.

The most common structures for magnetic hyperthermia induced drug delivery are derived from earlier designs of carriers for drug delivery. Perhaps the most significant of these developments was the combination of organic and inorganic materials; specifically, the magnetic responsive properties of inorganic nanoparticles were combined together with the loading and release properties of organic structures with controllable drug delivery properties.

When exposed to appropriate magnetic fields (amplitudes of kA/m and frequencies from tens to hundreds of kHz) magnetic nanoparticles (np's) can be induced to generate heat, either from hysteresis losses or from Néel or Brownian relaxation processes [3]. Such a local temperature increase is able to induce the release of the drug from a structure containing magnetic nanoparticles, however several studies suggest that magnetic nanoparticles in a liquid media may produce and sustain a significant macroscopic temperature increase (up to 10°C with respect to the basal one) in their immediate vicinity when heated by electromagnetic fields. This localized heating around the nanoparticles can change some biological processes and cause thermally-induced damages to the surrounding tissues and pain. All these thermal effects can be accepted in cancer therapy and it can be even possible to take advantage from them (i.e. cell apoptosis), but they may be not still accepted for a chronic disease treatment, such as inflammatory diseases.

In this context, it has been recently reported a pilot study [4] where it has been demonstrated the feasibility of a smart controlled delivery through a magnetic field with intensity significantly lower (amplitude << kA/m) than the usual ones reported in literature. In this way, the drug delivery can be controlled by a mechanical oscillation induced on the magnetic nanoparticles by the field, instead of heat generation. Therefore, a controlled release will be obtained without a macroscopic temperature increase. This represents a clear advantage: the tissues are not affected neither by the temperature nor by the eddy currents induced by the field, thus preventing damage and safety secondary effects [5]. Based on these preliminary and encouraging results, the main objective of this work is based on an in-depth evaluation of the potentiality of low amplitude magnetic fields as a tool for drug controlled release induced by a mechanical destabilization of magnetic phospholipid delivery systems.

To prove the impact of the mechanical effect induced through a low amplitude external alternating magnetic field (AMF) on the magnetic nanocarriers release, structures that are not sensitive to thermal effects have been required. In particular, nonphase transition magnetic liposomes (nPTmMLs) were investigated. nPTmMLs refer to magnetic liposome that not display a phase transition temperature (PTm) within temperature range of interest [6]. Since these liposomes are extremely stable with respect to physiological temperature, in vivo they do not exhibit a PTm to which they become permeable and leakly, so they may be administered as depot dosage form to treat chronic diseases. Hydrogenated soybean phosphatidylcholine (HSPC) and 20 mol% cholesterol to phospholipid molar ratio (PTm ~ 50°C), were employed to prepare 200 nm sized nPTmMLs entrapped commercially available carboxymethyl-dextran coated magnetite np's, (Fe_3O_4, fluidMAG-CMX, Chemicell GmbH) by the thin film hydration method as reported in [7]. Enhancement of cargo release upon exposure to magnetic field, at 37°C has been measured as the self-quenching decrease of the 5(6)-carboxyfluorescein, (CF) entrapped in the liposome pool. The fluorescent dye release has been evaluated for untreated nPTmMLs (4°C) and upon exposure (AMF 20 kHz, amplitude<100A/m) as well as for SHAM samples (release under null H field conditions, obtained using currents flowing in opposite directions in the wires, and placing the samples in the same position of the exposed ones). Details of the experimental setup are reported in [4] and here briefly recalled in fig. 1a). These studies were carried out under controlled temperature conditions (37.0±0.5°C) by the use of a thermal bath and measured by means of a thermocouple. Temperature experiments were also carried out by incubating the nPTmMLs in a water bath at 42°C, in order to verify that physiologically acceptable temperature induced no CF leakage from nPTmMLs, and at 50°C which represent temperature conditions able to get a positive release pattern (positive control). Dynamic light scattering (DLS) experiments were performed to assure integrity of the vesicles upon treatments. Physical stability of nPTmMLs stored at 4°C was investigated over four weeks to know how long the np's stay inside the vesicles.

Release results are reported in fig.1b as a function of time up to 15 h of treatments. As expected, it was not possible to release any encapsulated CF from nPTmMLs simply by submitting these liposomes to physiologically acceptable heat (37-42°C). Instead, 20 kHz AMF of intensity below 100 A/m was able to control, at 37°C, the release from nPTmMLs. There is a statistically significant difference of about 20% between the nPTmMLs exposed to the field and the ones with no field. This outcome indicates that for this type of liposomes some other tools of release induction are necessary. AMF provide an interactive triggered agent. A controlled release can be obtained in non- thermal manner, hence under the hypothesis of a mechanical coupling of the np's with the induced magnetic field inside the nPTmMLs. The possibility to remotely control the release of a drug, targeted in situ, opens the way to intriguing and new capabilities of biomedical applications. Positive control experiments verified that a rapid and quantitative drug release from the investigated vehicles appears only when their phase transition temperature of 50°C was reached.

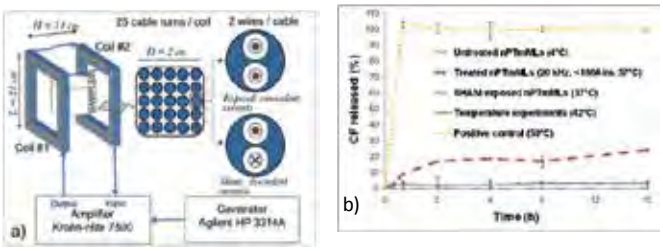

Fig. 1. Details of the experimental setup and final outcomes.
a: schematics of the exposure setup configuration where geometry of the coils are represented. L = 21cm side of the coil; H = 11 cm distance between coils; D = 2 cm side section of the coil. Current configurations for exposure (concordant) and sham (discordant) are sketched. Supply-chain of the generator, amplifier, and system is depicted. b: experimental comparison of the release of the fluorescent dye (CF) loaded in the nPTmMLs both for exposure condition (AMF, 20 kHz, 60 A/m) and for the SHAM, at increasing exposure times. A positive control at 50°C, nPTmMLs stored at 4°C and temperature experiments at 42°C are also reported.

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ZEBRAFISH TUMOR MODELS: NOVEL TOOLS FOR DRUG DEVELOPMENT

M. SPITZNER[1], S. Burkart[1], E. Alghisi[2], V. Anelli[3], C. Santoriello[4], M. Mione[1]

[1]Institute of Toxicology and Genetics, Karlsruhe Institute of Technology, Karlsruhe, Germany

[2]Hematology and Blood Disease and Stem Cell Transplantation, Department of Clinical and Experimental Sciences, University of Brescia Medical School, Brescia, Italy

[3]Departments of Surgery and Medicine, Weill Cornell Medical College and New York Presbyterian Hospital, 1300 York Avenue, New York, New York 10065, USA

[4]Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, Massachusetts 02138, USA

Despite intensive studies cancer is still difficult to treat. Major problems are drug efficiency and specificity while side effects limit the therapeutic range. Development of new targeting approaches requires appropriate models that closely resemble human tumors, allow for reliable drug administration and dosage control, enable easy and fast delivery and support screening techniques.

As today, drug efficacy and targeting are mostly tested in cell assays and established in mouse models. While cell assays can provide reliable drug administration and dosage control, mouse models closely mimic human tumors. However, drug administration and dosage control is difficult and analysis of targeting efficiency and treatment effect are highly limited in mouse models.

The zebrafish model can combine advantages of both cell culture and mouse models. As zebrafish are vertebrates and spontaneously develop tumors, they are suitable for cancer studies. Furthermore, they develop externally which allows for easy analysis of early events. Zebrafish larvae are translucent which facilitates whole organism life imaging even at a cellular level and pairs have large clutch sizes ideal for screening approaches. Additionally, drugs can easily be administered through their swimming water and dosage and uptake reliably controlled.

In our lab we developed diverse tumor models in zebrafish that closely resemble the human diseases. These include a model for myeloid leukemia1, melanoma2 and, currently under establishment, a model for glioma. All three models show cellular abnormalities already at larval stages and as adults develop tumors that mimic the corresponding human tumor type.

The leukemia model is based on expression of the human oncogenic version of HRAS under the promoter of fli1, a transcription factor required for development of the hemogenic endothelium. Two days old larvae develop an enlarged caudal hematopoietic tissue (CHT) due to increased proliferation of hematopoietic cells and delay in myeloid-erythroid maturation, while juvenile fish reveal pathologically increased number of myeloid progenitors.

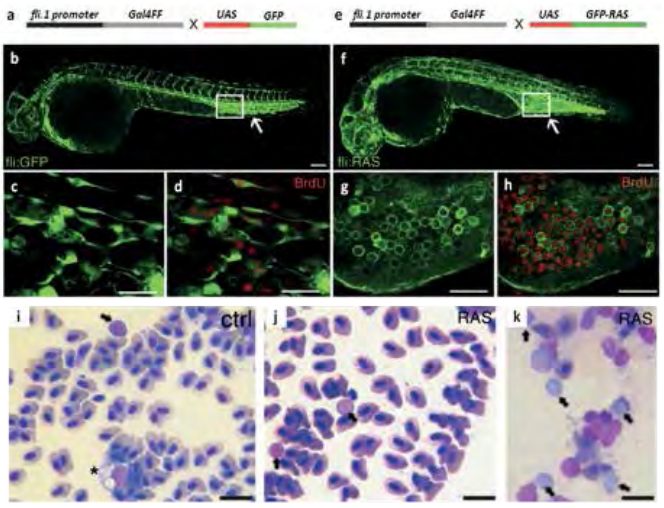

Figure 1: Oncogenic HRAS expression under the fli1-promoter leads to hyper proliferation of hematopoietic cells. (a, e) Schematic representation of the outcross between the tg(fli1:Gal4FFubs3) transgenic line and tg(UAS:GFP) line (fli:GFP shown in (b-d)) or tg(UAS:eGFP-H-RASV12)io06 (fli:Ras shown in (f-h)). (b, f) Micrograph of a live 30hpf embryo with GFP expression in the vasculature and in hematopoietic cells. A region (boxed) of CHT (arrow) is enlarged in (c, g), whereas in (d, h), the red immunofluorescence staining denotes labeling for BrdU showing in (h) almost all cells are proliferating. (i-k) May-Grunwald-Giemsa staining of peripheral blood of 3–4-week-old control fli:Gal4FF and mosaic fli:RAS zebrafish. While the control (i) contains erithrocytes, rare mp (arrow), rare macrophages (asterisk) and lymphocytes (not shown) blood of mosaic fli-RAS fish (j-k) contains a large number of mp, mostly type 3 (arrows).

The melanoma model depends on oncogenic HRAS expression under the kita promoter. Larvae appear with a highly increased number and less organized localization of melanocytes. In adults at the age of 1-3 months these fish develop pigmented as well as in rare cases unpigmented tumors.

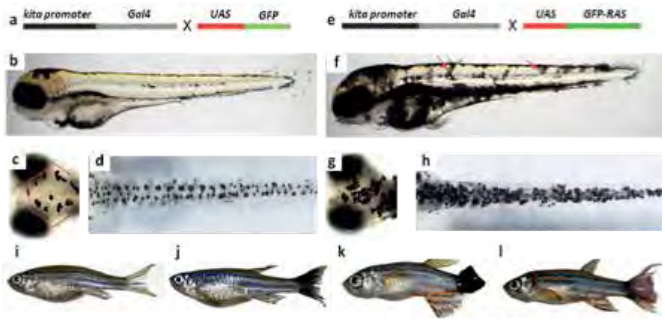

Figure 2: Oncogenic HRAS expression under the kita-promoter induces melanoma develop-ment. (a, e) Schematic representation of the outcross between the tg(kita:Gal4TA4)hzm1 transgenic line and tg(UAS:GFP) line (kita:GFP) or tg(UAS:eGFP-H-RASV12)io06 (kita:Ras). (b) 3dpf kita:GFP with dorsal view of head (c) and lower body (d). (f) 3dpf kita:Ras with dorsal view of head (g) and lower body (h) (i-h) different phenotypes in 2-month old kit:Ras zebrafish: a) control fish; b) black caudal fin; c) large hyper-pigmented tumor; d) hypo-pigmented tumor.

The glioma model uses the zic4 promoter to drive expression of oncogenic HRAS in the dorsal region of the central nervous system. Larvae develop increased brain size due to hyperproliferation as well as histological abnormalities. Juvenile fish appear with brain tumors that resemble the mesenchymal phenotype of human gliomas.

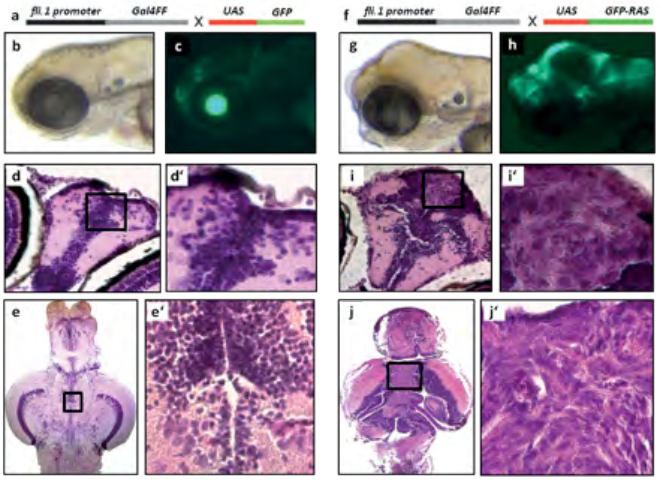

Figure 3: Oncogenic HRAS expression under the zic4-promoter induces glioma development. (a, f) Schematic representation of the outcross between the tg(zic4:Gal4TA4)hzm5 transgenic line and tg(UAS:GFP) line (zic4:GFP) or tg(UAS:eGFP-H-RASV12)io06 (zic4:Ras). (b-c) lateral view of 3dpf zic4:GFP as brightfield (b) and GFP (c). (g-h) lateral view of 3dpf zic4:Ras with enlarged brain and GFP fluorescent area (h) (d-e) H&E stained paraffin section of 5dpf (d) and 2mpf (e) zic4:GFP with enlarged view (d'-e') showing glial cells (arrows) and neurons (astreix). (i-j) H&E stained paraffin section of 5dpf (i) and 2mpf (j) zic4:Ras with enlarged view (i'-j') showing masses of elongated cells.

The strong larval phenotype of these models allows for analysis of drug effects already at larval stage and thus for efficient drug screening.
The glioma model also possess characteristics eligible for screening approaches including its enlarged brain size at 3 days of age suitable for microscopic evaluation. Additionally, zebrafish contain a blood-brain-barrier similar to mammals making this model ideal for drug targeting studies for the development of glioma treatment.
Our models resemble human tumor phenotypes. Additionally, their early phenotypes in larvae combined with the supportive characteristics of zebrafish including large clutch size, external development and especially translucent early development as well as easy drug administration and dose control make these models ideal for screenings for therapeutically active drugs and novel targeting approaches.

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CROSSLINKED POLY ETHYLENEIMINE IONOMER-ZN2+ COMPLEXES FOR DELIVERY OF CHEMOTHERAPEUTIC AGENTS

ALI MOHAMMAD TAMADDON[1], Samira Sadat Abolmaali[1], Gholamhossein Yousefi[1], Rasoul Dinarvand[2]
[1]Center for Nanotechnology in Drug Delivery, Shiraz University of Medical Sciences, Shiraz 71345, Iran; [2]Nanotechnology Research Centre, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 14174, Iran

A functional polyelectrolyte nanostructure was developed for delivery of water soluble chemotherapeutic agents. The complexes of poly ethyleneimine ionomer (PEI-g-mPEG) and Zn2+ were utilized as a template for crosslinking reaction with dithiodipropionic acid. Atomic force microscopy confirmed formation of soft, discrete and uniform nanoparticles. The particles demonstrated neutral zeta potential and mean hydrodynamic diameter of 162 ± 10 nm in phosphate buffered saline solution as determined by dynamic light scattering spectrometry. The ionic character of the polyamine allowed active loading of methotrexate (MTX) at a relatively high capacity ~ 57% w/w at optimum condition. The delivery system exhibited some suitable properties such as the hydrodynamic size of 117 ± 16 nm, polydispersity index of 0.22 and a prolonged swelling-controlled release profile over 24 h. The MTX loaded system showed more specific cytotoxicity against human HepG2 liver carcinoma cells if compared to free MTX at relatively high concentrations (above 1 µM). The enhanced anti-tumor activity in-vitro might be attributed to endocytic entry of the system as confirmed in the fluorescence microscopy experiment.

HYPERBRANCHED POLYDENDRONS WITH POTENTIAL NANOTHERAPY OPPORTUNITIES

LEE TATHAM[1], Fiona Hatton[2], Hannah Rogers[2], Jane Ford[2], Marco Siccardi[1], Steve Rannard[2] & Andrew Owen[1]
[1]Department of Molecular and Clinical Pharmacology, University of Liverpool, UK
[2]Department of Chemistry, University of Liverpool, UK

Dendrimers are symmetrical, perfectly branched macromolecules consisting of a core, branching points and surface groups. This architecture allows for high surface functionality and subsequently affords the materials significant scope. Dendrimers are therefore utilised in a number of biomedical applications. However, the major drawback in the use of dendrimers is the lengthy and costly synthesis required to prepare them. Dendrimers can either be synthesised via convergent or divergent routes. Each method requires a multi-step synthesis, which becomes more costly and complicated as the generation number increases. Here, we use dendrimer-like materials that combine aspects of dendrimer and branched polymer strategies to produce dendritic polymers containing linear dendritic hybrids. Hyperbranched polydendrons have distinct architectural differences with other dendronised polymer and linear-dendritic copolymer hybrids. Hyperbranched polydendrons utilise low generation dendron initiators to produce high molecular weight branched polymers and uniform nanostructures with controlled functionality, whilst maintaining facile synthesis [1].
Nanomedicines generally utilise 2 approaches, orally delivered solid drug nanoparticles (SDNs) and injected drug carriers [2-4]. Oral administration remains the preferential method of drug delivery due to improved patient compliance, reduced reliance on trained healthcare workers and reduced cost. However, nanocarriers are rarely dosed orally but may be worth exploring further for certain diseases (e.g. HIV). This work describes the pharmacological assessment of two different series of hyperbranched polydendron material sets and their suitability towards drug delivery applications. The hydrophobic dye, fluoresceinamine, was encapsulated into nine different hyperbranched polydendron materials, five with varying Generation 2 dendron to PEG ratios as initiators and four pH responsive materials with varying Generations of dendron initiator and polymer compositions. Nanoparticle sizes ranged from 45 - 249 nm and were shown to be stable in biological buffers for >80 days.

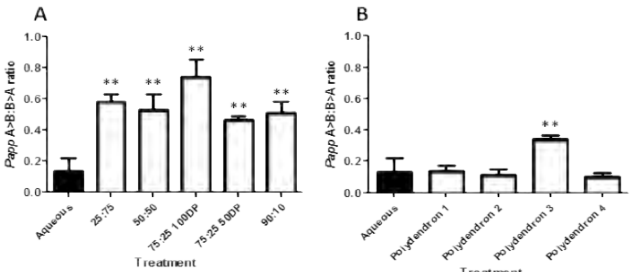

*Figure 1. Comparative apparent permeability (Papp) of fluoresceinamine encapsulated in hyperbranched polydendron materials of (A) varying G2 dendron:PEG ratios (B) pH responsive hyperbranched polydendrons, and as an aqueous (DMSO spiked) solution across Caco-2 monolayers after 4 hours incubation at 37oC 5% CO2. *, P <0.05; **, P <0.001; and ***, P <0.001 (ANOVA) (n=3).*

The transcellular permeation of the hyperbranched polydendron materials with encapsulated fluoresceinamine was assessed across differentiated Caco-2 monolayers as a model of absorption through the intestinal epithelium. Caco-2 cells are frequently used by the pharmaceutical industry [5] and have previously been assessed for nanoparticles [6]. HPLC analysis was used to determine the movement of fluoresceinamine from the apical to the basolateral compartment of the transwell plate (A>B; modelling gut to systemic circulation permeation) and conversely, from the basolateral to the apical compartment (B>A, modelling permeation from the systemic circulation to the gut). The apparent permeability results (Papp), calculated as the ratio of A>B:B>A provides a relative indication of apparent oral absorption. The results in Figure 1, suggest all the G2:PEG materials and the pH responsive 'Polydendron 3' material enhanced the apparent permeability of fluoresceinamine across the Caco-2 monolayer.
Subsequently, materials 75:25 100 DP and 'Polydendron 3' were investigated for cellular accumulation in Caco-2 and differentiated THP-1 cells as a model for macrophage accumulation [7]. The relative toxicity of each of the materials was also assessed using an adenosine triphosphate (ATP) assay (Fig 2.).

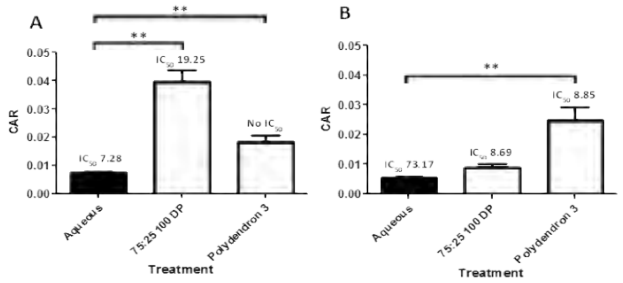

*Figure 2. Cellular accumulation of fluoresceinamine in (A) Caco-2 and (B) A-THP-1 cells. Treated cells were incubated for 24 hours at 37oC 5% CO2 with either 10 µM (final concentration) hyperbranched polydendron formulated or aqueous (DMSO spiked) fluoresceinamine. *, P <0.05; **, P <0.001; and ***, P <0.001 (ANOVA) (n=3).*

Greater cellular accumulation of fluoresceinamine was observed for both nanomaterials, in both cell types compared to the aqueous preparation of fluoresceinamine. Increased cytotoxicity was also seen in A-THP-1 cells compared to the aqueous. This increased toxicity may be attributed to the enhanced accumulation of fluoresceinamine into the cells, as the dye itself imparts toxicity. It is interesting to highlight that the pH responsive 'Polydendron 3' material had the greatest accumulation of fluoresceinamine in A-THP-1 cells. These functionalised particles, designed to collapse and release their contents below pH 6, may explain the enhanced fluoresceinamine accumulation in the macrophage-like cells upon treatment with this material.

The release of fluoresceinamine from the hyperbranched polydendron materials was investigated using an 8 kDa cut-off microdialysis. The release was compared with 2 aqueous preparations of fluoresceinamine; a DMSO dissolved fluoresceinamine solution used to spike aqueous media and a methanol dissolved preparation that was subsequently dried and reconstituted using an aqueous media. The results presented in Figure 3 A & B, highlight a similar fluoresceinamine release profile over the 4 hour incubation from the hyperbranched polydendron materials. However, variation in release profiles was observed between the two aqueous preparations, possibly highlighting the solubility enhancing role of DMSO. This is also evident in Figure 3 B, sample 'AQ dried', in which a large proportion of the fluoresceinamine is unaccounted for, suggesting the fluoresceinamine may not have been fully solubilised during the incubation.

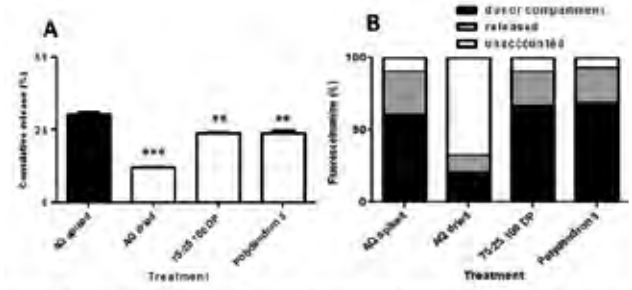

*Figure 3. Release rates of fluoresceinamine. (A) Comparison of cumulative release over 4 hours (B) Concentration of fluoresceinamine released, remaining in the donor compartment, and unaccounted for following 4 hours incubation at 37oC 120 rpm. *, P <0.05; **, P <0.001; and ***, P <0.001 (ANOVA) (n=3).*

In summary, we have shown that hyperbranched polydendrons can enhance the transcellular permeability and cellular accumulation of the encapsulated hydrophobic dye fluoresceinamine. Similar fluoresceinamine release profiles were observed in selected hyperbranched polydendron materials and a DMSO spiked aqueous preparation. This work highlights the potential of the nanomaterials for oral drug delivery and possible targeted delivery for specific pharmacological objectives. Future work is focused on understanding the mechanisms that underpin these observations.

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GLUTHATION AND CHROMATIN STRUCTURES CHANGE BY THE EFFECT OF SIZE AND ZETA POTENTIAL OF SILVER NANOPARTICLES

SHIMA TAVAKOL[a,b], Sharmin Kharrazi, Seyed Mahdi Rezayat Sorkhabadi[a,e,f]

[a]Student's Scientific Research Center, Tehran University of Medical Sciences, Tehran, Iran.

[b]Department of Medical Nanohtecnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran.

[e]Department of Toxicology & Pharmacology, School of Pharmacy, Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS), Tehran, Iran

[f]Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.

Sh_tavakol@razi.tums.ac.ir

Silver nanoparticles (AgNPs) are widely used as antibacterial agent and recently their anticancer potential has been suggested. This study focuses on the effect of size, zeta potential and ROS production of bare and citrate-coated AgNPs on cellular and bimolecular behavior. 3 different AgNPs with different size and Zeta potential were synthesized. The samples and their effect on cells and biomolecules were studied by means of UV-Vis, DLS, MTT assay, DPPH assay, circular Dichroism and DAPI staining. Based on our results it might be concluded that nanoparticles with equal size and more negative charge induced lesser harmful effects on biomolecules while larger particle with equal charge are more assimilated to organelles.

INTRODUCTION

Infection is one of the major problems in the healthcare. Recently, silver nanoparticles (AgNP) have been widely used due to their antibacterial properties. Although, use of AgNPs is expected to continue, antibacterial resistance to AgNP via plasmid gene of pMG101 is suggested. Increase of cytosolic calcium, impairment of respiratory chain in mitochondria and afterward disturbance in synthesis of ATP, breaking of DNA, protein carbonilation and lipidic membrane peroxidation are likely to be the effective mechanisms of bactericidal. Size provides important control over many of the physical and chemical properties of nanoscale materials and their effect on biological behavior. NPs with smaller particle size and higher surface/volume ratio can enter easier into the cells and mitochondria. Higher surface/volumes ratio results more active sites on their surface to interact with cell's organelles or biomolecules [1].

Zeta potential of the nanoparticles is another critical factor in determining their effect on biological behavior and can lead to different cell responses as compared to particle size. Zeta potential is electrical charge on the surface of material surrounded in the

medium. Higher Zeta potential results in higher stability and assimilation to the body because substances with high zeta potential do not tend to adhere or clump to each other. In the present work, we aimed to investigate the effect of particle size, Zeta potential and ROS production of 3 different AgNPs on cell viability, bacteriocidal efficacy, chromosomal structure and glutathione's conformation.

MATERIALS AND METHOD

AgNPs were synthesized using a simple wet chemical method by chemical reduction of Ag+ ions in water, in presence and absence of tri sodium citrate as stabilizer. Particle size, Zeta potential and ROS production were studied using UV-Visible spectroscopy, DLS and DPPH assay. Cell viability of HUVEC and bacteriostatic activity of NPs against resistant E.Coli and S. Aeurogenosa were measured by MTT assay and micro dilution methods, respectively. For investigation of effect of NPs on chromatin nucleus and on secondary structure of Glutathione, DAPI staining and Circular dichroism were done.

RESULTS AND DISCUSSION

Particle size and Zeta potential data showed that NPs with different concentrations of citrate had approximately equal Zeta potential (-23 and -21 mV) however, AgNP/HC had smaller particle size than AgNP/LC and AgNP/LC was in the particle size range of bare AgNP (17.2 and 17.8 nm). Zeta potential of AgNP was significantly lower than coated NPs (-9 mV). It might be said that citrate coating can slow down transformation and result in decrease of agglomeration and formation of more stable particles as compared to poor citrate particles.

Results of DPPH assay showed that three AgNPs induced non-significant free radical values as compared to each other at 2 ppm. However AgNP induced higher free radical as compared to control group. At 28 ppm, there was a significant increase of free radical of coated and bare particles as compared to control group but this difference was not significant between AgNP, AgNP/HC and AgNP/LC at 28 ppm. At 2 ppm concentrations, AgNP induced more ROS. This might be related to the poor coating on this NP. It is demonstrated that charge of NPs might be influence ROS production. Positively and neutrally charged NPs can induce more intracellular ROS production than negatively charged ones [3].

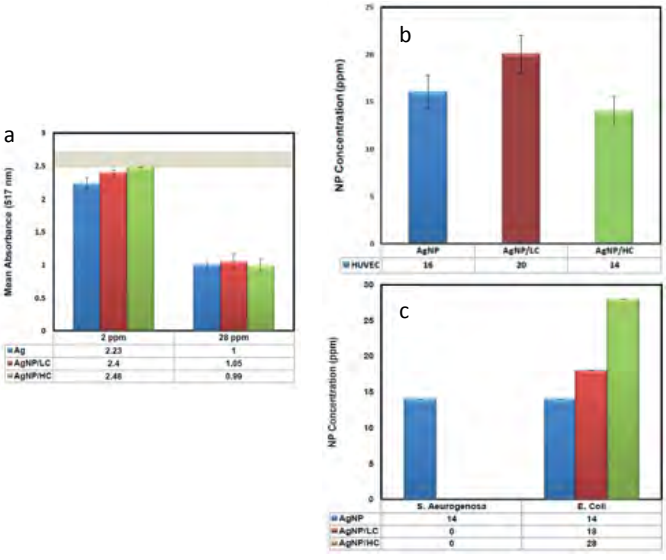

Fig. 2: a) ROS production of NPs measured by DPPH assay. Results showed that at high concentration there is not significant fiffrences between ROS production (P ≥ 0.5): b) IC50 results of HUVEC treated by coated and bare NPs. AgNP/LC induced significantly higher cell viability than AgNP/HC (P ≤ 0.5). c) MIC results of S. Aeurogenosa and E.Coli treated by coated and bare NPs.Results showed that bare AgNP had higher bacteriostatic activity than coated AgNPs.

Although, cell viability of HUVEC treated by AgNP/LC was higher than others, bacteriostatic activity of AgNP was stronger than coated NPs. These effects might be related to potential of ROS production of NPs and eventulally NPs with lower negative charge damaged morphology of mitochondria while higher negatively charged ones did not. Net charge of mitochondrial outer membrane is negative and NPs with lower negative charge such as AgNP can get adsorbed easier onto membrane than higher negative charged particles such as AgNP/LC and thus disruptive effect on mitochondria membrane decreases. Earlier studies showed that AgNPs interact with thiol groups in inner mitochondria membrane and mitochondria dysfunction is an important mechanism towards apoptosis [2]. DAPI staining was done to evaluate apoptotic cells via lighter blue chromatin in nucleus at 2, 12 and 24 ppm concentrations of AgNPs. Results from DAPI staining showed that AgNP and AgNP/LC induced apoptotic cells with lighter blue chromatin staining and AgNP/HC had rarely influenced chromatin even at high concentration. Nuclear membrane pore (NMP) consists of nuclear pore complexes (NPCs). The charge near the pore's wall and the pore's center are negative and positive, respectively. Owing to higher negative charge on the pore's wall it might be speculated that negative particles (AgNP/LC and AgNP/HC) are repelled from the pore's wall as compared to weaker negative charged particle (AgNP). Even if negatively charged AgNP/HC can enter into nucleus, negatively charged DNA tends to interact with positively charged materials and its influence on DNA will be not significant.

To gain further detailed insight into the structural features of GSH, the secondary structure changes during incubation were characterized by Far-UV CD spectroscopy. During incubation process, GSH undergoes conformational changes from β-sheet to random-coil structure.

At 2 ppm that cell viability was about 100 %, stability of GSH structure treated with AgNP was as same as highest concentration of this NP. This indicated that however, particles at this concentration did not alter cell viability but they can affect biomolecules and use of these materials chronically will show adverse side effects even at concentrations that cell viability is 100 %. Stability of GSH structure decreases with increasing the citrate concentration, and therefore Zeta potential can consider for this result. AgNP/LC had equal size compared with AgNP but stronger electronegative and higher negatively Zeta potential, made it a more assimilate to control group and resulted in lesser influence on structure stability of GSH. At 2 and 12 ppm of AgNP/HC, more GSH random coli structure was seen as compared to two others groups and stability of GSH structure decreased. Strongly negative charge and chemical stability of NP inhibit AgNP/HC strongly interact to GSH.

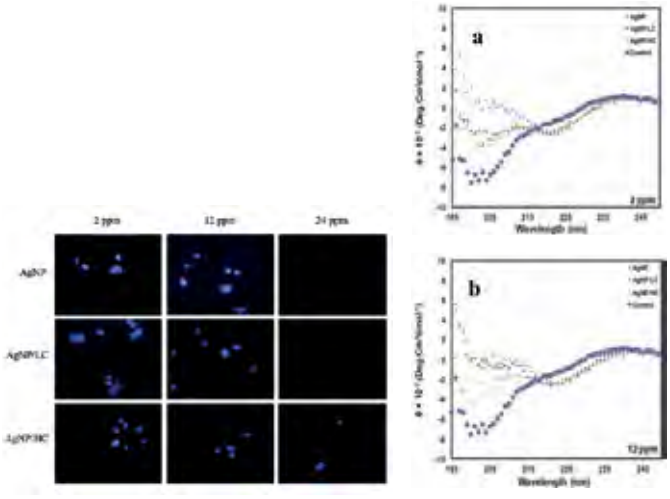

Fig. 3 a): Chromatin staining of HUVEC treated by coated and bare AgNPs. AgNP/HC had lower lighter chromatin than others and it resulted in lower broken chromatin in nucleus. b): Structural conformational GSH treated by three NPs. Results showed that AgNP/HC induced lesser changes in GSH structure than others.

CONCLUSIONS

Based on our results it might be concluded that nanoparticles with equal size and higher negatively charge induce lesser harmful effects on biomolecules while larger particle with equal charge are more assimilate to organelles. DAPI staining and circular dichroism results showed that AgNP and AgNP/HC induced highest and lowest breaking of chromosome and glutathione structural stability, respectively.

Acknowledgment: This work was supported by grant from Student's Scientific Research Center, Tehran University of Medical Sciences, Iran.

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CHARACTERIZING EPR-MEDIATED PASSIVE DRUG TARGETING USING CONTRAST-ENHANCED FUNCTIONAL ULTRASOUND IMAGING

BENJAMIN THEEK

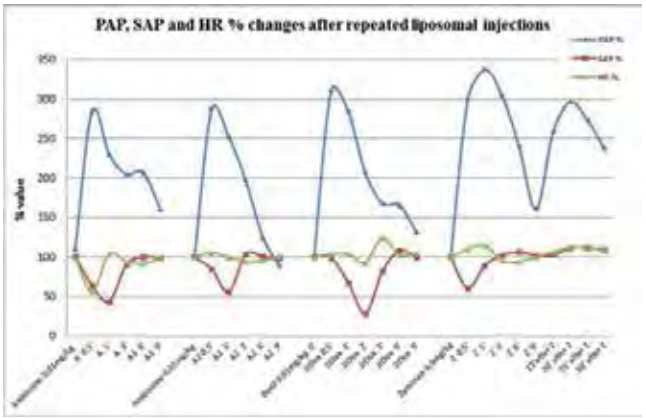
The Enhanced Permeability and Retention (EPR) effect is extensively used in drug delivery research. Taking into account that EPR is a highly variable phenomenon, we have here set out to evaluate if contrast-enhanced functional ultrasound (ceUS) imaging can be employed to characterize EPR-mediated passive drug targeting to tumors. Using standard fluorescence molecular tomography (FMT) and two different protocols for hybrid computed tomography-fluorescence molecular tomography (CT-FMT), the tumor accumulation of a ~10 nm-sized near-infrared-fluorophore-labeled polymeric drug carrier (pHPMA-Dy750) was evaluated in CT26 tumor-bearing mice. In the same set of animals, two different ceUS techniques (2D MIOT and 3D B-mode imaging) were employed to assess tumor vascularization. Subsequently, the degree of tumor vascularization was correlated with the degree of EPR-mediated drug targeting. Depending on the optical imaging protocol used, the tumor accumulation of the polymeric drug carrier ranged from 5 to 12% of the injected dose. The degree of tumor vascularization, determined using ceUS, varied from 4 to 11%. For both hybrid CT-FMT protocols, a good correlation between the degree of tumor vascularization and the degree of tumor accumulation was observed, within the case of reconstructed CT-FMT, correlation coefficients of ~0.8 and p-values of <0.02. These findings indicate that ceUS can be used to characterize and predict EPR, and potentially also to pre-select patients likely to respond to passively tumor-targeted nanomedicine treatments.

IMMUNOTOXICITY OF NANODRUGS, NANO-CARRIERS & IN VITRO - IN VIVO TESTING OF COMPLEMENT ACTIVATION RELATED PSEUDOALLERGY - CARPA

URBANICS, R.[1,2],*, Mészáros, T.[1,2], Vázsonyi, Cs.[1,2], Fülöp, T.[1,2], Krol,S.[3], Nemes, R.[4], Cerroni, B.[5], Paradossi, G.[5], Storm, G.[4,6], Metselaar, J. M.[6], Szebeni, J.[1,2]
[1] Nanomedicine Research and Education Center, Semmelweis University, Budapest, Hungary; [2] SeroScience Ltd., Budapest, Hungary; [3] Instituto Neurologico Carlo Besta, Milan, Italy; [4] Dept. Pharmaceutics, Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands; [5] Physical Chemistry, Universita degli Studi di Roma Tor Vergata, Italy; [6] Dept. Targeted Therapeutics, MIRA Institute, University of Twente, AE Enschede, The Netherlands;
*Corresponding author, E-mail: urbanicsr@gmail.com

Nanotechnology is delivering a variety of new therapeutic, diagnostic and combined activity nanoproducts with improved kinetic, targeting, efficacy and diminished toxicity properties. These compounds may provide the required complexity of the drugs to address the needs of todays medicine.

At the same time these advantageous characteristics are many times accompanied by a new type of side-effect, the Complement Activation Related PseudoAllergy – CARPA. This is an immediate hypersensitivity reaction, also called as anaphylactoid, idiosyncratic or infusion reaction and correlates with the activation of the complement (C) system. CARPA resembles the symptoms of Ig-E mediated anaphylactoid reactions, but without the presence of immunoglobuline Ig-E. Special feature is, that these reactions arise at first application of nanomaterials and are less severe or may be completely absent upon repeated exposures. The HSR occurs rapidly, it is dose dependent and usually shows spontaneous resolution. The occurrence rate is high (2-40%) and the reaction occasionally fatal. Fatal reactions are caused by cardiac and/or respiratory arrest, shock, or multi-organ failure. This reaction may be the major hurdle for nanoproducts reaching the clinical application. The importance of testing the newly developed nanodrugs, nanodiagnostics regarding this feature was recognized by FDA (Guidance for Industry; Immunotoxicology Evaluation of Investigational New Drugs, 2002) and also by the European Medicinal Agency: Reflection paper on the data requirements for intravenous liposomal products. It states that „Use of in vitro and in vivo immune reactogenicity assays such as complement (and/or macrophage/basophil activation assays) and testing for complement activation-related pseudoallergy (CARPA) in sensitive animal models should be considered to evaluate the extent of potential adverse event." (21 February 2013 Committee for Human Medicinal Products (CHMP).
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 dendrimeres, polyethylenimine block copolymers, gold nanoparticles or carbon nanotubes.
Domestic piglets are the most sensitive models to check the reactivity of nanoproducts. Rodents, mice and rats are insensitive in this regard, they need 2-3 magnitude higher dose injection to evoke pseudoallergic-like reactions. The pigs are so sensitive, than the most sensitive human beings. Pigs show all characteristics of the severe human reaction: cardiovascular and respiratory changes, blood cell alterations, skin changes. During severe CARPA reaction in pigs the pulmonary arterial pressure (PAP) usually reaching a 250-300% increase, this may be accompanied by systemic arterial pressure (SAP) increase/decrease, and with tachycardia and ECG alterations, dyspnoe and flush.
The tests are conducted on 12-14 week old, 20-35 kg weight domestic piglets. The animals are sedated in the stalls to minimize the stress, and during the experiment inhalation narcosis is maintained (2-3 % isoflurane in O_2).



The figure shows the PAP, SAP and HR changes after repeated injection of AmBisome and Doxil, both marketed liposomal drugs, and the positive reference material Zymosan. AmBisome evoked the same reaction after repeated injection, i.e. there is no tachyphylaxis.

Between the AmBisome and Doxil, the PEGylated steals liposome there is no cross reaction, but repeated Doxil injection evokes strong tachyphylactic effect. This feature, if present for a nanoproduct, with first low dose injection can be a way to prevent a severe CARPA reaction.

The main determinants of reactogeneity of nanoproducts are the size, the surface charges, the surface decorations and the shape of the substance. The injection/infusion speed can also influence the severity of reaction. The most characteristic and most often present symptom is the pulmonary arterial pressure increase. The PAP is measured by a Swan-Ganz balloon catheter in the pulmonary artery. The higher and steeper is this change, the more compromised the oxygen/CO2 exchange in the lung, causing choking sensation for a patient and eventually leading to panic reaction, further aggravating the situation.
Reactions of other nanoproducts, nanocarriers, nanodiagnostics, with different chemical compositions will be compared and the reactogeneity presented, like Chol-PEG liposomes, gold NPs, block copolymers, microbubbles will be presented on the poster.

Acknowledgement: This work was supported by EU project FP7-NMP-2012-LARGE-6-309820, NanoAthero and EU project FP7-Health-2013 CP-FP 602923-2, TheraGlio.

NANOMEDICINE: DELIVERY OF CETUXIMAB CONJUGATED-DOCETAXEL-LOADED MICELLES FOR TREATMENT OF TRIPLE NEGATIVE BREAST CANCER

RAJALETCHUMY VELOO KUTTY, David Tai Leong and Si-Shen Feng

This work aims to develop targeted therapy for Triple Negative Breast Cancer (TNBC) by using nanomedicine. TNBC is one of the most difficult cancers to cure due to a lack of targeted therapy and its insensitivity to common treatment compared to other types of breast cancer. We synthesized micelles from D-a-tocopheryl polyethylene glycol succinate (Vitamin E TPGS, or simply TPGS) which carry Docetaxel as an anticancer drug. The surface of the micelles was conjugated with Cetuximab to bind specifically to epidermal growth factor receptor (EGFR). MCF7, SKBR-3, MDA MB 468, MDA MB 231 and HCC38 cell lines, which are of estrogen/progesterone receptor (ER/PR), human epidermal growth factor receptor2 (HER2),positive breast cancer and Triple Negative Breast Cancer cell lines were employed to obtain proof-of concept experimental results for the advantages of such a design. The IC50 value of free drug (Taxotere) shows that TNBC cells are difficult to kill in comparison to the SKBR-3 and MCF7 where is it found to be 1.81,1.136,35.266,15.53 and 6.25 ug/ml for MCF7,SKBR-3, MDA MB 468, MDA MB 231 and HCC 38 respectively . However this can be overcome by using nanomedicine where the IC50 value obtained was 0.9134, 0.5994, 1.116, 1.52, 1.001 µg/mlfor MCF7, SKBR-3, MDA MB 468, MDA MB 231 and HCC38 cell lines respectively. The therapeutic effect was observed for the combination therapy of Cetuximab and Docetaxel on overexpressed and moderate expressed EGFR receptor cell where this nanomedicine was 205.6 and 223.77 fold more efficient than the free drug treatment for MDA MB 468 and MDA MB 231 cell lines respectively. Xenograft tumor model of NIR fluorescence imaging for TNBC confirmed the advantage of micelle formulation versus free dye where our micelles formulations shows higher uptake in tumor.

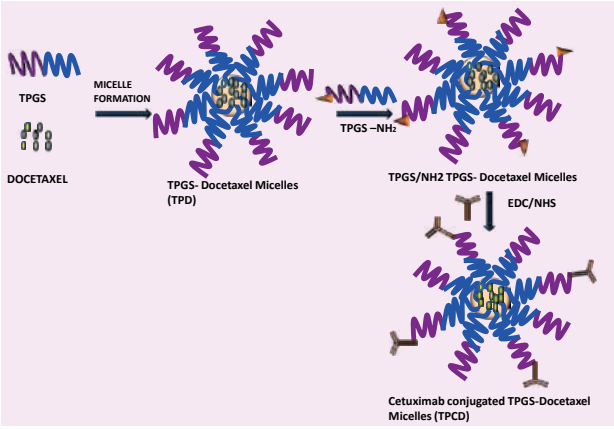


Figure 1: Schematic illustration of formulation of the Docetaxel-loaded micelles (TPD) and Cetuximab-conjugated, docetaxel-loaded micelles (TPCD).

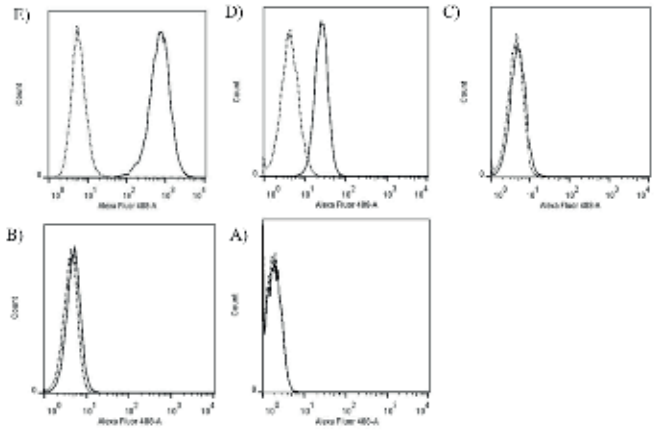


Fig.2. Representative flow cytometry for EGFR receptor expression of A) MDA MB 468, B) MDA - MB 231, C) HCC38, D) MCF 7 and E) SK-BR 3 cell lines

SYNTHESIS OF DEXTRAN-AMINE NANOPARTICLES FOR ANTICANCER TREATMENT AND DIAGNOSIS

I. WASIAK, A. Kulikowska, M. Michalak, T. Ciach
Warsaw University of Technology, Department of Chemical and Process Engineering, Laboratory of Biomedical Engineering

Cancer currently has a major impact on human health. Most of the anticancer treatments in widely use today were developed before 80s of the last century. Currently, there are numerous techniques that are used for cancer treatment, but each technique has its own limitations and adverse effects. The most common cancer treatments are surgery, radiation and chemotherapy. Chemotherapy is the treatment of cancer with one or more cytotoxic anti-neoplastic drugs. Chemotherapeutics drugs may destroy healthy tissue along with carcinomatous tissue. The cytotoxic effect of chemotherapeutic drugs is highest in bone marrow, gonads hair follicles and digestive tract all of which contain rapidly proliferating cells. The adverse effect of chemotherapy include fatigue, nausea, vomiting alopecia, gastrointestinal disturbance, impaired fertility and bone narrow suppression resulting in anemia, leucopenia and thrombocytopenia. Frequently challenge encountered by conventional chemotherapeutics include nonspecific systemic distribution of antitumor agents, inadequate drug concentration reaching the tumor site, intolerable cytotoxicity, limited ability to monitor therapeutic responses and development of drug resistance. Despite adverse effects and tumor resistance phenomena, anthracyclines are the most active and widely used chemotherapeutic agent for cancer treatment.
In the past years increasing scientific knowledge and technical innovations in the area of cell biology, biotechnology and medicine resulted in development of promising therapeutically approaches for cancer treatment. One of this novel approaches are nanomedicines. Nanomedicines can be defined as use of nanotechnology products in medicine, as drug carriers and diagnostic devices. Nanoparticles are a complex and innovative class of biopharmaceuticals. Specific delivery of chemotherapeutic compound to tumors by nanoparticles drug delivery system has a great potential to reduce the toxicity and avers effect related to most anticancer drug used today. As nanoparticles are highly sophisticated product they are able to: reduce the exposure of normal cells and tissues to cytotoxic drugs, accumulated only in carcinomatous tissue, increase drug concentration near cancer cells, prolonged half-life of the drug in biofluids, controlled release of encapsulated drug.
The main goal of presented research is to develop biodegradable polysaccharide nanometrical drug carrier for cancer treatment. In our studies we want to encapsulate anthracyclines (doxorubicin, daunorubicin) and carry them to cancer cells using passive and active targeting mechanism. The targeted delivery of discussed nanoparticles will be achieved due to the presence of glucose residues on the nanoparticles surface, based on Warburg's effect. The principle of this effect lays in about 200 times higher glucose demand in cancer cells than normal cells.

To reach this goal, dextran - amine nanoparticles were obtained. Optimal combination of polysaccharide and amine type, reagents concentration, their modification and component ratio were adjusted and defined. Our original method of nanoparticles synthesis (patent pending) is based on modified polysaccharide self assembly in water environment. First dextran molecules are partially oxidized (H2O2, IO4-) to form aldehyde groups along the chain. Oxidation process doesn't break the polysaccharide backbone but opens glucose rings along the chain. Then lipophilic side groups (aliphatic amines) can be attached to form Schiff base bond. In water, due to hydrophobic – hydrophilic forces amphiphilic molecules forms nanoparticles. Nanoparticles formation was verified by using light scattering technique. The formed nanoparticles exhibit a half life in water solution of 2-4 weeks, depending on the composition. They are very stable, biocompatible and homogeneous in size. Nanoparticles can be dried to the powder form and easily get dispersed again when immersed in water. By changing the ratio of polysaccharide and amine, and the degree of the polysaccharide oxidation we are able to control the size of obtained nanoparticles.

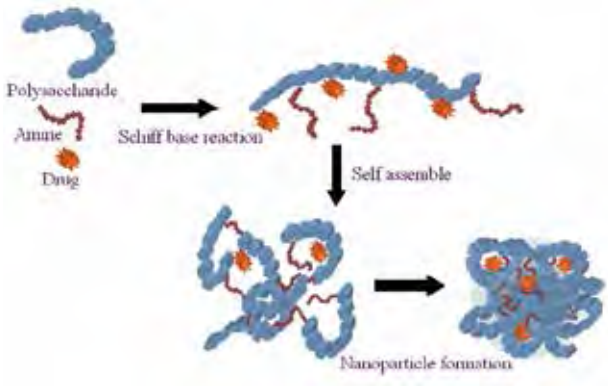


Fig.1. Graphic representation of polysaccharide – amine nanoparticles formation.

After relevant selection of reagents, nanoparticles from dextran 70 kDa with dodecylamine were considered as the most promising for anticancer nanocarriers. In such nanoparticles anthracyclines drug was encapsulated through covalently attached with effectives over 90% and drug loading capacity 4.5%.

LIPIDOID COCKTAIL: COMBINING SINGLE AND DOUBLE TAIL LIPIDOIDS FOR SYNERGISTIC TRANSFECTION

YIHANG WU[1], Linxian Li[1,2], Pavel A. Levkin[1,2], Gary Davidson[1]
[1]Institute of Toxicology and Genetics, Karlsruhe Institute of Technology, 76344 Karlsruhe, Germany.
[2]Institute of Organic Chemistry, University of Heidelberg, 69120 Heidelberg, Germany; E-mail: yihang.wu@kit.edu

INTRODUCTION

Cationic lipidoids are widely used for in vitro and in vivo gene delivery. However, the number of efficient and safe lipidoids is still limited. We use combinatorial chemistry to synthesize libraries of thioether lipidoids with different cationic head groups and with one or two hydrophobic tails. We have previously demonstrated that such lipidoids can deliver both plasmid DNA or siRNA into the cells. [1,2,3] In this study, we show that addition of certain single tail lipidoids synergise with their corresponding double tail lipidoid to enhance cellular transfection.

RESULTS AND DISCUSSION

We synthetized 17 double tail lipidoids and 17 single tail lipidoids using thiol-yne and thiol-ene click reaction chemistry. 6 amine head groups, 2 linkers and 5 alkyl thiols were used and Electrospray Ionization Mass Spectrometry (ESI-MS) was used to characterize the 34 lipidoids. Liposomes were prepared by mixing double tail and single tail lipidoids at five different ratios, keeping the overall ratio of lipidoids with DOPE at 1:1 molar ratio. HEK 293T cells were used and the transfection efficiency was calculated based on the percentage of cells transfected. The size and zeta potential of liposomes and lipoplexes were characterized by Dynamic Light Scattering (DLS). Intracellular DNA delivery and trafficking was further examined using confocal microscopy, after mixing different lipidoid formulations with cy-5 labelled LacZ plasmid. In total, 3 single tail lipidoids were found to synergise with their corresponding double tail lipidoids to enhance cellular transfection.

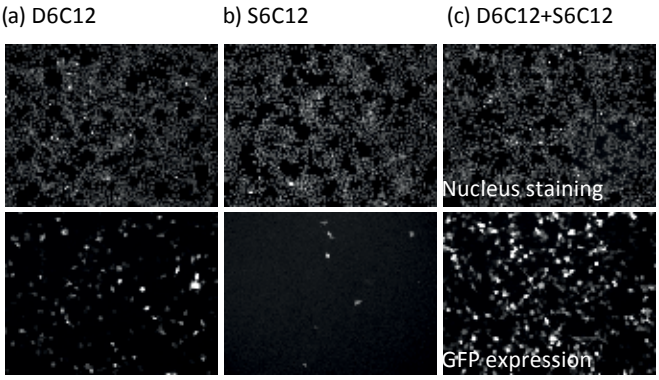


Fig. 1. Fluorescent microscope images of HEK 293T cells transfected with different lipidoid formulations/pGFP for 20h (a) Double tail lipidoid (D6C12), (b) Single tail lipidoid (S6C12) and (c) Their combination (D6C12+S6C12).

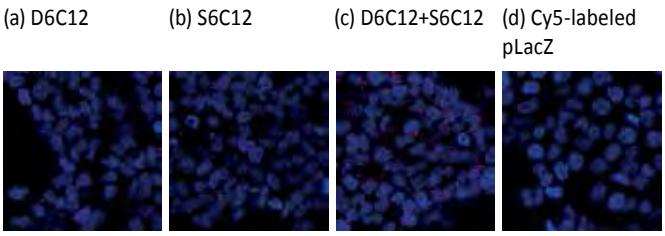


Fig. 2. Confocal microscope images of HEK 293T cells transfected with different lipidoid formulations/Cy5-labeled pLacZ for 2h (a) Double tailed lipidoid (D6C12), (b) Single tailed lipidoid (S6C12), (c) Their combination (D6C12+S6C12) and (d) Cy5-labeled pLacZ alone.

CONCLUSION

Our results show that combining single with double tail lipidoids increases uptake of lipoplexes as well as cellular transfection efficiency. These results indicated that different lipidoids with different number and length of tails may play different roles in forming liposomes and lipoplexes. We envision that lipidoid cocktails could be potentially used as a general strategy to enhance the efficiency of gene delivery.

REFERENCES

[1] Linxian Li, David Zahner, Yi Su, Christoph Gruen, Gary Davidson, Pavel A. Levkin, Biomaterials, 2012, 33, 8160–8166.
[2] Linxian Li, Fengjian Wang, Yihang Wu, Gary Davidson, Pavel A. Levkin, Bioconjugate Chem., 2013, 24 (9), 1543–1551.
[3] Kevin T. Love, Kerry P. Mahon, et al., Proc Natl Acad Sci, 2010, 107(5), 1864–1869.

EVALUATION OF HISTAMINE-FUNCTIONALIZED BLOCK COPOLYMER MICELLES AS DRUG DELIVERY SYSTEMS IN 2D AND 3D MODELS OF BREAST CANCER

YUNING ZHANG[1], Pontus Lundberg[2], Maren Diether[1], Christian Porsch[3], Michael Malkoch[3], Eva Malmström[3], Craig J. Hawker[2] and Andreas M. Nyström[1]*

[1]IMM Institute of Environmental Medicine, Nanosafety & Nanomedicine Laboratory, Karolinska Institutet, SE-17177, Stockholm, Sweden

[2]Materials Research Laboratory, University Of California, Santa Barbara, CA 93106, USA

[3]KTH Royal Institute of Technology, School of Chemical Science and Engineering, Department of Fibre and Polymer Technology, SE-10044, Stockholm, Sweden

Nanoparticle based drug delivery has been widely investigated under the last decade. One major issue remaining for effective nano-carrier development is finding ways to avoid endosomal and lysosomal entrapment of the drugs, and as a consequence retain the drugs efficacy in cells. Doxorubicin inhibits cancer cell proliferation via intercalating DNA and has been widely applied in clinic to treat several types of carcinomas, including breast cancer. In this research, a comprehensive library of polymer micelles were developed using block copolymers, poly(allyl glycidyl ether)-b-poly(ethylene glycol), which were synthesized and functionalized with different amounts of histamine, and octane or benzyl groups in order to instill a pH sensitive behavior to achieve endosomal/lysosomal escape via charge reversal of the histamine groups. These histamine groups are hydrophobic at neutral pH and transform to hydrophilic (positively charged) when encountering an acidic environment (Lundberg et al. Soft Matter, 2013), similar to the pH of endosomes or lysosomes. The octane and benzyl groups incorporated in copolymers can provide a sufficient hydrophobic core to stabilize the core-shell structure and enhance doxorubicin loading. In the following experiments, we established 2D and 3D cultures of breast cancer cells to evaluate the polymer nanoparticle library as drug delivery system. The efficacy studies performed via MTT assays indicate that DOX-NPs exhibit stronger efficacy in short time (12-24 h) treatment compared to free DOX. Further, intracellular tracking of the drug reveals that DOX from the 50% histamine containing NPs can accumulate in the nuclei as rapidly as free DOX, within 2h, and this NP also demonstrated enhanced mitochondria localization. These findings on drug localization studies indicate a small endosomal/lysosomal escape property of the nano-carriers. In addition, a 3D spheroid model of MDA-MB-231 was established and applied to the NP evaluation to investigate the DOX-NP composition associated toxicity and cargo distribution in 3D spheroids. The results are consistent with previous results suggesting that 50% histamine containing NPs, especially the one from the low histamine /low PEG molecular weight group is the most promising carriers in the library and it is potentially suitable for drug delivery application.

CLINAM 6/13 SATELLITE SESSIONS
SWISS NATIONAL SCIENCE FOUNDATION
DEUTSCHE FORSCHUNGSGEMEINSCHAFT
SWISS COMPETENCE CENTRE TEDD

CLINAM Satellite Session 1

This is a Satellite Event within the CLINAM 6/13 Summit, organized by the Swiss National Science Foundation and the Deutsche Forschungsgemeinschaft. It is held on Tuesday, June 25 in Hall Osaka-Samarkand as Session 19 (10 -13 am) within the Summit Programme. All participants of the Summit are invited to join this Session that gives excellent overview on the activities of the programmes in Germany and Switzerland.

Biological Responses to Nanoscale Particles

The Priority Programme1313 of the Deutsche Forschungsgemeinschaft (DFG) aims at • the manufacturing and characterization of NPs • the transition of NPs into an interaction with the biological environment • the impact of NPs on fundamental biological functions.

The Swiss Research Programme 62 of the Swiss National Science Foundation aims at • developing new intelligent materials and combinations of such with new functions • establishing their application potential for various sectors of industry • serving as a model for future cooperation between SNSF and CTI.

The Swiss National Research Programme 64 of the Swiss National Science Foundation aims at • identifying opportunities arising from the use of nanomaterials for health care, the environment and natural resources. • revealing the potential risks that nanomaterials pose • developing tools that maximize the advantages of nanomaterials and minimize the risks for humans and the environment • enhancing expert knowledge and competencies for developing innovative nanomaterials and assessing risk in Switzerland.

10.10 ***German Priority Programme SPP 1313***

Chair **Prof. Dr. rer. nat. Dr. h.c. Reinhard Zellner,** Senior-Professor of Physical Chemistry, University of Duisburg- Essen and Coordinator of SPP1313, Essen (D)

Impact of the Nanoparticle - Protein Corona on Biomedical Applications
Prof. Dr. Roland Stauber, Molecular and Cellular Oncology/*ENT Department*/University Medical Center of the Johannes-Gutenberg University Mainz (D)

Quantitative Fluorescence Studies of Nanoparticles interacting with Proteins and Cells
Prof. Dr. G. Ulrich *Nienhaus,* Karlsruhe Institute of Technology (KIT) and Center for Functional Nanostructures (CFN), Karlsruhe (D)

The Fate of Nanoparticles in Vivo
PD Dr. Peter Nielsen, Head Department of Biochemistry and Molecular Cell Biology Universitätsklinikum Hamburg-Eppendorf, Hamburg (D)

11.05 ***Swiss National Research Program 62***

Chair **Prof. Dr. Louis Schlapbach,** President of NRP 62, Berne (CH)

Nanoparticle Transport Across the Human Placenta
Dr. Peter Wick, Group leader, Empa Swiss Federal Laboratories for Material Science and Technology, Laboratory for Materials-Biology Interactions, St. Gallen, Switzerland

Mechano-sensitive Nanocontainers for Innovative Targeted Drug Delivery
Dr. med. Till Saxer, Cardiology Department, University Hospitals of Geneva, Switzerland

Polymer Nanoparticles for Drug-delivery and Localized Synthesis of Antibiotics.
Dr. Nico Bruns, Chemistry Department of Chemistry, University Basel (CH)

12.05 ***Swiss National Research Program 64***

Chair **Prof. em. Dr. Peter Gehr**, University of Berne, President of NRP 64, Berne (CH)

On the Relationship between Cell Entry and Nanoparticle Toxicity
Prof. Dr. Francesco Stellacci, Institute of Materials, EPFL Lausanne (CH)

Biomedical Nanoparticles as Immune-modulators
PD Dr. med. Christophe von Garnier, Department of Clinical Research, University of Berne (CH)
Transport of Nanoparticles after Release from a Biodegradable Implant
Prof. Dr. med. vet. Meike Mevissen, Division Veterinary Pharmacology & Toxicology, Vetsuisse University of Berne, Berne (CH)

CLINAM Satellite Session 2

This is a Satellite Event within the CLINAM 6/13 Summit, organized by the Swiss Competence Centre TEDD in Zürich, Switzerland. It is held on Wednesday, June 26 in Hall Rio as Session 30 (09 -13 am) within the Summit Programme. All participants of the Summit are invited to join this Session that gives excellent overview on the research and findings of the Swiss Competence Centre TEDD and their related researchers.

CLINAM Satellite (Hall Rio)

Tissue Engineering for Drug Development and Substance Testing

Chair **Prof. Dr. Ursula Graf-Hausner**, Zürich University of Applied Sciences, Wädenswil (CH)

09.00 **3D Tissue Models for Drug Development and Substance Testing – an Overview**
Prof. Dr. Ursula Graf-Hausner, Zürich University of Applied Sciences, Wädenswil (CH)

Innovative Technologies for 3D Cell Culture - Biomimetic Materials
Dr. Martin Ehrbar, Zürich University, Zürich (CH)

Bioprinting – a Promising Approach for 3D Tissue and Organ Fabrication
Marc Thurner, CEO of regenHu Ltd., Villaz-St.-Pierre (CH)

3D Liver Microtissues to Test Nanoparticle Toxicity
Dr. Jens Kelm, CSO of InSphero AG, Zürich (CH)

Break (10.00 – 10.30)

Infectious Disease Model in Cell Cultures
Dr. Bruno Schnyder, University of Applied Sciences and Art, Western Switzerland, Sion (CH)

A Novel Approach to Print an Air-blood Tissue Barrier
Prof. Dr. Barbara Rothen-Rutishauser, Chair Bionanomaterials, and **Dr. Corinne Jud**, Adolphe Merkle Institute, Université de Fribourg, Marly (CH)

Co-culture of Human 3D Liver and Skin Equivalents in a Dynamic Two-tissue Microcirculation Chip
Dr. Uwe Marx, Program Head "Multi-Organ-Chips", Technical University, Berlin and CEO TissUse, Berlin (D)

3D Microtissues for Regenerative Medicine and their Application in Cardiovascular Surgery
Prof. Dr. Dr. Simon Philipp Hoerstrup, Head Cardiovascular Research and Swiss Center for Regenerative Medicine, Zürich University, Zürich (CH)

PORTRAITS OF THE EXHIBITORS

BASEL INKUBATOR
Hochbergerstrasse 60c
4057 Basel
+41 61 633 30 10
info@basel-inkubator.ch
www.basel-inkubator.ch

BASEL INKUBATOR is a venture of the University of Basel, the University of Applied Sciences of Northwestern Switzerland FHNW, the Canton of Basel-Stadt, and "EVA - the Basel life sciences start-up agency". It opened its doors in January 2010 and was established to support high-tech spin-offs of the Universities in the region. Other applicants are welcome as long as there is space available. The location at the Stücki Business Park ensures an optimal access with public transport as well as by car.

Start-up projects are supported already in a very early phase. Typically, the first contact is already established before the decision to create a start-up is made. The BASEL INKUBATOR provides an affordable infrastructure (offices and laboratories) and coaching in various fields. A start-up company can stay up to three years within the BASEL INKUBATOR. The aim is that a company can successfully build up in this environment until either first sales are established or a major investment round is successfully closed which provides the financial resources to further develop.

Currently the BASEL INKUBATOR houses 5 life sciences companies, 4 ICT projects, 3 Med Tech company and one Nano-/Greentech company. There is apparently a positive trend to convert research result into an own business. The common infrastructure fosters a productive interaction where the start-up companies profit from each other's experiences.

The BASEL INKUBATOR is part of a interwoven network in the Basel area which is pushing the spirit of innovation in all domains. Additional needs of start-up companies like e.g. financing are covered within this network.

Please feel free to contact the BASEL INKUBATOR via info@basel-inkubator.ch.

OFFICE
Baldiri Reixac 15
08028, Barcelona
Spain

EMAIL
judith.sendra@endornanotech.com

WEB
www.endornanotech.com

Endor Nanotechnologies

In Endor we develop and manufacture healthcare products based on nanotechnology. Our products differ because its innovative technology and are oriented to oncologic and dermatological sectors.

Our technology consists in the conjugation of inorganic nanoparticles to selected active molecules. That way we create innovative materials with unique biological properties.

We apply our versatile and high technologic potential R&D platform in nanotechnology to develop products oriented to very diverse sectors, from oncology to dermatology.

The Biomedicine Unit evaluates the nanomaterial efficacy and safety in order to complete the preclinical development, for oncologic and dermatologic candidates.

We have the facilities and know-how to fully characterize the active ingredient behavior in biological media, study how they interact with cells, what cell mechanisms are involved and how to ensure product security. The objective is to get the best candidate from the security and efficacy points of view.

R&D team is specialized in nanomaterials design & synthesis and pharmaceutical development. Managing team has a wide experience in tech companies management and business development of innovative products. We are a multidisciplinar team with a shared global strategy, what allow us to be flexibles, efficient, and boost open innovation through different departments.

IBM Research

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The World is Our Laboratory - No matter where discovery takes place, IBM researchers push the boundaries of science, technology and business to make the world work better. Today, IBM researchers are redefining where discovery happens by stepping outside of the laboratory and challenging the status quo to solve some of the world's most complex problems. From monitoring energy and water desalinization in the deserts of the Middle East to using nanopolymers to fight bacteria, IBM Research is a global community of forward-thinkers working in 12 research laboratories distributed over 6 continents towards one common goal: progress.

In 1956, IBM Research established its first West Coast laboratory in San Jose, helping to create what would eventually become Silicon Valley. In 1986, IBM Research - Almaden became home to a rapidly growing team of scientists and researchers. IBM Research - Almaden boasts a rich history of breakthroughs that include the distributed relational database; the ability to position individual atoms; the first data mining algorithms; the IBM Microdrive – the world's smallest disk drive; racetrack memory; and innovations in data storage technology. Today, the researchers there are focused on new breakthroughs in areas as diverse as nanomedicine, services science and storage at the atomic scale.

IBM Research – Australia was established in October 2010, and is located in the University of Melbourne precinct, on the fringe of the Melbourne Central Business District. The lab began operation in the first half of 2011 and encompasses the IBM Research Collaboratory in Life Sciences - Melbourne, which is located at the Victorian Life Sciences Computation Initiative (VLSCI). Projects at IBM Research – Australia include work in natural resource management, disaster management, bio-nanotechnology, life sciences and healthcare.

Izon Science

- Izon Science designs and manufactures precision instrumentation for multi-parameter measurement of nano- and micro- sized particles.

- Izon instruments use unique nanopore-based detection to enable the size, charge and concentration of 50nm 20 micron sized particles to be measured on a particle-by-particle basis.

- The level of detail, accuracy and repeatability in measurement enables Izon's systems to meet the high standards required for nanomedicines going to clinical trials and into routine medical use.

- Izon originated in New Zealand and now sells its products in 35 countries. It has its European headquarters in Oxford, UK and its US headquarters are in Cambridge, MA.

APPLICATIONS

The underlying measurement technique known as Tunable Resistive Pulse Sensing (TRPS) has been applied to enable high resolution analysis of a wide range of particle types in fields including:

- **Drug Delivery / Nanomedicine Development**
  E.g. Liposomes, Nanobubbles, Polymeric Drug Delivery

- **Virus Quantification / Vaccine Production**
  E.g. Viral Vaccines, Adenovirus, Lentivirus

- **Microvesicle Research & Haematology**
  E.g. Microparticles & Exosomes, While Blood Cells, Platelets

PRODUCTS

Two instruments based on TRPS technology are currently commerically available:

- **qNano** is a compact benchtop device for highly precise physical characterization (size, zeta-potential, concentration) of a wide range of particle types.

- **qViro-X** is a purpose-built virus analysis instrument ideal for assessment of viral titre and aggregation. It meets stringent decontamination requirements ideal for manufacturing and quality control environments.

CONTACT DETAILS:

Asia Pacific 8C Homersham Place PO Box 39168, Burnside Christchurch 8053 New Zealand
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Europe / Middle East / Africa The Oxford Science Park Magdalen Centre, 1 Robert Robinson Ave Oxford OX4 4GA, UK
Tel: +44-1865-784-630, Fax: +44-1865-784-631, Email: uk-info@izon.com

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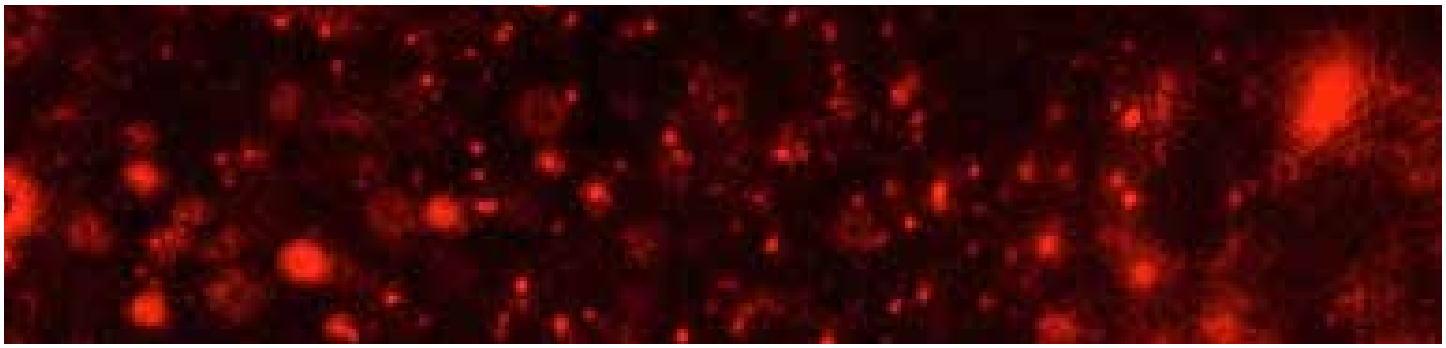
NanoSight

Making the small things count.



NanoSight delivers the world's most versatile and proven multi-parameter nanoparticle analysis in a single instrument. NanoSight visualizes, measures and characterizes virtually all nanoparticles. Particle size, concentration, aggregation and zeta potential can all be analyzed while a fluorescence mode provides speciation of labeled particles. NanoSight provides real time monitoring of the subtle changes in the characteristics of particle populations with all of these analyses uniquely confirmed by visual validation.

NanoSight has installed over 600 systems worldwide with users including BASF, GlaxoSmithKline, Merck, Novartis, Pfizer, Proctor and Gamble, Roche and Unilever together with the most eminent universities and research institutes. NanoSight's technology is validated by 800+ third party papers citing NanoSight results and by the ASTM Standard E2834, consolidating NanoSight's leadership position in nanoparticle characterization.

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Gräubernstrasse 12–14
4410 Liestal / Switzerland

Phone +41-61-927 47 47
Fax +41-61-927 47 00
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Nanosurf is a leading provider of easy-to-use atomic force microscopes (AFM) and scanning tunneling microscopes (STM). Our products and services are trusted by professionals worldwide to help them measure, analyze, and present 3D surface information. Our microscopes excel through their compact and elegant design, their easy handling, and their absolute reliability.

With FluidFM™ and ARTIDIS®, Nanosurf is now offering two unique products for life science (single cell biology) and clinical applications (tissue diagnostics):

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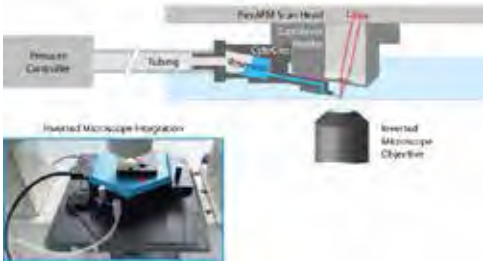
Fluid Force Microscopy combines the unique possibilities of nanofluidics by Cytosurge with the positional accuracy and force sensitivity of the Nanosurf FlexAFM atomic force microscope to provide a whole new level of control and application possibilities in single-cell biology and beyond. An inverted microscope, the FlexAFM Inverted Microscope Option, a FlexAFM system, and special hollow cantilevers are required to perform Fluid Force Microscopy.



FluidFM is an exclusive add-on product to the Nanosurf FlexAFM and features:

- Optical access to the sample and highly accurate pressure, force, and position control
- Optimized experimental workflows: (1) cell adhesion and spectroscopy mapping, (2) spatial manipulation, (3) deposition and lithography, (4) injection and extraction
- Complete system integration, operation, and handling via intuitive touchscreen control software

ARTIDIS®

A new tool for nanomechanical tissue diagnostics

Nanomechanical investigations of tissues such as breast, cartilage, and retina, have opened new ways to better understand and diagnose diseases like osteoarthritis [1] and cancer [2].

ARTIDIS® (Automated Reliable Tissue DIagnosticS) — jointly developed by Nanosurf, the Biozentrum of the University of Basel, and the Swiss Nanoscience Institute — provides nanomechanical investigation of tissues, including stiffness and adhesion measurements, and its statistical and quantitative analysis.

When a patient is suspected of cancer, time-consuming tissue analyses have thus far been necessary to obtain a reliable diagnosis. With ARTIDIS®, lengthy waiting times can now be reduced from days to hours. Nanomechanical measurements on suspect tissues provide a characteristic “fingerprint”, which allows classification into healthy tissue, benign material, or malignant tumor [2].

References
1. Loparic M. *et al.* (2010), Biophysical Journal 98, 2731–2740.
2. Plodinec M. *et al.* (2012), Nature Nanotechnology 7, 757–765.

First devices are currently being installed in expert laboratories to acquire data on various tissue types and to develop a user-friendly diagnostic instrument.

Polymun Scientific - Biopharmaceuticals and Liposomes Contract Development and Manufacturing

Polymun Scientific GmbH is a family-owned company in Klosterneuburg, Austria, founded in 1992. Our core activities are contract development and manufacturing of biopharmaceuticals as well as liposomal formulations of APIs and vaccine antigens.
Polymun operates in accordance with current GMP guidelines and holds an Austrian production license thus meeting all EU requirements for drug manufacturing.
Polymun employs a team of more than 50 dynamic and highly qualified scientists, technologists and support staff. 45% hold an academic title, and about 45% have graduated from technical schools or colleges. With quality and reliability as a pre-requisite basis, innovation and creativity are strongly encouraged at Polymun. We are a flexible partner focused on the requirements of our clients.

Liposomal formulations

Polymun offers the development of liposomal formulations for all kinds of active pharmaceutical ingredients and vaccine antigens. We manufacture GMP-material, including all necessary documentation, and assist in planning of clinical trials.
License agreements for Polymun's patented liposome production technology are offered for the respective substance on an exclusive basis. Contracts can be arranged step by step - proof of concept, in-depth analysis, GMP-material production, product license - or all in one. Industrial applicability is the focus throughout each project.
Polymun's technology enables the industrial realization of pharmaceutical and cosmetic products for liposomal drug formats. The production technology is suitable for a broad range of substances formulated by passive entrapment, active loading or membrane incorporation.
The main characteristics of our technology are: Scalability, sterility, production of homogeneous, uniform vesicles, excellent batch to batch consistency and long term stability due to a mild procedure.

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The core competence of Polymun is the development and GMP-compliant manufacture of bio-pharmaceuticals, using both mammalian and microbial cell technology. Polymun offers all steps from the gene to clinical grade material for innovator and generic products.
Contract manufacturing includes the preparation of IMPD and CMC documents. Based on our own experience we are able to support planning of phase I-III clinical trials.
As a small, independent and private company Polymun has no conflict of interests thus granting a high degree of flexibility and security for its clients including the possibility of technology transfer.

Capacity: Mammalian cells	- Stirred tank: 15l, 240l, 2 x 2,500l
	- Ultrasonic cell retention: up to 100l continuous perfusion culture
Yeast / bacteria	- Stirred tank: 50l, 750l
Downstream purification	- Process systems up to 600l/hr and 6 bar

Contact Details:

Polymun Scientific Immunbiologische Forschung GmbH, Donaustr. 99, 3400 Klosterneuburg, Austria
Tel: +43 2243 25060 300 Fax: +43 2243 25060 399 office@polymun.com www.polymun.com

SeroScience Ltd.

SeroScience Ltd. (www.seroscience.com) is a well-known Hungarian biotech company specializing in **immunotoxicology** and **liposomal formulation services**. SeroScience is a pioneer in studying pseudoallergic reactions (infusion reactions) to **nanomedicinal formulations**.

Immunotoxicology is a fast growing segment of drug safety testing and the most dominant toxicity of biologics (therapeutic antibodies, targeted liposomes, therapies based on nucleic acid) as well as imaging agents. Immunotoxicology is mandatory in drug development. **Nanomedicine** develops formulations in the nanoscale and is the most promising segment of present day drug development.

The Company's services contain both *in vitro* and *in vivo* testing. The pseudoallergy test panel is the **most complete testing portfolio in the market**.

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Biosafety testing

Medical devices, transplants, implants, pharmaceuticals and blood products require safety testing for their compatibility with human tissue and blood. The testing requirements are guided by regulatory agencies and notified bodies like FDA, EMA and ASTM.

Nanomedicine (e.g. liposomal drugs, polymer-protein conjugates, imaging agents, drug carrier nanosystems) and antibody therapeutics are in the frontline of modern pharmacotherapy but also carry unique toxicity issues. Stimulation of the immune system can cause hypersensitivity or infusion reaction, a major and potentially lethal haemoincompatibility. Infusion reactions can represent a real allergy (involving IgE) or a pseudoallergy (no IgE). The latter may arise at least in part, as a consequence of activation of the complement (C) system = C activation-related pseudoallergy (CARPA).

TECOmedical offers a range of biosafety assays and Test protocols for:

- Haemocompatability related to activation of the complement (C) system – Anaphylatoxins. Human and animal models.
- Complement C activation related to pseudoallergy (CARPA)
- Cytotoxicity

Toxicity testing: drug-induced liver and kidney injury

Predicting which drugs will prove toxic to the liver and kidney is an important aspect during drug development. Current standards for liver and kidney injury have severe limitations and are not sensitive and specific enough. In order to reduce the risk of failure of new medicines, the European Innovative Medicines Initiative (IMI) and C-Path's Predictive Safety Testing Consortium (PSTC), a program run in partnership with the Food and Drug Administration (FDA) are validating new biomarkers for liver and kidney injury. The new biomarkers are validated for clinical application, regulatory decision making in clinical drug development and in a translational context.

TECOmedical offers a range of assays for monitoring drug-induced liver and kidney injury in:

- Drug development toxicity studies using kidney and liver cell culture models
- Preclinical toxicity studies in animals (rat, mouse)
- Clinical toxicity studies in humans

TECOmedical offers customized test development based on ELISA technology, e.g. immunogenicity and host cell protein assays. As well as scientific and regulatory compliance.

to-BBB technologies BV

Contacts

Willem van Weperen
Chief Executive Officer

Address

J.H. Oortweg 19
2333 CH Leiden
The Netherlands

Telephone

+31 71 3322251

Fax

+31 84 8313409

Email

WillemVanWeperen@tobbb.com

Year Founded

2004

Financial Summary

Seed 2004-2006: ±€1M
Series-A 2007: ±€4M
Series-B 2011: ±€6M
Interim round 2013: ±€2.5M

Accum. grants/credits: ±€5M

Company Profile

to-BBB is a clinical stage blood-brain barrier company in the Netherlands with a proprietary technology (G-Technology®) to safely enhance brain drug delivery. Its lead product 2B3-101 is a brain-targeted formulation of Doxil/Caelyx, which has demonstrated higher brain levels but similar systemic exposure, resulting in therapeutic benefits in animal studies with 2B3-101. It is now achieving high therapeutic dose levels in a clinical phase I/IIa trial in patients with solid tumor brain metastases and glioma. Preliminary efficacy has been shown both intra- and extra-cranially. Upon determining maximum tolerated dose, the expansion phase of the trial will study preliminary efficacy in specific patient populations: 1) Breast Cancer brain metastases with or without trastuzumab (Herceptin). 2) Small Cell Lung Cancer brain metastases; 3) Melanoma brain metastases, and; 4) malignant glioma; The expansion phase will allow selection of most responsive tumor types for further development, while performing due diligence processes and negotiation discussions for licensing purposes.
to-BBB is developing several other products for brain diseases, either internally or in collaboration with pharma/biotech partners. Furthermore, it is evaluating oncology compounds that can benefit from the G-Technology by combining a slow release profile with enhanced brain delivery for metastatic disease including brain metastases or for primary brain tumors.

Management

Chief Executive Officer:
Willem van Weperen, MSc, MBA

Chief Scientific Officer/founder:
Pieter Gaillard, PhD

Chief Medical Officer:
Fredrik Lonnqvist, MD, PhD, Assoc. Prof.

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